The SaeRS Two-Component System Controls Survival of \textit{Staphylococcus aureus} in Human Blood through Regulation of Coagulase

Haiyong Guo\textsuperscript{1,2}, Jeffrey W. Hall\textsuperscript{2}, Junshu Yang\textsuperscript{2} and Yinduo Ji\textsuperscript{2*}

\textsuperscript{1} Department of Biological Science, School of Life Science, Jilin Normal University, Siping, China, \textsuperscript{2} Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St Paul, MN, United States

The SaeRS two-component system plays important roles in regulation of key virulence factors and pathogenicity. In this study, however, we found that the deletion mutation of \textit{saeRS} enhanced bacterial survival in human blood, whereas complementation of the mutant with SaeRS returned survival to wild-type levels. Moreover, these phenomena were observed in different MRSA genetic background isolates, including HA-MRSA WCUH29, CA-MRSA 923, and MW2. To elucidate which gene(s) regulated by SaeRS contribute to the effect, we conducted a series of complementation studies with selected known SaeRS target genes \textit{in trans}. We found coagulase complementation abolished the enhanced survival of the SaeRS mutant in human blood. The \textit{coa} and \textit{saeRS} deletion mutants exhibited a similar survival phenotype in blood. Intriguingly, heterologous expression of coagulase decreased survival of \textit{S. epidermidis} in human blood. Further, the addition of recombinant coagulase to blood significantly decreased the survival of \textit{S. aureus}. Further, analysis revealed staphylococcal resistance to killing by hydrogen peroxide was partially dependent on the presence or absence of coagulase. Furthermore, complementation with coagulase, but not SaeRS, returned \textit{saeRS/coa} double mutant survival in blood to wild-type levels. These data indicate SaeRS modulates bacterial survival in blood in coagulase-dependent manner. Our results provide new insights into the role of staphylococcal SaeRS and coagulase on bacterial survival in human blood.

Keywords: \textit{S. aureus}, survival, two-component system, SaeRS, coagulase

INTRODUCTION

\textit{Staphylococcus aureus} is an important pathogen that can cause various infections, including skin and soft tissue infection and systemic infections such as pneumonias, endocarditis, and toxic shock syndrome (Klevens et al., 2007; Gordon and Lowy, 2008). The pathogenicity of \textit{S. aureus} is attributable to its ability to produces different virulence factors, including a series of cell wall-associated proteins and a range of extracellular cytotoxins, proteases, DNases, and enterotoxins (Foster and Höök, 1998), which enable the bacteria to evade innate and or adaptive immune systems (Liu et al., 2005; Clauditz et al., 2006; Lacey et al., 2016) and induce disease.

Two-component signal regulatory systems (TCSs) collaborate with transcriptional regulators to regulate the expression of virulence factors, which in turn contribute to the pathogenesis of
S. aureus. The well-studied global and TCSs virulence regulators include Agr (Novick, 2003; Montgomery et al., 2010; Thoenedl and Horswill, 2013), ArlRS (Fournier et al., 2001; Bronner et al., 2004; Liang et al., 2005), SaeRS (Giraudo et al., 1999; Liang et al., 2006; Voyich et al., 2009; Nygaard et al., 2010; Zurek et al., 2014; Liu et al., 2016), SarA (Chien et al., 1999; Cheung et al., 2008; Li et al., 2016; Loughran et al., 2016), and MgrA (Ballal et al., 2009; Gupta et al., 2015).

The SaeRS TCS controls the expression of critical virulence genes. SaeRS up-regulates the transcription and expression of hla, hlb, hlgABC, lukED, and coa in vitro (Giraudo et al., 1999; Liang et al., 2006; Rogasch et al., 2006; Nygaard et al., 2010), as well as controls hla expression in vivo, as the sae null mutation significantly decreased the expression of α-toxin (hla) during infection (Goerke et al., 2001, 2005). The mutation of sae eliminated the expression of fnbA, but increased the expression of CP5 in S. aureus strain Newman (Steinhuber et al., 2003). As a consequence of SaeRS signaling pathway disruption, the cytotoxicity and ability of S. aureus to adhere to and invade epithelial cells (Liang et al., 2006) and endothelial cells (Steinhuber et al., 2003) is impaired. In addition, it has been demonstrated that the SaeRS system is an important virulence regulator in various animal models of infection (Goerke et al., 2001, 2005; Liang et al., 2006; Voyich et al., 2009; Montgomery et al., 2010; Cho et al., 2015; Zhao et al., 2015).

Staphylococcal coagulase is directly regulated by SaeRS (Liu et al., 2016) and is an important factor for distinguishing S. aureus and coagulase negative staphylococci. Coagulase converts host prothrombin to staphylothrombin, leading to activation of the protease activity of thrombin. It was predicted that coagulase could protect bacteria from phagocytic and immune defenses by causing localized clotting, however, there are contradictory reports regarding the role of coagulase in pathogenicity in animal models of infection (Baddour et al., 1994; Moreillon et al., 1995; Cheng et al., 2010).

In this study, we aimed to determine the role of SaeRS in survival capacity of S. aureus in human blood. We utilized two published SaeRS mutant strains, including hospital-acquired methicillin resistant S. aureus (HA-MRSA) WCUH29 and USA400 community-associated (CA)-MRSA MW2 isolates, generated a saeRS deletion mutant in a USA300 CA-MRSA 923 human isolate, and examined the impact of the saeRS mutation on bacterial survival in the blood. Using targeted complementation studies, gene deletions, and purification of recombinant proteins, we determined the SaeRS regulated coa gene, encoding coagulase, was a mediator of enhanced saeRS mutant survival in human blood. Hence, modulation of coa expression by SaeRS may contribute to S. aureus survival in human blood and bacteremia.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Growth Media
The bacterial strains and plasmids used in this study are listed in Table 1. Escherichia coli DC10B (gift of T.J. Foster) served as the host for all in vitro recombinant DNA (Monk et al., 2012).

E. coli transformants were selected on Brain Heart Infusion (BHI; Difco) agar containing erythromycin (100 µg/ml) or Luria-Bertani agar containing ampicillin (100 µg/ml). S. aureus was cultured in Trypticase Soy Broth (TSB; Difco) or on TSA agar at 37°C with appropriate antibiotics. All bacterial cell cultures were incubated with shaking at 220 RPM. S. aureus transformants were selected on TSA containing chloramphenicol (10 µg/ml) or erythromycin (5 µg/ml).

Construction of the saeRS and coa Gene Deletion Mutants, and the saeRS and coa Gene Complemented Strains
Deletion of saeRS and/or coa was carried out following the pKOR1 allelic exchange protocol as described (Sun et al., 2005; Baé and Schneewind, 2006) and primers sets saeRS-pKOR1-For/Rev and coa-pKOR1-R For/Rev listed in Table 2. The R-For primer was synthesized with a 5’ phosphate group. Each PCR fragment was purified and the two fragments were ligated together with T4 DNA ligase (Promega). The ligation product was mixed with BP Clonase, per manufacturer’s instructions, and plasmid pKOR1, incubated at 25°C overnight, then transformed into E. coli DC10B. The pKOR1-saeRSKO or pKOR1-coaKO plasmid was subsequently transformed into S. aureus 923, WCUH29, or MW2, respectively. Big colonies were re-streaked to fresh TSA plates and deletion of saeRS and/or coa was confirmed by diagnostic PCR.

In order to examine whether the expression of saeRS, coa in trans can complement the effect of the mutation of the respective endogenous gene, we constructed recombinant plasmids, including pYH4/saeRS, pYH4/sa1000, pYH4/efb, pYH4/coa by cloning the saeRS, sa1000, efb, fnbAB, or coa coding region (which was obtained by PCR) into the AscI and Pmel sites of pYH4 (Huang et al., 2004), and electroporated into the saeRS and/or coa knockout mutant, respectively, resulting in Sa371com, Sa371/pYH4-sa1000, Sa371/pYH4-efb, Sa371/pYH4-fnbAB, Sa371/pYH4-coa, and coa complementation strains in Table 1. The recombinant plasmid DNA were isolated from the complementary strains and confirmed by PCR and DNA sequencing (data not shown).

Cloning, Expression, and Purification of Coagulase-His Tagged Fusion Protein in Escherichia Coli
The coa gene was obtained by PCR amplification using the primers listed in Table 2, cloned into pET24b, and resulted in pET24b-coa. Plasmid pET24b-coa was introduced into a BL21(DE3) strain. The resulting strain was grown in LB medium at room temperature; the expression of coagulase was induced when the culture media reached OD600 nm equal to 0.6 by addition of 1 mM IPTG (isopropyl-b-D-thiogalactoside) and incubation pursued for 4 h. The coagulase-his tagged protein was purified using Ni-NTA agarose column (Novagen) and examined using 12% SDS-PAGE and Coomassie Blue staining as described (Yang et al., 2015). The purification of SarZ-6xHis was carried out as described using the previously constructed pET24b-sarZ plasmid (Liang et al., 2011).
TABLE 1 | Bacterial strains and plasmids used in this study.

| Strain or plasmid | Relevant characteristics | References |
|-------------------|--------------------------|------------|
| DC10B             | Dam~ E. coli            | Monk et al., 2012 |
| BL21              | Recombinant protein expression strain E. coli |   |
| WCUH-29           | Human clinical MRSA isolate, sigB^{+}/srbU^{+} | NCIMB40771; Hall et al., 2017 |
| WCUH-29/pYH4      | WCUH29 with empty pYH4; Erm^{R} | Sun et al., 2005 |
| Sa371             | WCUH29 saeS allelic replacement mutant with tetA gene; Tc^{R} | Liang et al., 2006 |
| Sa371/pYH4        | WCUH29 saeS::tetA with pYH4; Tc^{R}, Erm^{R} | Liang et al., 2006 |
| Sa371.com         | WCUH29 saeS::tetA with pYH4-saeS; Tc^{R}, Erm^{R} | Liang et al., 2006 |
| Sa371/pYH4-1000   | WCUH29 saeS::tetA with pYH4-sa1000; Tc^{R}, Erm^{R} | This study |
| Sa371/pYH4-efb    | WCUH29 saeS::tetA with pYH4-efb; Tc^{R}, Erm^{R} | This study |
| Sa371/pYH4-fnbAB  | WCUH29 saeS::tetA with pYH4-fnbAB; Tc^{R}, Erm^{R} | This study |
| Sa371/pYH4-coa    | WCUH29 saeS::tetA with pYH4-coa; Tc^{R}, Erm^{R} | This study |
| WCUH29::coa       | WCUH29 coa deletion mutant | This study |
| Sa371::coa        | WCUH29 saeS and coa double mutant; Tc^{R} | This study |
| WCUH29::coa/pYH4  | WCUH29 coa deletion mutant with pYH4; Erm^{R} | This study |
| WCUH29::coa/pYH4-coa | WCUH29 coa deletion mutant with pYH4-coa; Erm^{R} | This study |
| Sa371::coa/pYH4   | WCUH29 saeS and coa double mutant with pYH4; Tc^{R}, Erm^{R} | This study |
| Sa371::coa/pYH4-coa | WCUH29 saeS and coa double mutant with pYH4-coa; Tc^{R}, Erm^{R} | This study |
| Sa371::coa/pYH4-saeRS | WCUH29 saeS and coa double mutant with pYH4-saeRS; Tc^{R}, Erm^{R} | This study |
| 923               | USA300 CA-MRSA          | Montgomery et al., 2010 |
| 923::saeRS        | 923 saeRS deletion mutant | This study |
| 923::coa          | 923 coa deletion mutant | This study |
| 923::saeRS::coa   | 923 saeRS and coa double deletion mutant | This study |
| 923::pYH4         | 923 with pYH4; Erm^{R} | This study |
| 923::saeRS::pYH4  | 923 saeRS deletion mutant with pYH4; Erm^{R} | This study |
| 923::saeRS::pYH4-saeRS | 923 saeRS deletion mutant with pYH4-saeRS; Erm^{R} | This study |
| 923::coa::pYH4    | 923 coa deletion mutant with pYH4; Erm^{R} | This study |
| 923::coa::pYH4-coa | 923 coa deletion mutant with pYH4-coa; Erm^{R} | This study |
| 923::saeRS::pYH4-coa | 923 saeRS and coa double deletion mutant with pYH4-coa; Erm^{R} | This study |
| 923::saeRS::pYH4-saeRS | 923 saeRS and coa double deletion mutant with pYH4-saeRS; Erm^{R} | This study |
| MW2               | USA400 CA-MRSA human clinical isolate | Herold et al., 1998 |
| NW2::saeRS::spec  | MW2 saeRS allelic replacement with spectinomycin resistant gene; Spec^{R} | Voyich et al., 2009 |
| NW2::saeRS::pYH4  | NW2 saeRS::spec with pYH4; Erm^{R}, Spec^{R} | This study |
| NW2::saeRS::pYH4-saeRS | NW2 saeRS::spec with pYH4-saeRS; Erm^{R}, Spec^{R} | This study |
| MW2::coa          | MW2 coa deletion mutant | This study |
| MW2::saeRS::spec::coa | MW2 saeRS and coa double mutant; Spec^{R} | This study |
| MW2::pYH4         | MW2 with pYH4; Erm^{R} | This study |
| MW2::coa::pYH4    | MW2 coa deletion mutant with pYH4; Erm^{R} | This study |
| MW2::saeRS::pYH4-coa | MW2 coa deletion mutant with pYH4-coa | This study |
| MW2::saeRS::spec::coa::pYH4 | MW2 saeRS and coa double mutant with pYH4; Erm^{R}, Spec^{R} | This study |
| MW2::saeRS::spec::coa::pYH4-coa | MW2 saeRS and coa double mutant with pYH4-coa; Erm^{R}, Spec^{R} | This study |
| MW2::saeRS::spec::coa::pYH4-saeRS | MW2 saeRS and coa double mutant with pYH4-saeRS; Erm^{R}, Spec^{R} | This study |
| S. epidermidis    | Coagulase negative | Microbiology teaching lab |
| S. epidermidis::pYH4 | S. epidermidis with pYH4; Erm^{R} | This study |
| S. epidermidis::pYH4-coa | S. epidermidis with pYH4-coa; Erm^{R} | This study |

PLASMIDS

| Plasmid            | Relevant characteristics | References |
|--------------------|--------------------------|------------|
| pYH4               | Shuttle vector with Tc inducible promoter; Erm^{R} | Huang et al., 2004 |
| pYH4-sa1000        | sa1000 cloned downstream of pYH4 tet promoter; Erm^{R} | Liang et al., 2006 |
| pYH4-efb           | efb (sa1003) cloned downstream of pYH4 tet promoter; Erm^{R} | Liang et al., 2006 |
| pYH4-coa           | coa cloned downstream of pYH4 tet promoter; Erm^{R} | This study |

(Continued)
TABLE 1 | Continued

| Strain or plasmid | Relevant characteristics | References |
|-------------------|--------------------------|------------|
| pYH4-frnAB        | fnbAB cloned downstream of pYH4 tet promoter; Erm<sup>R</sup> | This study |
| pYH4-saeS         | saeS cloned downstream of pYH4 tet promoter; Erm<sup>R</sup> | Liang et al., 2006 |
| pYH4-saeRS        | saeRS cloned downstream of pYH4 tet promoter; Erm<sup>R</sup> | This study |
| pKOR1             | Temperature sensitive inducible allelic exchange plasmid for S. aureus; Cm<sup>R</sup> | Bae and Schneewind, 2006 |
| pKOR1-coa         | pKOR1 with in-frame coa upstream/downstream deletion region; Cm<sup>R</sup> | This study |
| pKOR1-saeRS       | pKOR1 with in-frame saeRS upstream/downstream deletion region; Cm<sup>R</sup> | This study |
| pET24b            | saz cloned his-tag expression vector PET24b | Liang et al., 2011 |
| pET24b-coa        | coa cloned his-tag expression vector PET24b | This study |

TABLE 2 | Oligonucleotides used in this study.

| Primer         | Sequence (5′-3′) |
|---------------|-----------------|
| saeRS-K0-pKOR1-L-F | CACGATCAAGTAAATGGTGTCAT |
| saeRS-K0-pKOR1-L-R | GTAACATTACAAAAATTAGACATTAGCTCATAATC |
| saeRS-K0-pKOR1-R-F | GGGGACCACCTTTGACTAAAGAAGCTGG |
| saeRS-K0-pKOR1-R-R | CGCACTGAGTGAGTCGTTATGCTTATTTTG |
| saeRfor | GGGGACAAGTTTGTACAAAAAAGCAGGCTG |
| CoaLRev | AATTTTTAATTCCTCAGAAAGGTAAATGGCC |
| CoaIfor | GTGTTGTTGTTCATCGCTTACTCG |
| Coa-Rev-AscI | TTGGCCTCGCTATTATGTTACG |
| coaBamHIFor | TTGGATCATCGAAAGGTAAATGCTCGT |
| coaXholRev | CGCGTCAGTGGTTTTATCGCTACG |
| FnBrB-For-RBS | AGGAGGTTTTAACATGAAAGAAGCAGGCT |
| T7promoterfor | GATCTAAGCGGCTAGGTA |
| T7terminatorrev | CAATCAAGTCG |

Blood Survival Assay

Strains were cultured in TSB with appropriate antibiotics. Inducer anhydrotetracycline (ATc) was added when indicated to overnight cultures. Following 18 h of culturing, the bacteria were washed twice in sterile PBS and suspended to an OD of 0.14 using a Behring photometer in PBS. Fresh venous human whole blood was collected using heparin containing Vacutainer tubes (BD) from outwardly healthy adult donors. The blood was then immediately used in the assay as described (Liu et al., 2005; Hall et al., 2015, 2017). The percentage of surviving bacteria was calculated as \((\text{CFU}_{\text{timepoint}}/\text{CFU}_{\text{initial input}})\times100\). Human blood collection was approved by the University of Minnesota Institutional Review Board.

Hydrogen Peroxide Survival Assay

To determine the contribution of coagulase to S. aureus survival when challenged with oxidative stress, overnight cultures were washed with PBS and ∼2 × 10<sup>8</sup> CFU were incubated at 37°C in a 1.5% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)/PBS solution for 60 min (Liu et al., 2005). Serial dilutions were plated on TSA for enumeration of surviving CFU. Percent survival was calculated as surviving CFU/ input CFU multiplied by 100, (#CFU/ #CFU)*100.

Data Analysis

Independent samples were statistically analyzed using a Student’s t-test with an alpha level ≤0.05 considered significant. For data figures with more than two independent samples, a one-way ANOVA analysis with a post-hoc Tukey HSD-test was used to determine if there was statistical significance between samples with an alpha level ≤0.05 considered significant.

RESULTS

The Deletion Mutation of saeRS Enhanced S. aureus Survival in Human Blood

Our previous studies have demonstrated that the AirSR(YhcSR) system contributes S. aureus survival in human blood (Hall et al., 2015, 2017); and our preliminary study found that AirSR probably positively regulates the transcription of saeRS (data not shown). It has been well-documented that the SaeRS system is a key regulator of virulence factors that contribute to evade innate immune system and pathogenicity in a variety of animal models of S. aureus infection (Steinhuber et al., 2003; Liang et al., 2006; Zurek et al., 2014; Cho et al., 2015). These data led us to speculate that SaeRS system is likely involved in the function of AirSR in survival in the blood. To test this possibility, we deleted saeS and examined the impact of the deletion on bacterial survival in human blood. Unexpectedly, we found that the null mutation of
saeS in HA-MRSA WCUH29 significantly increased survival in the blood throughout the 3 h experiment (Figure 1A).

To determine whether the genetic backgrounds of S. aureus affect SaeRS's mediated bacterial survival in the blood, we created a saeRS deletion mutant in a USA300 CA-MRSA 923 strain and acquired a saeRS allelic replacement mutant of USA400 CA-MRSA MW2 (Voyich et al., 2009). Consistent with the results of WCUH29, the saeRS null mutation remarkably increased the percentage of CFUs that survived in the blood for all strains (Figures 1B, C). Moreover, in trans SaeRS complemented strains had similar survival percentages as the wild-type controls (Figures 1A, B), indicating a specific effect of SaeRS on bacterial survival in human blood.

The Introduction of Constitutive Coagulase Expression System Eliminated the Effect of the saeRS Null Mutation on Bacterial Survival in Human Blood

Previous studies revealed that the SaeRS system is a critical positive regulator of important virulence factors, including fibronectin-binding proteins (fnbB, fnb), fibrinogen-binding proteins (efb), coagulase (coa), and toxins (hla, hlb; Giraudo et al., 1999; Liang et al., 2006; Sun et al., 2010). We hypothesized the deletion of SaeRS prevented the expression of virulence factor with the result being increased bacterial survival in blood. In order to identify which SaeRS regulated gene(s) are involved in this phenomena, we tested the impact of fnbAB, efb, sa1000, or coa overexpression in trans on bacterial survival in blood in the saeRS mutants. The constitutive expression of coagulase restored the percentage of bacteria that survived to wild-type control levels and decreased the survival capacity of S. aureus compared to the saeRS null mutant control (Figures 2A–C). In contrast, the overexpression of FnbAB, Efb, or SA1000 did not eliminate the enhanced survival phenomena of the saeRS knockout mutant (Figure 2A).

The Deletion Mutation of coa Increased the Survival of S. aureus in Human Blood

To confirm the role of coagulase in SaeRS’s involvement in bacterial survival in the blood, we created coa deletion mutants in WCUH29, 923, and MW2 strains. Each new coa mutant was complemented and we tested each strain’s ability to coagulate rabbit plasma. The coa deletion mutants of 923 and MW2 strains...
exhibited negative coagulation, whereas the coa deletion mutant of WCUH29 strain formed partial coagulation (Table 3). The 923 and MW2 wild type, coa null, and complemented strains were examined in our survival assays using human blood. Similar to the results of saeRS null mutants, the deletion of coa enhanced the survival capacity of S. aureus 923 and MW2 after 1 h of infection (Figures 3A,B). The in trans coa complementation restored the survival level to the wild-type control (Figures 3A,B), indicating coagulase production in blood is detrimental to the survival of S. aureus.

The Heterologous Expression of Coagulase or Addition of Recombinant Coagulase Inhibited Survival Ability in Human Blood

To further confirm the role of coagulase in survival, we determined the effect of heterologous expressing coagulase in S. epidermidis on survival in the blood. Compared with the control, the induction of coa expression significantly decreased the survival of S. epidermidis in the blood throughout the duration of the experiment (Figures 4A,B). To define the role of coagulase in survival of S. aureus, we cloned, expressed, and purified recombinant coagulase (rCoa). The purity of purified rCoa was examined using SDS-PAGE (Figure 4C); the activity of purified rCoa was confirmed in a coagulation assay using human blood (Figure 4D). The addition of rCoa significantly decreased the survival ability of USA300 CA-MRSA 923 in human blood throughout the period of the experiment in a dose-dependent manner (Figure 4E). In contrast, the addition of control protein, recombinant SarZ, had no impact on survival compared to the negative control (Figure 4E).

Coagulase Is Associated with Susceptibility to Hydrogen Peroxide Killing

Coagulase is able to bind host prothrombin to form staphylothrombin, which in turn activates the protease activity of thrombin. Although it has been predicted that coagulase could protect bacteria from phagocytic and immune defenses by causing localized clotting, the role of coagulase in pathogenicity is contradictory (Baddour et al., 1994; Moreillon et al., 1995; Cheng et al., 2010). Reactive oxygen species (ROS) is a key element used by phagocytic cells to kill phagocytosed bacteria (Liu et al., 2005; Clauditz et al., 2006). To explore the potential mechanism of coagulase in survival, we examined
whether the deletion mutation of coa alters the susceptibility to the ROS H₂O₂. The deletion mutation of coa increased the bacterial survival compared to the 923/pYH4 control strain (68 vs. 53%, Figure 5A), whereas the epismally coa complemented strain had significantly decreased survival compared to the coa deletion mutant (68 vs. 51%). Similarly, heterologous expression of coagulase reduced the ability of coagulase negative *S. epidermidis* to tolerate H₂O₂—mediated killing compared to the *S. epidermidis* pYH4 control (74 vs. 93%, Figure 5B).

**The Involvement of SaeRS in Survival of *S. aureus* in Human Blood Is Coagulase Dependent**

The above studies demonstrated both SaeRS and coagulase are involved in controlling *S. aureus* survival in human blood. To further understand whether the role of SaeRS is attributable to its positive regulation of coa, we created *saeRS* or *coa* double mutants in 923 and MW2 strains and examined the effect of expression of either *coa* or *saeRS* in trans on survival of the double mutant in blood. Similar to the results of *coa* or *saeRS* single gene null mutant, the *saeRS/coa* double mutant in 923 resulted in a significant increase of bacterial survival in the blood (Figure 6). The expression of coagulase in trans complemented the survival ability of the *saeRS/coa* double mutants to their wild-type control level, whereas the expression of SaeRS in trans had no impact on the survival ability of the *saeRS/coa* double mutants (Figure 6). Similar results of *saeRS/coa* double mutant of MW2 were observed (data not shown).

### DISCUSSION

Our results clearly indicate the roles of SaeRS and coagulase in *S. aureus* survival in human blood. Despite the fact that SaeRS plays a critical role in bacterial invasion of host cells, cytoxicity, and pathogenicity of *S. aureus* in animal models of infection (Liang et al., 2006; Voyich et al., 2009), it did not appear to enhance survival in human blood. Conversely, SaeRS inhibited the survival of *S. aureus* in human blood, as demonstrated by experiments with the *saeRS* null mutants and by complementation studies. Surprisingly, the expression of *coa* in trans could eliminate the enhanced survival of the SaeRS null mutant in blood, which was demonstrated with *coa* complementation studies in the *saeRS* single and *saeRS/coa* double mutants. Consistently, coagulase decreases the bacterial survival in the blood, as supported by experiments with the *coa* null mutants, *coa* heterologous expression strains, recombinant coagulase, as well as by complementation experiments. This finding is at odds with the assumption coagulase could protect bacteria from immune defenses by causing localized clotting. Conversely, our data suggest localized clotting mediated by coagulase may be detrimental to the survival of *S. aureus* in blood. Interestingly, our data indicate that coagulase is somehow associated with *S. aureus* susceptibility to hydrogen peroxide killing.

**TABLE 3 | Coagulase activity of wild type strains, saeRS or coa null mutants, and saeRS or coa complemented strains.**

| Strains            | Coagulation | Strains            | Coagulation |
|--------------------|-------------|--------------------|-------------|
| WCUH-29            | +           | 923                | +           |
| WCUH-29Δcoa        | ±           | 923Δcoa            | –           |
| Sa371Δcoa          | –           | 923ΔsaeRSΔcoa      | –           |
| WCUH-29/pYH4       | +           | 923/pYH4           | +           |
| WCUH-29Δcoa/pYH4   | ±           | 923Δcoa/pYH4       | –           |
| WCUH-29Δcoa/pYH4   | +           | 923Δcoa/pYH4-coa   | +           |

**FIGURE 3 | Effect of coa deletion mutation and complementation on survival of *S. aureus* in human blood.** Percent survival of wild-type *S. aureus* USA300 CA-MRSA 923 (A), USA400 CA-MRSA MW2 (B), and coa deletion mutants and its complementation in freshly collected heparinized human blood with appropriate antibiotics. Bacteria were cultured overnight, diluted, inoculated into blood, and incubated at 37°C in a rotisserie incubator. Percent survival = (%CFUinput/ΔCFUinput)×100. The data represents the mean ± SEM of at least six independent experiments.
Our findings in this study only pinpoint the role of SaeRS in control of S. aureus survival in human blood through regulation of coagulase production. This suggests that SaeRS may play different roles in different stages of host cell-pathogen interactions. Several key steps of infection include bacterial colonization, proliferation, evasion of innate defenses, and cytotoxicity (Ji et al., 1999; Okumura and Nizet, 2014; Janhsen et al., 2016; McGuinness et al., 2016). It was clearly demonstrated that SaeRS is critical for S. aureus to adhere to and invade human epithelial and endothelial cells through regulation of adhesins and invasins (Giraudo et al., 1999; Liang et al., 2006; Sun et al., 2010). Fibronectin-binding proteins (FnBPs) are one kind of major adhesins and invasins, which enable to assemble Fn to connect the host and bacterial cells via binding its receptor α5β1-integrin (Sinha et al., 1999; Dziewanowska et al., 2000; Fowler et al., 2000). Moreover, SaeRS is crucial for S. aureus to mediate the expression of toxins, which are required for cytotoxicity in vitro cell culture (Liang et al., 2006; Liang and Ji, 2007) and pathogenicity in different models of infection (Kernodle et al., 1997; Ji et al., 1999; Schwan et al., 2003).

Our results indicate that the SaeRS system controls the capacity of S. aureus survival in human blood, which contradicts to previous report that the saeRS mutation decreased survival of MW2 strain in human blood (Voyich et al., 2009). To rule out possible effect of genetic background of different S. aureus isolates, we utilized the saeRS null mutant and its parental control MW2 (which are kindly provided by Dr. Voyich) and conducted survival assays. Consistent with the results of the SaeRS mutants of WCUH29 and 923 isolates, the saeRS mutant of MW2 exhibited the similar elevated survival capacity compared to its wild-type control. This contradiction, we believe, is mainly due to the different survival ability of wild type control MW2 in the blood samples. In our studies, <10% of the MW2 cells were able to survive in human blood after 1–3 h; in contrast more than 100% of MW2 cells survived after 3 h of incubation in human blood in the previous report (Voyich et al., 2009). This inconsistence is possibly attributable to the differences in the human blood, as different individuals likely possess different levels of antibodies against S. aureus, which would affect the opsonophagocytic killing process (Pier and Elcock, 1984; Chen et al., 2013; Humphries et al., 2015). Another explanation is that in our experiments S. aureus from the stationary phase were utilized, while Voyich et al used the mid-exponential phase of bacterial cells. It is possible that the temporal regulation of coa
FIGURE 5 | Effect of Coa on bacterial resistance to H$_2$O$_2$. (A) Percent survival of wild-type USA300 CA-MRSA 923, coa deletion mutant and complementation strain after exposure of H$_2$O$_2$. (B) Percent survival of wild-type S. epidermiditis and coa expression strain in trans after exposure of H$_2$O$_2$. The bacterial strains were cultured with appropriate antibiotics. Approximately 5 × 10$^9$ CFU were incubated in sterile PBS with 1.5% hydrogen peroxide at 37°C for 1 h. The percent survival was calculated as ($\#$CFU$_{final}$/\#CFU$_{input}$)×100. The data represents the mean ± SEM of at least four independent experiments.

Regarding coagulase, our results clearly showed that the addition of rCoa to the blood enhanced the susceptibility of S. aureus to phagocytic killing. Moreover, altering the expression of coagulase affects bacterial sensitivity to hydrogen peroxide killing. It is necessary to further determine the mechanism in future studies. Our finding is supported with previous reports demonstrating that coagulase is not a virulence factor in several infection models as the coa null mutation had no impact on pathogenicity in a rat endocarditis model of infection (Baddour et al., 1994; Moreillon et al., 1995). Moreover, coagulase negative staphylococci (CNS) have become a major nosocomial pathogen (Becker et al., 2014). Additionally, CNS infections cause renal abscesses and lethality in a mouse model of blood stream infection (Wang et al., 2015). On the other hand, our results are inconsistent with recent report that indicated coagulase is a critical virulence factor for abscess formation and bacteremia in a mouse model of blood stream infection (Cheng et al., 2010). This inconsistence is probably due to the difference of animal models of infection and the variation of genetic background for S. aureus isolates in these studies. Moreover, in this study we found that unlike 923 and MW2 isolates, the deletion mutation of coa could not abolish the coagulation activity of WCUH29, suggesting that other factors, such as von Willebrand factor binding protein, are likely involved in coagulation activity as reported (Thomer et al., 2013).

In conclusion, we identified that the SaeRS two-component system plays an important role in survival of S. aureus in human blood via regulation of coagulase production. Coagulase alleviates the survival capacity of S. aureus in human blood. These data suggest that coagulase might be used as an alternative strategy to treat S. aureus-induced bacteremia.

**ETHICS STATEMENT**

This study was carried out in accordance with the recommendations of NIH guidelines with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the University of Minnesota Institutional Review Board.

**AUTHOR CONTRIBUTIONS**

HG, JH, and YJ performed the experiments. HG, JH, and YJ designed the experiments. HG, JH, JY, and YJ analyzed the data. HG, JH, and YJ wrote the manuscript.
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