**Staphylococcus aureus** is a commensal bacterium and pathogen. Identifying biomarkers for the transition from colonization to disease caused by this organism would be useful. Several **S. aureus** small RNAs (sRNAs) regulate virulence. We investigated presence and expression of 8 sRNAs in 83 **S. aureus** strains from 42 patients with sepsis or septis shock and 41 asymptomatic colonized carriers. Small pathogenicity island sRNAs sprB and sprC were clade specific. Six sRNAs had variable expression not correlated with clinical status. Expression of RNAIII was lower in strains from septic shock patients than in strains from colonized patients. When RNAIII was associated with expression of sprD, colonizing strains could be discriminated from strains in patients with bloodstream infections, including patients with sepsis and septic shock. Isolates associated with colonization might have sRNAs with target expression different from those of disease isolates. Monitoring expression of RNAIII and sprD could help determine severity of bloodstream infections.

*Staphylococcus aureus* causes many community-acquired, healthcare–related, and nosocomial infections in humans. This bacterium is a commensal organism (part of the normal microflora), but it can also infect the body at various sites. Diseases caused by **S. aureus** differ greatly, ranging from skin lesions to invasive infections. A total of 20%–30% of the healthy population is colonized with **S. aureus** in the nostrils (1), and a substantial percentage of **S. aureus** bacteremia originates from endogenous colonies in the nasal mucosa (2,3).

Clinical expression of sepsis covers a continuum of manifestations; the most serious form is septic shock. In this state, vascular offense and systemic inflammation lead to endangered cardiac function and decrease in blood pressure that cause impaired oxygen delivery, organ failure, and death. Sepsis-related deaths and lack of mitigating clinical approaches attest to our limited understanding of the complex host–**S. aureus** interactions.

**S. aureus** has high rates of transmission and increased levels of antimicrobial drug resistance and produces many virulence factors (4). To coordinate expression of virulence genes during infection, **S. aureus** uses 2-component systems, transcription factors (5) and regulatory or small RNAs (sRNAs), which function as positive (6) or negative (7) virulence determinants. There are ≈160 sRNAs in the Staphylococcal Regulatory RNA database (8). Although their functions have not been extensively investigated, some sRNAs are known to regulate virulence factors. Quorum sensing is mediated by the accessory gene regulator (agr, and RNAIII is the effector (9). Staphylococcal infection severity is based on host factors and bacterial pathogenesis (10). We investigated differences in sRNA gene content and expression levels in **S. aureus** strains isolated from patients with bloodstream infections and from asymptomatic carriers.

**Materials and Methods**

**Isolates and Sample Collection**

We obtained clinical isolates from a prospective study of all patients given a diagnosis of **S. aureus** bloodstream infections in 2006 at the Rennes University Hospital (Rennes, France), a tertiary referral hospital in western France. We selected patients with nonsevere sepsis or septic shock (11). Patients with severe sepsis (sepsis with organ dysfunction or tissue hypoperfusion improving after fluid therapy and not requiring vasopressors) were not included because their clinical status might too closely resemble nonsevere sepsis or shock. To prevent other confounding factors, immunodeficient patients were excluded: those infected with HIV; those with congenital immunodeficiency, malignant hemopathy, organ or stem cell transplants; those receiving systemic corticosteroid therapy for >3 weeks; and those undergoing another immunosuppressive treatment.

We extracted data from medical records. A nosocomial bloodstream infection was defined as either a bloodstream
infection diagnosed in a patient hospitalized for >48 hours before symptom onset or a bloodstream infection in a patient receiving chronic hemodialysis or peritoneal dialysis. For each patient, we calculated at admission the Charlson Comorbidity Index and the Simplified Acute Physiology Score (12). We also collected 41 isolates from asymptomatic carriers: 23 from medical students in Rennes; 7 from healthcare workers sampled during their medical visit at a hospital in Lausanne, Switzerland; and 11 from the National Reference Laboratory for Staphylococci in Lyon, France. The study was approved by review board at Rennes University Hospital.

**Multilocus Sequence Typing and S. aureus Protein A Typing**

*S. aureus* protein A (*spa*) typing was performed by using primers spa-1113f (5′-TAAGACGATCCCTC-GTGAGCG-3′) and spa-1514r (5′-CAGCAGTGAGTCC-GTTTTGGTT-3′). Sequences were determined by using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and a 3730xl DNA analyzer (Applied Biosystems). The *spa* repeats and types were determined by using BioNumerics (Applied Maths, Sint-Matens-Latem, Belgium) and the Ridom Spa Server (http://www.spaserver.ridom.de/). The *spa* types with similar profiles were grouped within similar lineages. Multilocus sequence typing (MLST) was performed according to procedures of Enright et al. (13). The PCR products were sequenced by using a 3730xl DNA analyzer, and sequence types (STs) were determined by using BioNumerics and the MLST database (http://www.mlst.net/). MecA1 and MecA2 primers were used to amplify a 1,102-bp gene fragment, which was used to identify mecA. Isolates were screened for *tst* and *pvl* by using real-time PCR. PCR was used to detect small pathogenicity island (P1) RNAs (spr); sprA1/2, sprB, sprC, sprD, sprX, ssrA, 6S RNA, and rsaE. Primers used are listed in online Technical Appendix Table 1 (http://wwwnc.cdc.gov/EID/article/22/9/15-1801-Technapp1.pdf). All PCR products were analyzed by using 2% agarose gel electrophoresis.

**Bacterial Cultures, RNA Isolation, and Expression Analysis**

*S. aureus* strains were grown in Luria-Bertani medium and then harvested. Cells were isolated by centrifugation and dissolved in a solution of 33 mmol/L sodium acetate, 17 mmol/L sodium dodecyl sulfate, and 1 mmol/L EDTA (pH 5.5). The cells were then mixed with glass beads and lysed by using a Fast Prep Apparatus (MP Biochemicals, LLC, Santa Ana, CA, USA).

RNAs were isolated by using water-saturated phenol (pH 5.0). RNAs were precipitated and washed with ethanol. Northern blotting of RNA markers was conducted by loading 10 µg of total RNA onto 8 mol/L urea, 8% polyacrylamide gels. Gels were subjected to electrophoresis and blotted onto nylon membranes at 30 V for 1.5 h in 0.5× Tris-HCl, borate, EDTA buffer.

Prehybridization and hybridization were performed by using ExpressHyb solution (Clontech, Mountain View, CA, USA) and 32P-labeled DNAs (online Technical Appendix Table 2). Signals were detected by using phosphorimaging (Biocompare, South San Francisco, CA, USA) and quantified. Expression levels of sRNAs in strains were monitored by using quantitative PCR and specific primers (online Technical Appendix Table 3). cDNAs were produced by using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Using the comparative cycle threshold method (Applied Biosystems), we normalized sRNA counts against transfer–messenger RNA (tmRNA) and *S. aureus* reference strain L102.

**Bacterial Protein Extracts and Western Blotting**

For preparation of protein extracts, bacteria were grown until the desired optical density (OD) at 600 nm was reached. Cells were centrifuged at 8,000 × *g* for 10 min at 4°C and suspended in lysis buffer (10 mmol/L Tris-HCl[pH 7.5], 20 mmol/L NaCl, 1 mmol/L EDTA, and 5 mmol/L MgCl2) in the presence of a protease inhibitor cocktail tablet containing 0.1 mg/mL lysisostaphin. The cells were then dissolved in 1× Laemmli buffer containing 10% β-mercaptoethanol and heated at 90°C for 5 min. Samples were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 8% polyacrylamide gels and transferred to polyvinylidene fluoride membranes at 100 V for 1 h. Membranes were blocked in Tris-buffered saline containing 5% milk.

Second immunoglobulin-binding protein (Sbi), which is an immune evasion protein, was detected by using specific antibodies as described (14), and SaeR protein was detected by using specific antibodies. Incubation with primary antibodies against Sbi (diluted 1:10,000) or antibodies against SaeR protein (diluted 1:5,000) was performed at room temperature for 2 h. Blots were incubated with antirabbit IgG peroxidase-conjugated secondary antibodies for 1 h; washed in Tris-buffered saline, 0.05% Tween; developed in ECL Western Blotting Detection Reagent (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA); and exposed on an ImageQuant LAS4000 Imaging System (GE Healthcare Bio-Sciences). Quantifications were performed by using the ImageQuant System. Levels of Sbi or SaeR protein were normalized against levels of total proteins.

All statistical tests and graphic representations were performed by using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA). Quantitative values were compared by using the Mann-Whitney U test. A p value <0.05 was considered significant.
Results

Characteristics of Patients with *S. aureus* Bloodstream Infections

A total of 42 patients (17 with septic shock and 25 with nonsevere sepsis) were included in this study (Table 1). Patients with nonsevere sepsis were more likely than those with septic shock to have nosocomial bloodstream infections (p = 0.02) and a lower Simplified Acute Physiology Score (p = 0.01). The mortality rate was also significantly higher for patients with septic shock than for patients with nonsevere sepsis (41.2% vs. 8.0%; p = 0.01). The clonal distribution of isolates (Table 2, http://wwwnc.cdc.gov/EID/article/22/9/15-1801-T2.htm) was similar to that reported for France in the European Antimicrobial Resistance Surveillance System (15).

Genotyping of Strains from Patients with Invasive Diseases and Asymptomatic Carriers

We used MLST and spa typing to analyze 83 *S. aureus* isolates from 42 blood cultures for patients with bloodstream infections or 41 nasal samples from asymptomatic carriers. Isolates clustered into 17 STs (Figure 1, panel A, http://wwwnc.cdc.gov/EID/article/22/9/15-1801-F1.htm). Of 83 strains analyzed, none had genes encoding the Panton-Valentine leukocidin, which is associated with increased virulence of certain strains. Toxic shock syndrome toxin genes were detected in infectious and methicillin-susceptible *S. aureus* colonizing strains that belonged to ST5 and ST30.

Among 37 methicillin-susceptible *S. aureus* colonizing strains, ST398 was the most common (n = 6) (online Technical Appendix Table 4). Only 4 strains isolated from nasal samples were methicillin-resistant *S. aureus* (MRSA), and these belonged to ST8 and ST22 (Table 2). Most (n = 10) MRSA isolates were ST8 (Table 2). MRSA prevalence was ~10% in healthy colonized healthcare workers and students and ~2% in the general population (17). The prevalence of MRSA in infectious samples (~21%), all positive for *mecA*, was consistent with the overall prevalence of staphylococcal infections in France (18).

We detected a predominance of the ST8 MRSA clone, which is the predominant pandemic MRSA clone in France and has *sea* and *lukED* genes. Isolates from patients with bloodstream infections and carriers were evenly distributed among the STs (Table 2). As reported by Feil et al. (19), genetic distances between the 8 group 1 STs (mean ± SD 25. ± 10.6) were longer than those between the 9 group 2 STs (20.0 ± 7.4), which is consistent with earlier emergence of group 1 isolates.

Specificities of sRNAs for Bacterial Clades

We used PCR to identify a subset of sRNAs that were specific for conserved sequences. Eight sRNAs were selected from core and accessory genomes for determining their distribution among strains. We chose sRNAs according to their presence in the accessory genome because this presence implies variability in their presence/absence among strains and their putative roles in virulence. We included the few sRNAs ubiquitously detected in bacteria. Housekeeping tmRNA and 6S RNA genes, which were detected in many bacterial species, were found in all strains. All strains also contained RNAIII, which is the quorum-sensing effector (9).

In addition, we detected 5 sRNAs expressed on PIs: sprA (s5n_3580), sprB (s5n_3600), sprC (s5n_3610), sprD (s5n_3800), and sprX (s5n_3820) (8). Because of absence of PIs *phiSa3* and vsaβ in group 1, all 5 sRNAs on PIs were detected only in group 2 STs (Table 2). We found that sprA was rarely detected in ST398 strains, sprB was not detected in all group 1 strains and STs, sprC was not detected in group 1 STs except ST398 strains, sprD was detected in all but 5 strains from both groups, and sprX was detected in all strains except ST398. Detection of sprD and sprX in most STs from both groups reflects evolution of *S. aureus*, which has been punctuated by successive acquisitions and losses of genetic elements. The presence of sprB and sprC among *S. aureus* infectious isolates indicates *S. aureus* phylogeny

| Characteristic                      | Sepsis, n = 25 | Septic shock, n = 17 | p value |
|------------------------------------|----------------|---------------------|---------|
| Male sex, %                        | 84             | 84                  | 1.00    |
| Age, y (range)                     | 62.7 (15–97)   | 68.1 (33–84)        | 0.30    |
| Nosocomial bacteremia              | 80             | 41.1                | 0.02    |
| MRSA                               | 32             | 5.8                 | 0.06    |
| Diabetes mellitus                  | 16             | 29.4                | 0.45    |
| Alcohol abuse                      | 12             | 35.3                | 0.12    |
| Charlson Comorbidity Index         | 1.4 (0–5)      | 2.1 (0–5)           | 0.06    |
| Endovascular device                | 52             | 29.4                | 0.21    |
| SAPS II                            | 43.2 (14–61)   | 60.9 (38–126)       | 0.01    |
| Delayed antibiotic therapy         | 4.6 (0–42)     | 3.1 (0–10)          | 0.42    |
| Infective endocarditis             | 12             | 17.6                | 0.70    |
| C-reactive protein                 | 186.4 (32–427) | 250.5 (67–445)      | 0.17    |
| Polynuclear neutrophils            | 14,588 (4,700–33,000) | 14,977 (4,230–26,000) | 0.84    |
| Mortality rate                     | 8              | 41.2                | 0.01    |

*Values are % or no. (range). MRSA, methicillin-resistant *S. aureus*; SAPS II, Simplified Acute Physiology Score.
and strain clonality (Figure 1, panel B). Strain genotyping showed that the sample analyzed reflected diversity of staphylococcal infections at the national level in France.

**PI-Encoded RNA Expression**

Because of low amounts (10–100 CFU/mL of blood) of bacteria isolated from patients with bloodstream infections (20), *S. aureus* isolates must be cultured before assessing sRNA expression. We selected 16 strains for subsequent analyses: 5 from nasal carriers, 6 from patients with sepsis, and 5 from patients with septic shock. Each group of samples contained strains from the same sequence types (ST5, ST8, and ST25) (Table 2). We intentionally included strains belonging to the same ST (ST8), with strains from nasal carriers, from patients with sepsis, and from patients with septic shock. Strain selection was also dictated by availability in our collection. For these 16 isolates, we assessed sRNA expression levels at OD$_{600}$ nm = 2 (early exponential), OD$_{600}$ nm = 4 (late exponential), and OD$_{600}$ nm = 8 (stationary) growth phases (Figure 2; online Technical Appendix Figure). Growth curves for all isolates were superimposable (Figure 3, panel A).

We compared overall sRNA expression levels among the infectious subgroups. For all strains, tmRNA was constitutively expressed, and there were no differences in expression among strains (Figure 2). This finding is consistent with the status of the tmRNA gene as a housekeeping gene involved in ribosome rescue (21). The 6S RNA was also constitutively expressed, and there were no differences in expression among strains. Expression of the 5 Spr RNAs varied widely among the strains (Figure 2). We found that sRNA showed different expression profiles within the same ST (online Technical Appendix Figure), which indicated the complexity and variability of sRNA-driven gene regulation in *S. aureus*.

Expression of sprD was heterogeneous in strains from asymptomatic carriers but more homogeneous in infected patients (Figure 2). These results were inferred

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**Figure 2.** Expression of 5 sRNAs and tmRNA in 16 *Staphylococcus aureus* isolates from patients with bloodstream infections (nonsevere sepsis or septic shock) and asymptomatic colonized carriers (carriage), Rennes, France. A) sprA; B) sprB; C) sprC; D) sprD; E) sprX; F) tmRNA. Isolates were derived from ST5, ST8, and ST25. Expression was measured by using Northern blotting after strain isolation and culture. Total RNAs were obtained during early exponential (E), late exponential (L), and stationary (S) growth phases. Horizontal lines indicate medians of expression for each growth phase and sRNA. tmRNA was used as reference sRNA for subsequent quantitative PCR analyses that monitored expression levels of other sRNAs because its expression is stable for all isolates at each growth phase. Black dots indicate carriage strains at E growth phase; black squares indicate carriage strains at L growth phase; black triangles indicate carriage strains at S growth phase; inverted black triangles indicate strains causing sepsis at E growth phase; black diamonds indicate strains causing sepsis at L growth phase; circles indicate strains causing sepsis at S growth phase; squares indicate strains causing shock at E growth phase; triangles indicate strains causing shock at L growth phase; inverted triangles indicate strains causing shock at S growth phase. ST, sequence type; sRNA, small RNA; spr, small pathogenicity island RNA; tmRNA, transfer–messenger RNA.
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Discrimination of Isolates by Expression Levels of Sbi
SprD and RNAIII negatively regulate expression of Sbi by blocking translation through binding with the Sbi mRNA (6,14). We performed Western blotting for 61 isolates in growth phase E by using polyclonal antibodies against intracellular and membrane proteins to monitor Sbi levels (Figure 5, panel A). As reported by Smith et al. (22), we found that molecular weights of Sbi detected in isolates were variable (mean ≈50 kDa) and the amount of Sbi varied among isolates (Figure 5, panel A). Individual assessments showed significantly lower Sbi levels in isolates from patients with bloodstream infections than in patients with nasal colonization (p = 0.04).

Discussion
A set of 83 S. aureus strains with known genotypes was collected from asymptomatic carriers and patients with nonsevere sepsis or septic shock. We used this collection for a prospective study of the presence and expression of certain sRNAs in the core and accessory genome. We also monitored expression of Sbi, an immune evasion protein whose expression is negatively controlled by the sRNAs SprD and RNAIII (14) and SacR, a positive regulator of Sbi. In clinical and carriage staphylococcal strains, the presence of ≥2 PI-encoded sRNAs, sprB (srn_3600) and sprC (srn_3610), was indicative of the presence of PIs and prophages. These PI-encoded sRNAs, particularly sprB, could...
be used as probes to improve genotyping studies. These sRNAs probably appear during the transition between ST22 and ST25 (Figure 1, panel A). The sRNA sprB is absent in most group 1 isolates. In some strains, we cannot rule out that sequence variations among the sRNA genes might hamper their amplification.

Molecular typing can identify genetic diversity of strains, which is required for epidemiologic surveillance of infections. Bacterial strain typing methods include generation of DNA banding patterns, sequencing, and hybridization. Bacterial genotyping has benefited from identification of novel locus-specific typing markers. The sRNAs might be useful probes for genotyping bacteria because their overall content varies considerably, even among closely-related strains. Furthermore, because several sRNAs are encoded by mobile genetic elements, these sRNAs reflect the acquisition/loss of virulence factors encoded by these elements and molecules that confer antimicrobial drug resistance. In phylogenetic studies, selected sRNAs located in accessory genomes might shed light on genomic diversity.

The immunologic ability of patients to eradicate pathogens is a major determinant of infection outcome, and patients with septic shock are often immunocompromised (23). Our data suggests that, for staphylococcal bloodstream infections, one must also consider attributes of infecting strains, including at least an immune evasion molecule and sRNAs RNAIII and sprD. The effector of the agr quorum-sensing system was expressed at lower levels in strains isolated from patients with bloodstream infections, especially those with septic shock, than in asymptomatic carriers. Therefore, low RNAIII levels might identify S. aureus isolates that are responsible for bloodstream infections, even after isolation and culture. agr-defective S. aureus is a frequent cause of bloodstream infections (24). Our observations concur with the previous identification of inactivating mutations in the S. aureus agr virulence regulator, which have been associated with poor outcomes in for patients with bloodstream infections (3). Coupling the expression levels of RNAIII and SprD could discriminate colonization from infection and also provide useful information about severity of bloodstream infections. However, this possibility must be confirmed with a larger set of clinical isolates.

Because sprD and RNAIII negatively control the expression of the Sbi immune evasion protein (14), we also monitored Sbi expression in the isolates. Sbi is an immune evasion factor (25) involved in the S. aureus-induced inflammatory response (26). Cell wall–anchored Sbi proteins are essential components for S. aureus survival in the commensal state (27). Detection of additional Sbi proteins in strains isolated from asymptomatic carriers than in those from patients with septic shock is consistent with their role during colonization and in immune tolerance. Expression of Sbi at the transcriptional level is positively regulated by SaeRS, which is composed of the histidine kinase SaeS.
and the response regulator SaeR (28). Expression of Sbi is controlled by ≥3 regulators: negatively by 2 sRNAs, and positively by a 2-component system. This system indicates why RNAIII and sprD levels are not inversely correlated with Sbi levels in isolates tested. Carriage strains have higher SaeR levels than the sepsis strains, which is consistent with higher Sbi levels in the carriage strains than sepsis strains (Figure 5).

The transition from commensalism to infection in *S. aureus* is an essential but complex process. From a clinical standpoint, most *S. aureus* infections are derived from previous colonizers (29). When those strains switch to invasiveness, the transition may be related to regulatory network expression changes, including within sRNAs. Sequencing has identified changes in regulatory functions of strains isolated from a person who initially was a carrier and then showed development of a fatal bloodstream infection (30), which suggested that molecular evolution might play a key role in this process. Our results suggest that certain sRNAs from the gene regulatory network in a human pathogen will provide insights into commensal-to-pathogen transitions. These sRNAs could be used as surrogate markers for severity of staphylococcal infections and as biomarkers for prophylaxis and monitoring of *S. aureus* infection. Comparison of frequency and expression of selected sRNAs in *S. aureus* isolates that colonize or become infectious might be a way to identify associations between sRNA expression and disease patterns.

In vitro expression levels of some *S. aureus* sRNAs might not reflect their in vivo levels (31). Nevertheless, direct analysis of expression levels of bacterial sRNAs directly in blood from patients with bloodstream infections is technically difficult because of low levels of bacteria collected. We compared sRNA expression in fresh isolates from patients with the same isolates after they were frozen for 3 weeks. We found no differences in RNAIII and sprD expression between isolates obtained directly from patients and isolates that had been frozen. Subsequent investigations will address the functional and clinical relevance of expression patterns of RNAIII and sprD. Broadening our investigations to include additional sRNAs might identify biomarkers that predict staphylococcal disease severity in infected patients. In addition to their roles as biomarkers, sRNAs could also be targets for innovative therapeutic approaches.

**Figure 5.** Expression levels of Sbi and SaeR proteins of *Staphylococcus aureus* isolated from patients with bloodstream infections and asymptomatic carriers, Rennes, France. A) Western blot of Sbi protein for 61 strains during early exponential growth phase. A protein sample from strain 19 was loaded on each gel and used as an internal control to prevent intensity variations of the bands between each experiment. Carriage: lane 1, 1; lane 2, 10; lane 3, 15; lane 4, 19; lane 5, L102; lane 6, 1911; lane 7, 0026; lane 8, 0310; lane 9, 2203; lane 10, 2155; lane 11, 2752; lane 12, 2167; lane 13, 1954; lane 14, 1955; lane 15, 21; lane 16, 11; lane 17, L45; lane 18, 8; lane 19, 23. Sepsis: lane 1, 49; lane 2, 18; lane 3, 88; lane 4, 82; lane 5, 46; lane 6, 71; lane 7, 59; lane 8, 75; lane 9, 91; lane 10, 86; lane 11, 9; lane 12, 43; lane 13, 20; lane 14, 52; lane 15, 85; lane 16, 104; lane 17, 54; lane 18, 58; lane 19, 61; lane 20, 101; lane 21, 62; lane 22, 74. Shock: lane 1, 26; lane 2, 44; lane 3, 17; lane 4, 14; lane 5, 42; lane 6, 30; lane 7, 34; lane 8, 45; lane 9, 57; lane 10, 89; lane 11, 27; lane 12, 11; lane 13, 28; lane 14, 430; lane 15, 069; lane 16, 886. B) and C) Sbi/total protein. D) SaeR/total protein. SaeR is a positive regulator of Sbi protein. p values (by Mann-Whitney U test) for significant differences are shown. Experiments were performed in triplicate. Horizontal lines indicate medians. To exclude loading variations between samples, values were normalized against total protein levels. AU, arbitrary units; Sbi, second immunoglobulin-binding.
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References

1. van Belkum A, Verkaik NJ, de Vogel CP, Boelens HA, Verveer J, Nouwen JL, et al. Reclassification of Staphylococcus aureus nasal carriage types. J Infect Dis. 2009;199:1820–6. http://dx.doi.org/10.1086/599119
2. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of Staphylococcus aureus bacteraemia. Study Group. N Engl J Med. 2001;344:11–6. http://dx.doi.org/10.1056/NEJM200101043440102
3. Smyth DS, Kafer JM, Wasserman GA, Velickovic L, Mathema B, Holzman RS, et al. Nasal carriage as a source of agr-defective Staphylococcus aureus bacteraemia. J Infect Dis. 2012;206:1168–77. http://dx.doi.org/10.1093/infdis/jsq483
4. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28:603–61. http://dx.doi.org/10.1128/CMR.00134-14
5. Rosenstein R, Gotz F. What distinguishes highly pathogenic Staphylococci from medium- and non-pathogenic? Curr Top Microbiol Immunol. 2013;358:33–89. http://dx.doi.org/10.1007/978-3-662-45793-1_286
6. Chabelskaya S, Gaillot O, Felden B. A Staphylococcus aureus small RNA is required for bacterial virulence and regulates the expression of an immune-evasion molecule. PLoS Pathog. 2010;6:e1000927. http://dx.doi.org/10.1371/journal.ppat.1000927
7. Romilly C, Lay C, Tomasini A, Caldelari I, Benito Y, Hammann P, et al. A non-coding RNA promotes bacterial persistence and decreases virulence by regulating a regulator in Staphylococcus aureus. PLoS Pathog. 2014;10:e1003979. http://dx.doi.org/10.1371/journal.ppat.1003979
8. Sassi M, Augagneur Y, Mauro T, Ivaín L, Chabelskaya S, Hallier M, et al. SRD: a Staphylococcus regulatory RNA database. RNA. 2015;21:1005–17. http://dx.doi.org/10.1261/rna.049346.114
9. Novick RP, Geissinger E. Quorum sensing in staphylococci. Annu Rev Genet. 2008;42:541–64. http://dx.doi.org/10.1146/annurev.genet.42.110807.091640
10. Powers ME, Babek Budeberg J. Igniting the fire: Staphylococcus aureus virulence factors in the pathogenesis of sepsis. PLoS Pathog. 2014;10:e1003871. http://dx.doi.org/10.1371/journal.ppat.1003871
11. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med. 2013;39:165–228. http://dx.doi.org/10.1007/s00134-012-2769-8
12. Christensen S, Johansen MB, Christiansen CF, Jensen R, Lemseshow S. Comparison of Charlson Comorbidity Index with SAPS and APACHE scores for prediction of mortality following intensive care. Clin Epidemiol. 2011;3:205–11. http://dx.doi.org/10.2147/CLEP.S20247
13. Erridge MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol. 2000;38:1008–15.
14. Chabelskaya S, Bordeau V, Felden B. Dual RNA regulatory control of a Staphylococcus aureus virulence factor. Nucleic Acids Res. 2014;42:4847–58. http://dx.doi.org/10.1093/nar/gku119
15. Dauwalder O, Lina G, Durand G, Bes M, Meugnier H, Jarlier V, et al. Epidemiology of invasive methicillin-resistant Staphylococcus aureus clones collected in France in 2006 and 2007. J Clin Microbiol. 2008;46:3454–8. http://dx.doi.org/10.1128/JCM.01050-08
16. Cooper JE, Feil EJ. The phylogeny of Staphylococcus aureus: which genes make the best intra-species markers? Microbiology. 2006;152:1297–305. http://dx.doi.org/10.1099/mic.0.28620-0
17. Robert J, Tristan A, Cavelie L, Decousser JW, Bes M, Etienne J, et al. Panton-Valentine leukocidin--positive and toxic shock syndrome toxin 1--positive methicillin-resistant Staphylococcus aureus: a French multicenter prospective study in 2008. Antimicrob Agents Chemother. 2011;55:1734–9. http://dx.doi.org/10.1128/AAC.01221-10
18. Le Moing V, Alla F, Doco-Lecompte T, Delahaye F, Piroth L, Chirouze C, et al. Staphylococcus aureus bloodstream infection and endocarditis: a prospective cohort study. PLoS ONE. 2015;10:e0127385. http://dx.doi.org/10.1371/journal.pone.0127385
19. Feil EJ, Cooper JE, Grundmann H, Robinson DA, Erridge MC, Berendt T, et al. How clonal is Staphylococcus aureus? J Bacteriol. 2003;185:3307–16. http://dx.doi.org/10.1128/JB.185.11.3307-3316.2003
20. Kiehn TE, Wong B, Edwards FF, Armstrong D. Comparative recovery of bacteria and yeasts from lysis-centrifugation and a conventional blood culture system. J Clin Microbiol. 1983;18:300–4.
21. Keiler KC. Mechanisms of ribosome rescue in bacteria. Nat Rev Microbiol. 2015;13:285–97. http://dx.doi.org/10.1038/nrmicro3438
22. Smith EJ, Visai L, Kerrigan SW, Speziale P, Foster TJ. The Sbi protein is a multifunctional immune evasion factor of Staphylococcus aureus. Infect Immun. 2011;79:3801–9. http://dx.doi.org/10.1128/IAI.05075-11
23. Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. Lancet Infect Dis. 2013;13:260–8. http://dx.doi.org/10.1016/S1473-3099(13)70001-X
24. Painter KL, Krishna A, Wigneshweraraj S, Edwards AM. What role does the quorum-sensing accessory gene regulator system play during Staphylococcus aureus bacteraemia? Trends Microbiol. 2014;22:676–85. http://dx.doi.org/10.1016/j.tim.2014.09.002
25. Burman JD, Leung E, Atkins KL, O’Seaghdha MN, Lango L, Bernado P, et al. Interaction of human complement with Sbi, a staphylococcal immunoglobulin-binding protein: indications of a novel mechanism of complement evasion by Staphylococcus aureus. J Biol Chem. 2008;283:17579–93. http://dx.doi.org/10.1074/jbc.M800265200
26. Gonzalez CD, Ledo C, Giai C, Garofalo A, Gomez MI. Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus. Nat Rev Microbiol. 2014;12:49–62. http://dx.doi.org/10.1038/nrmicro3161
28. Rogasch K, Ruhmling V, Pane-Farre J, Hoper D, Weinberg C, Fuchs S, et al. Influence of the two-component system SaeRS on global gene expression in two different *Staphylococcus aureus* strains. *J Bacteriol*. 2006;188:7742–58. http://dx.doi.org/10.1128/JB.00555-06

29. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis*. 2005;5:751–62. http://dx.doi.org/10.1016/S1473-3099(05)70295-4

30. Young BC, Golubchik T, Baty EM, Fung R, Larner-Svensson H, Votintseva AA, et al. Evolutionary dynamics of *Staphylococcus aureus* during progression from carriage to disease. *Proc Natl Acad Sci U S A*. 2012;109:4550–5. http://dx.doi.org/10.1073/pnas.1131219109

31. Song J, Lays C, Vandenesch F, Benito Y, Bes M, Chu Y, et al. The expression of small regulatory RNAs in clinical samples reflects the different life styles of *Staphylococcus aureus* in colonization vs. infection. *PLoS ONE*. 2012;7:e57294. http://dx.doi.org/10.1371/journal.pone.0037294

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**Including:**

- *Escherichia coli* O157 Outbreaks in the United States, 2003–2012
- Underrecognition of Dengue during 2013 Epidemic in Luanda, Angola
- Health Care–Associated Infection Outbreak Investigations in Outpatient Settings, Los Angeles County, California, USA, 2000–2012
- Differentiation of Acute Q Fever from Other Infections in Patients Presenting to Hospitals, the Netherlands
- Risk for Mycobacterial Disease among Patients with Rheumatoid Arthritis, Taiwan, 2001–2011
- Phylogeography of Influenza A(H3N2) Virus in Peru, 2010–2012
- Susceptibility of Carrion Crows to Experimental Infection with Lineage 1 and 2 West Nile Viruses
- Response Strategies against Meningitis Epidemics after Elimination of Serogroup A Meningococci, Niger
- Influenza A Viruses of Human Origin in Swine, Brazil
- Prevalence of Hepatitis E Virus Infection in Pigs at the Time of Slaughter, United Kingdom, 2013
- Development of Framework for Assessing Influenza Virus Pandemic Risk
- Seasonal Patterns of Buruli Ulcer Incidence, Central Africa, 2002–2012
- Human–Bat Interactions in Rural West Africa
- Occupational Exposure to Dromedaries and Risk for MERS-CoV Infection, Qatar, 2013–2014
- Cutaneous *Legionella longbeachae* Infection in Immunosuppressed Woman, United Kingdom
- *Bartonella* spp. and *Coxiella burnetii* Associated with Community-Acquired, Culture-Negative Endocarditis, Brazil
- Detection and Full-Length Genome Characterization of Novel Canine Vesiviruses
- Smallpox Vaccination of Laboratory Workers at US Variola Testing Sites
- Enterovirus A71 Meningoencephalitis Outbreak, Rostov-on-Don, Russia, 2013
- Extensive Nosocomial Transmission of Measles Originating in Cruise Ship Passenger, Sardinia, Italy, 2014
- Transmission Models of Historical Ebola Outbreaks
- Multidrug-Resistant Tuberculosis in Patients for Whom First-line Treatment Failed, Mongolia, 2010–2011
- Geographic Distribution and Expansion of Human Lyme Disease, United States
- Infections with *Candidatus* Neoehrlichia mikurensis and Cytokine Responses in 2 Persons Bitten by Ticks, Sweden
- Ribavirin for Chronic Hepatitis Prevention among Patients with Hematologic Malignancies
- Macrolide-Resistant *Mycoplasma pneumoniae*, United States
- Rabies Postexposure Prophylaxis for Travelers Injured by Nonhuman Primates, Marseille, France, 2001–2014
- Genome of Emerging Norovirus GII.17, United States, 2014
- Measles Vaccination Coverage and Cases among Vaccinated Persons

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