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Role of *Staphylococcus aureus* colonization in burn patients

Susanne Sütterlin [a,b,*], Marie Lindblad [c,d], Eva Tano [e], Sara Frosth [f], Filip Farnebo [g], Torgny Schennings [g], Jan-Ingmar Flock [h], Fredrik Huss [c,d]

[a] Department of Women's and Children's Health, Paediatric Inflammation, Metabolism and Child Health Research, Uppsala University, Sweden
[b] Department of Pediatrics, Uppsala University Hospital, Sweden
[c] Department of Surgical Sciences, Plastic Surgery, Uppsala University, 75185 Uppsala, Sweden
[d] Burn centre, Department of Plastic and Maxillofacial Surgery, Uppsala University Hospital, 75185 Uppsala, Sweden
[e] Department of Medical Sciences, Section of Clinical Bacteriology, Uppsala University, 75185 Uppsala, Sweden
[f] Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences (SLU), Box 7036, 75007 Uppsala, Sweden
[g] Department of Plastic and Craniofacial Surgery, Karolinska University Hospital, K1 Molekylär medicin och kirurgi, K1 MMK Rekonstruktiv plastikkirurgi, 17176 Stockholm, Sweden
[h] Department of Microbiology, Tumour and Cell Biology, Karolinska Institutet, Box 280, 17177 Stockholm, Sweden

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SUMMARY

Objectives: Staphylococcus aureus is an important pathogen in burn patients and contributes to mortality, however, the role of colonization with S. aureus in the course of the disease is less well described.
Methods: The study aimed to determine the frequency of S. aureus colonization in 80 patients treated in a national burn center in Uppsala, Sweden, during the first ten days of hospitalization in relation to length of stay, number of days before antibiotic treatment started and mortality; additionally, epidemiological relationship and phylogeny were analyzed.
Results: A total of 38/80 (47.5%) patients tested positive for S. aureus upon admission, while 47 out of 65 patients who completed the 10-day study period (72%) were colonized with S. aureus. Patients who were colonized at admission tended to stay longer at the burn center, particularly when admitted with more severe conditions corresponding to a ʀBaux score > 70 (p=0.05, R2=0.09). Patients carrying isolates of phylogroup 2 received antibiotic treatment approximately one day later than patients with isolates belonging to phylogroup 1 ($p < 0.05$, $R^2 = 0.09$).
Conclusions: The study findings emphasize that screening for S. aureus colonization in burn patients upon admission, particularly in critically injured patients, could prove beneficial in optimizing antibiotic therapy.

Introduction

Burn injuries constitute a significant health problem in many areas of the world, with bacterial infections contributing to up to 50% of all deaths following burn trauma.[1,2] Thermal injuries damage the skin barrier that is colonized with bacterial species belonging to the skin flora, and thus making the burn wound the most important origin of invasive infections in burn-injured patients.[3] Gram-positive bacteria like *Staphylococcus aureus* belong to the normal flora of the healthy skin, and avoidance of growth of these opportunistic pathogens within the burn eschar is considered critical in preventing invasive infections.[4] A study conducted by Krishnan *et al.*, on burn patients in the United Kingdom with large, burned surface areas, showed that 16.2% of the mortality cases were associated with non-resistant *S. aureus* infections and 9.3% with Methicillin-Resistant *S. aureus* (MRSA).[5]

Bacterial biofilm infections have been recognized as the starting point for the establishment of deeper tissue and systemic infections.[6] Several epidemiological studies have shown that *S. aureus* is the first pathogen to colonize the burn eschar.[4,7] Exposure of planktonic *S. aureus* to burn serum and oxidative stress resulted in the repression of the *agr* system, which is an autoinducing signal that leads to increased production of biofilm and cell-cell aggregation.[6] *S. aureus* seems to possess an initial advantage in colonizing the burn wounds, as studies using a rodent model have shown its ability to outgrow the wound pathogen *Pseudomonas aeruginosa* within the first day of *in vivo* infections, subsequently facilitating the aggregation and biofilm formation of *P. aeruginosa*.[8,9] However, after three days, the quantity of *P. aeruginosa* surpasses that of *S. aureus* in

* Correspondence to: Department of Women's and Children's Health Uppsala University, 75185 Uppsala, Sweden.
E-mail address: susanne.sutterlin@kbh.uu.se (S. Sütterlin).

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the same rodent model,[9] or establishes a stable co-existence with other gram-negative rods.[10]

Colonization of *S. aureus* from the nares, axilla or perineum has long been recognized as a significant source of infection in the wound eschar. Three categories of nasal carriage of *S. aureus* have been described: those with persistent carriage (10–35%) in which individuals carry a single strain for extended periods, intermittent carriage (20–75%) characterized by carrying different strains for shorter durations, and non-carriers (5–50%) who do not carry *S. aureus* at all.[11] Upon admission, approximately 60% of burn wounds are colonized with coagulase-negative Staphylococci, while *S. aureus* can be found in 19 to 43% of all wounds.[4,7]

In the present study, our objective was to determine the frequency of *S. aureus* colonization among 80 patients undergoing treatment at the burn center in Uppsala, Sweden. The colonization status of these patients was evaluated during their initial ten-day period at the ward, considering factors such as length of stay, number of days before antibiotic treatment initiation, and mortality. Additionally, we investigated the epidemiological relationship and phylogeny of *S. aureus* isolates through the utilization of whole genome sequencing (WGS).

Materials and methods

Setting and study design

The study was conducted at the Uppsala University Hospital's burn center in Sweden. The center compromises of an intensive care ward unit, intermediate care, surgery, and an outpatient clinic, all located on the same floor. The ward consists of seven beds with a capacity of accommodating three to four intensive care patients.

Patients with acute (< 24 h) burn injuries, older than 18 years, and expected length of stay > 7 days were offered to participate in the study during a period of 29 months (2014–2016). Patients with known comorbidity caused by malignant, immunosuppressive disease, and suspected or known risk of hepatitis or HIV were excluded. Informed consent was obtained from all patients or their next of kin. Initially, 90 patients were included, but ten patients were subsequently excluded due to failed initial sampling or death during acute handling of the burn injuries. Therefore, the final study group consisted of 80 patients. Follow-up sampling was possible for 65 patients, while 15 patients were excluded due to death, early discharge or sampling failure (Fig. 1). The study was approved by the local Institutional Review Board (IRB No: 2013/359 and amendment IRB No: 2021–03889).

Microbial samples were collected using swabs from the nares, throat, and perineum upon admission, on day three, and between days seven to ten of hospital stay. Additional microbial samples were taken based on clinical indications, including wound swabs, blood stream cultures, tracheal secretion specimens, and urine samples. Prior to further statistical analysis, the samples were categorized into three sampling periods: admission, day one to five, and day six to ten. Patients with at least one positive sample during all periods were classified as persistently colonized, those with positive samples during one or two sampling periods were categorized as non-persistently colonized, and patients without any culture-positive sample were classified as never colonized. The frequency of *S. aureus* isolation per patient was calculated for the entire study period by determining the percentage of *S. aureus*-positive specimens out of the total number of specimens collected.

Furthermore, the following data was extracted from the patients' records: age, gender, date/time of injury, length of stay at burn center (LOS), days elapsed after admission before the initiation of the first antibiotic treatment, mortality, type of burn (flame, scald, contact *etc.*), body mass index, percent total body surface area burned (TBSA), medications, and current diseases at admission. The revised Baux score (rBaux)[10] was calculated as the sum of TBSA, age, and when inhalation injury was present the sum was increased with 17.

Bacteriology sampling and culture

Specimens from the nares, throat, perineum, and wounds were collected using cotton swabs and transported in charcoal medium (Copan Diagnostics, Murrieta, CA, USA) and immediately sent to the Department of Clinical Microbiology, Uppsala University Hospital. All screening specimens were cultured on a *S. aureus* ID (SAID) agar plate (bioMérieux, Marcy-lÉtoile, France) which is a selective medium for *S. aureus*. Additionally, the specimens were also plated on blood agar (Columbia blood agar base, Oxoid Ltd., Basingstoke, Hants, UK, with 5% defibrinated horse blood). Other specimens, such as bloodstream, urine, and tracheal secretions, were sampled and processed according to standard clinical routine procedures. All suspicious *S. aureus* colonies were subjected to species identification using standard clinical routine procedures and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) using Maldi Biotyper Microflex, Bruker (Bruker Corporation, Billerica, Ma, USA) where appropriate. All *S. aureus* isolates were stored at −80° in a glycerol solution.

Genetic epidemiology

Bacterial genomic DNA was extracted and purified using a Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, Wisconsin, USA) according to the manufacturer's recommendations for Gram-positive bacteria, with the exception that DNA was rehydrated with 10 mM Tris-HCl (pH 8.0). The quality of the extracted DNA was controlled by gel electrophoresis and spectrophotometry. DNA concentrations were measured using Quant-iT dsDNA BR assay and a Qubit instrument (Invitrogen, Waltham, Massachusetts, USA). After standardizing the DNA extracts, the samples were transferred to the National Genomics Infrastructure (Stockholm, Sweden) for library preparation and whole-genome sequencing (WGS). Fragmented DNA was end-repaired, A-tailed, adapter-ligated, and amplified using Nextera DNA Library Prep kit (Illumina, San Diego, California, USA). Sequencing was performed on a NovaSeq SP-300 platform, generating 150 bp paired-end reads.

The obtained reads were analyzed using the Ridom SeqSphere$^+$ version 8.3.5 software.[12] Raw reads were *de novo* assembled through a pipeline using SKESA version 2.4.0[13] with default settings, which included downsampling to 180 x coverage and assembly remapping and polishing by BWA mapping algorithm BWA-MEM.[14] Prior to assembly, read data quality and adapter content were assessed by FastQC version 0.11.7 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and if adapters were found, adapter trimming was performed by Trimmomatic version 0.36.[15] Additionally, Mash Screen version 2.1[16] was run to check for possible contamination. Multi-locus sequencing typing (MLST) profiles were assigned in Ridom SeqSphere$^+$[12] using the scheme for *S. aureus* available at https://pubmlst.org/organisms/staphylococcus-aureus. New MLST profiles (ST 7444–7451) with isolate identities: 37566–37574, have been deposited in the PubMLST database (https://pubmlst.org/organisms/staphylococcus-aureus). The Ridom SeqSphere$^+$ software[12] was also utilized for core genome (cg) MLST analysis and to construct minimum spanning trees (MST:s). The cgMLST scheme for *S. aureus* used consisted of 1861 targets. Isolates with a maximum of 24 allelic differences were designated as belonging to the same MST cluster. All sequenced isolates were assigned to 7-gene sequence types according to Enright *et al.* and to phylogenetic lineages according to Feng *et al.,* Monecke *et al.*, and Cooper *et al.*[12–15] Furthermore, the isolate's Agr quorum-sensing system was typed in accordance with Strauss *et al.* as implemented in Ridom SeqSphere$^+$

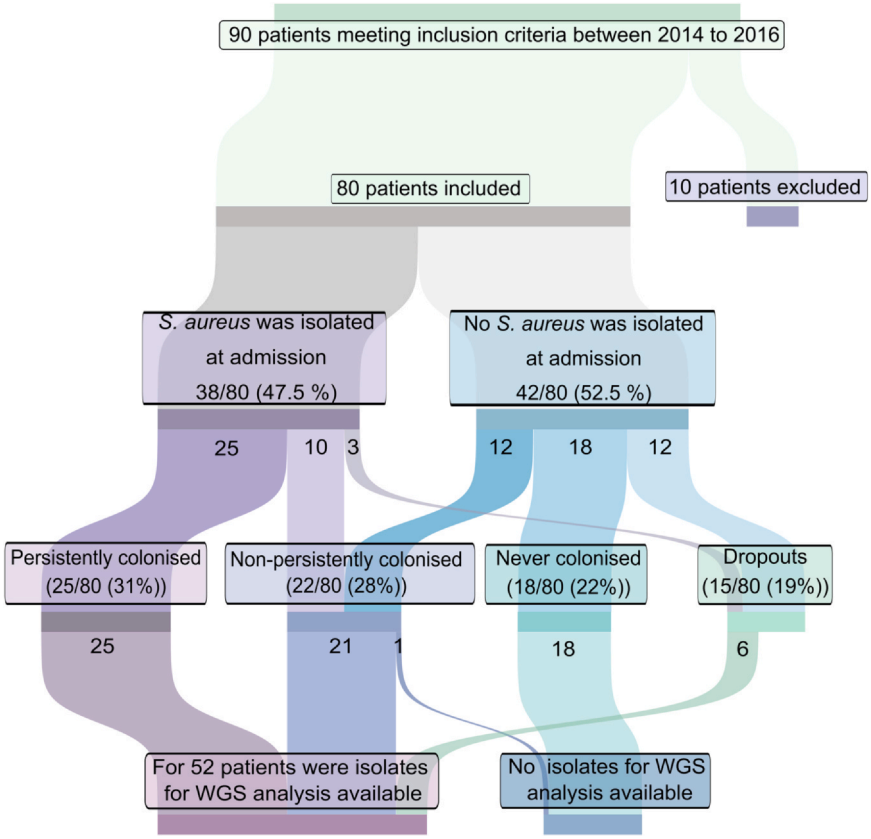

Fig. 1. Summary of the study group with a study inclusion flow chart illustrating the group sizes.

software.[16] All sequence data is publicly available through the European Nucleotide Archive (www.ebi.ac.uk/ena) under accession number PRJEB63216.

Statistical analyses

Statistical analyses of the descriptive data were performed using basic R (version 4.1.1, 2021), and visualizations were created with ggplot2 (version 3.4.0). Continuous variables were presented as medians with minimum-maximum range, and frequencies were calculated for categorical variables. Logistic regression analysis was used to predict the length of stay at the burn center, antibiotic-free days following admission to the burn center and mortality (dependent variables) based on *S. aureus* colonization at admission, type of colonization and isolation frequency (independent variables). Likewise, the influence of age, body mass index and rBaux score on *S. aureus* colonization and isolation frequency was estimated. Furthermore, odds ratios and probabilities were calculated using Fisher's exact test for categorical data, where appropriate. Probabilities of < 0.05 were regarded as statistically significant.

Results

Patient characteristics

The median age of the study population was 51 years (19–93), with a male predominance (66%, 53/80). The median body mass index was 27 kg/m^2 (range: 15–47), and most patients (68%, 54/80) had pre-existing medical conditions, with 44% (35/80) on prescribed medications prior to admission. The mortality rate was 16% (13/80), with six patients passing away before day six from admission. Patients stayed a median of 13 days (range: 1–94) at the burn center. Of the patients, 40/80 (50%) continued inpatient care at other hospitals near their residence, while 27/80 (34%) received continued medical care at Uppsala University Hospital. Most patients (82%, 66/80) sustained flame injuries (82%, 66/80), with 25% (20/80) also experiencing inhalation injury. The median total body surface area burned (TBSA) was 20% (range: 4–92), and the median rBaux score was 77 (range: 28–150).

S. aureus *colonization and sampling*

At admission, 47,5% 38/80 of patients were colonized with *S. aureus*, primarily in nares, axillae, or perineum (92%, 35/38). The remaining 52,5% (42/80) had negative cultures upon arrival. Admission samples were available for 80 patients; however, 15 patients were excluded from further analysis due to death or missed follow-up sampling. Of these, three were colonized at admission, and 12 were not colonized at admission. Follow-up sampling was therefore available for 65 patients, of whom 35 were colonized at admission. Among these, 71% (25/35) showed persistent colonization, while 29% (10/35) had a non-persistent pattern. Patients not colonized at admission had a 29% (12/42) likelihood of acquiring colonization during hospitalization, with 43% (18/42) remaining consistently culture negative. Patients colonized at admission were significantly more likely to remain colonized during day 1–5 (94%, 33/35 *versus* 34, 10/30; OR 31, $p < 0.05$) and days 6–10 (86%, 30/35 *versus* 24%, 7/30; OR 18, $p < 0.05$) compared to those not colonized at admission.

At admission, *S. aureus* was predominantly detected in screening specimens from nares (71%, 27/38), throat (21/38, 55%), and perineum (11/38, 29%). Persistently colonized patients were significantly more likely to have *S. aureus* detected in screening samples at admission (OR 41, $p > 0.05$). However, no significant difference was observed between screening and clinical samples during follow-up (days 1–5: OR=0.3, p=0.6; days 6–10 (OR 6, p=0.2). Non-persistently

colonized patients also show no statistically significant differences across sample types at any time point (admission: OR 4, p=0.09; days 1–5: OR 0.3, p=0.1; days 6–10: OR 0.9, p=1).

A total of 1390 specimens were collected, with screening samples accounting for 45% (626/1390). At admission, the median number of samples taken was similar between patients colonized and those not colonized at admission (4, range: 3–10 *versus* 4, range: 2–13; p=0.6, R^2=0.004). However, over the entire study period, fewer samples were collected from patients who were never colonized (16, range: 7–39) compared to those with non-persistent colonization (19, range: 8–26) or persistent colonization (21, range: 11–36); p=0.07, R^2=0.05).

Therefore, an isolation frequency was calculated as the rate of culture-positive samples out of the total number of samples and expressed as a percentage. Patients not colonized at admission had a significantly lower isolation frequency during the study period (36%, 8–82 compared to those colonized at admission (0%, range: 0–40; $p < 0.05$). Additionally, patients with none-persistently colonization had a significantly lower isolation frequency compared to those with persistent colonization (20%, range: 9–46 *versus* 47%, range: 19–82; $p < 0.05$).

S. aureus *colonization and outcome variables*

No significant associations were found between *S. aureus* colonization at admission or type of colonization and the patients' age, body mass index, or rBaux score. However, patients colonized with *S. aureus* at admission tended to have longer stays at the burn center compared to non-colonized patients (median stay: colonized 17 days *versus* non-colonized 9.5 days, p=0.08, R^2=0.02). This trend was more pronounced in patients with severe injuries (rBaux score > 70), where colonized patients had significantly longer stays (median stay: colonized 26.5 days, non-colonized 11 days, p=0.05, R^2=0.09). While the rBaux score, driven mainly by TBSA, strongly influenced length of stay, colonization status at admission had no significant effect on the length of stay for patients with TBSA > 20% (p=0.7, R^2=0.005). Similarly, no statistical relationship was observed between colonization status at admission and the timing of antibiotic therapy initiation or type of colonization. However, patients with lower *S. aureus* isolation frequencies tended to have shorter antibiotic-free periods (p=0.1, R^2=0.04). Colonization status had no significant impact on mortality, regardless of whether the patients had more severe injuries, patients with a rBaux score > 70 or a TBSA > 20% (Fig. 2).

S. aureus *colonization and results from sequence typing*

General comments on the sequence data

High-quality reads were obtained for 255 isolates from 52 patients, with species verification confirmed all as *S. aureus*. The sequences had a median coverage of 181x (range: 34 – 711), and covered a median of 99.2% (range: 97.0 – 99.7) of the cgMLST-scheme. A median of 5 isolates per patient (range: 1 - 18) were available for analysis.

Multi-locus sequence typing

7-gene multi-locus sequence typing successfully assigned 254/255 isolates to 31 sequence types (ST), with one isolate assigned to clonal complex (CC) CC30 due to the absence of the *arcC* locus. The majority of isolates clustered into nine clonal complexes: CC1 (n = 2), CC5 (n = 15), CC8 (n = 4), CC15 (n = 7), CC22 (n = 4), CC30 (n = 4), CC45 (n = 10), CC97 (n = 4), and CC121 (n = 1). Eleven additional sequence types were represented by single patients (ST7, ST12, ST20, ST59, ST101, ST375, ST3075, ST7447, ST7448), except ST395 (n = 2), which appeared in two patients. Seven patients carried two distinct strains (ST1 and ST5; ST30 and ST101; ST5 and ST15; ST5 and ST45; ST97 and ST630; ST30 and ST7446; ST34 and ST7448), while two patients carried three distinct strains(ST45 and ST59 and ST395; ST5 and ST121 and ST188).

Sequence analysis and relation to colonization and outcome variables

All patients showed unique cgMLST profiles, with a minimal allele difference of 91 between patients. Phylogenetic analysis assigned 43% (20/46) isolates to phylogroup 1a/b, 65% (30/46) to phylogroup 2, and three patients carried isolates from both groups. The five bloodstream isolates belonged to phylogroup 2 (4/5) and one to phylogroup 1a/b (1/5). Patients not colonized at admission were more likely to acquire isolates from phylogroup 2 during hospitalization (9/11 *versus* 2/11, OR 0.2, $p < 0.05$) compared to those already colonized (phylogroup 2: 4/9 *versus* phylogroup 1: 5/9; OR 0.2, p=0.08). Length of stay at the Uppsala burn center was comparable between groups colonized with respective lineage (p=0.7, R^2=0.003). However, patients colonized with phylogroup 1 isolates started antibiotics approximately one day earlier than those with phylogroup 2 isolates (lineage 1 *versus* 2: 2 (range: 0–11) days *versus* 3 (range: 0–11) days; 3.3 ± 3.1 days *versus* 4.4 ± 3.1 days; $p < 0.05$, R^2=0.09). Among phylogroup 2 isolates, patients with CC5 and CC15 strains had longer periods of antibiotic-free days (5, range: 1–10 and 5.5, range: 2–9) compared to CC22 and CC45 strains (2, range: 2–10 and 2, range: 0–4). Of the eight deceased patients with WGS data, seven carried phylogroup 2 isolates, indicating a 5.3 times higher likelihood of death with phylogroup 2 (7/8 *versus* 1/8; OR=5.3, p=0.1).

Agr types were determined for 254/255 isolates, with the following distributions: *agr* I (26/52 patients), *agr* II (19/52 patients), *agr* III (3/52 patients). Four patients harbored isolates with multiple *agr* types (*agr* I and II; *agr* I and III; *agr* III and IV; and *agr* I, II and IV). One isolate lacked *agr* type assignment but belonged to a cluster with *agr* I isolates. No statistically significant associations were found between *agr* type and outcome variables, including length of stay, antibiotic-free days, or mortality. Patients with *agr* II isolates had a median delay of four days (range: 1–10) before antibiotic treatment initiation compared two days *agr* I (range: 0–11), and *agr* III (range: 0–2). *Agr* III patients tended to have a longer length of stay, but this observation was based only on three patients. (Fig. 3–5, Tables 2 and 3).

Discussion

The present study investigated the colonization of burn-injured patients with *S. aureus* during the first ten days after admission to the burn intensive care unit at Uppsala University Hospital, Sweden. Nearly half of the patients who were admitted were colonized with *S. aureus* (47.5%) and stayed almost twice as long at the burn center compared to patients not colonized with *S. aureus*. All patients carried unique isolates, most likely representing community-acquired *S. aureus*. Isolates from the bloodstream and from deceased patients belonged more often to phylogroup 2. Remarkably, patients carrying *S. aureus* of phylogroup 2 received their first antibiotic treatment about one day later compared to patients colonized with *S. aureus* of phylogroup 1.

The colonization frequency of burn-injured patients upon admission (47.5%) reflected the prevalence of *S. aureus* carriage in the community, as reported in previous studies, confirming the common occurrence of *S. aureus* colonization.[4,7,17] Consistent with previous studies, 31% of the patients showed a persistent colonization pattern, and nasal samples were the most reliable in detecting this *S. aureus* colonization pattern.[18] Patients without persistent growth of *S. aureus* showed a scarcer isolation frequency, confirming the previously described transient character of this type of colonization.[11] According to cgMLST, all patients carried unique isolates, suggesting no evidence of clonal spread of *S. aureus* during the study.

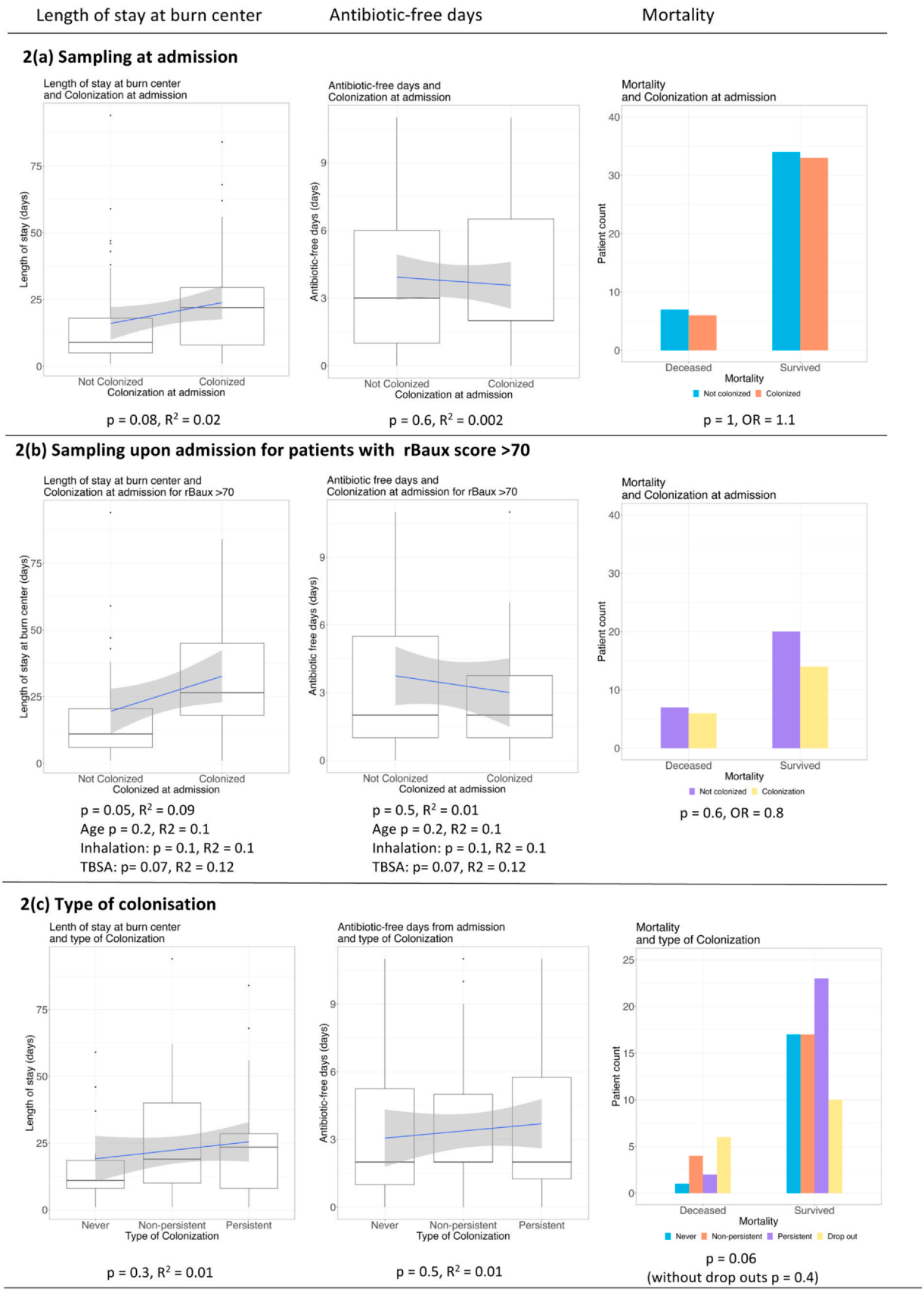

Fig. 2. Summary of the results for *S. aureus* colonization in relation to outcome variables for all patients (3a), for severe injured patients with rBaux > 70 (3b) and type of colonization (3c). Boxplots show interquartile range, median and maximum or minimum values, respectively; linear regression lines are shown with 95% confidence level intervals.

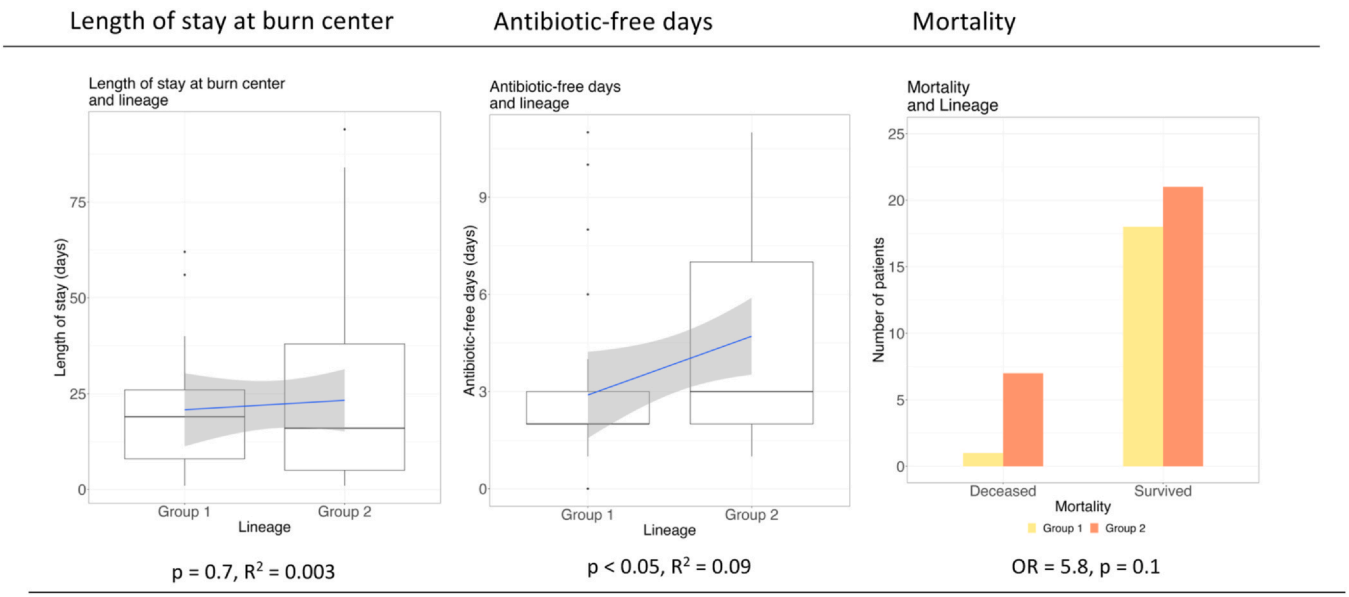

Fig. 3. Summary of outcome variables and *S. aureus* lineage group 1a/b, and group 2 in relation to outcome variables. Boxplots show interquartile range, median and maximum or minimum values, respectively; linear regression lines are shown with 95% confidence level intervals.

Contemporaneously, an *Acinetobacter baumannii* outbreak coincided with the study at the burn center which lead to high awareness of hygiene measures amongst the staff.[19] These circumstances benefited the representativeness of the present study and gave a valuable base for general conclusions from the study results. Although this study focused on *S. aureus* colonization, we also reviewed data from clinical cultures collected during routine care to explore co-colonization with Gram-negative bacteria. However, the lack of standardization in sampling and evaluation of culture plates limited our ability to objectively analyze these data.

Staphylococcus aureus colonization upon admission was associated with a prolonged length of stay at the burn intensive care unit. Especially patients with rBaux score over 70 had a distinct prolonged treatment period at the intensive care unit when colonized with *S. aureus* at admission; however, it did not contribute to increased mortality. Particularly, nasal carriage of *S. aureus* has been shown to contribute to endogenous spread of bacteria to damaged skin or surgical sites and contribute to establishing infections.[20] While Manson *et al.* and Issler-Fisher *et al.* demonstrated that the frequency of bacterial colonization with *S. aureus* from diverse locations like catheters and wounds was associated with prolonged stay in the intensive care unit, this study highlights the role of endogenous microbial flora in disease progression.[21,22] Burn injured patients with a rBaux score over 70 have a high disease burden due to the injury, with a predicted mortality risk of at least 10%.[23] The study suggests that *S. aureus* colonization further increases the disease burden, indicating the potential benefits of implementing screening strategies for *S. aureus* in this patient group to optimize antimicrobial treatment.

Staphylococcus aureus is a typical opportunistic pathogen in humans, however, there is limited understanding of the genetic and phenotypic mechanisms leading to a change between a commensal and pathogenic life style. *S. aureus* CC45 is one of the most frequently found isolates in nasal carriage and bloodstream isolates, but no significant genetic differences between the commensal and pathogenic isolates could be found in a large study on nasal and invasive *S. aureus* CC45 by Roe *et al.*[24] The most prevalent clonal complex in the present study was CC5, a lineage that is known to cause severe infections with a significant amount of treatment failure due to acquired antibiotic resistance to methicillin. In a large collection of *S.*

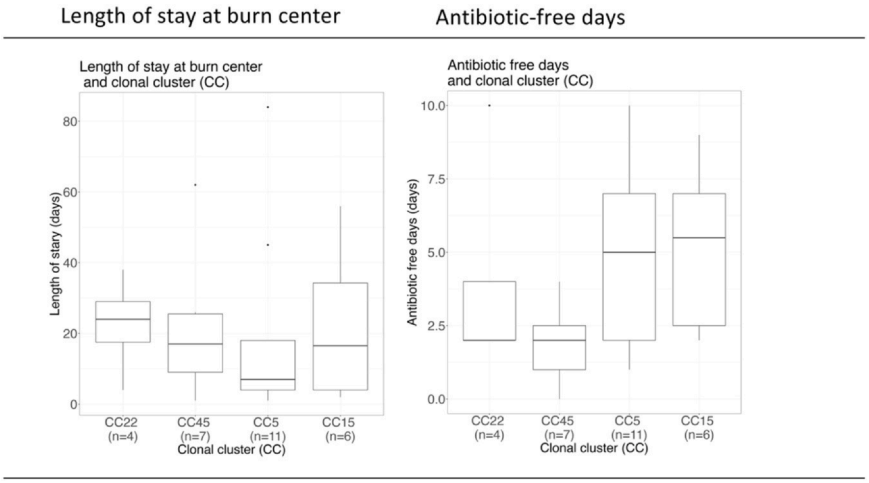

Fig. 4. Summary of the most frequently represented *S. aureus* clonal clusters CC22, CC45, CC5 and CC15 in relation to length of stay at the burn center and antibiotic-free days. Boxplots show interquartile range, median, and maximum or minimum values, respectively.

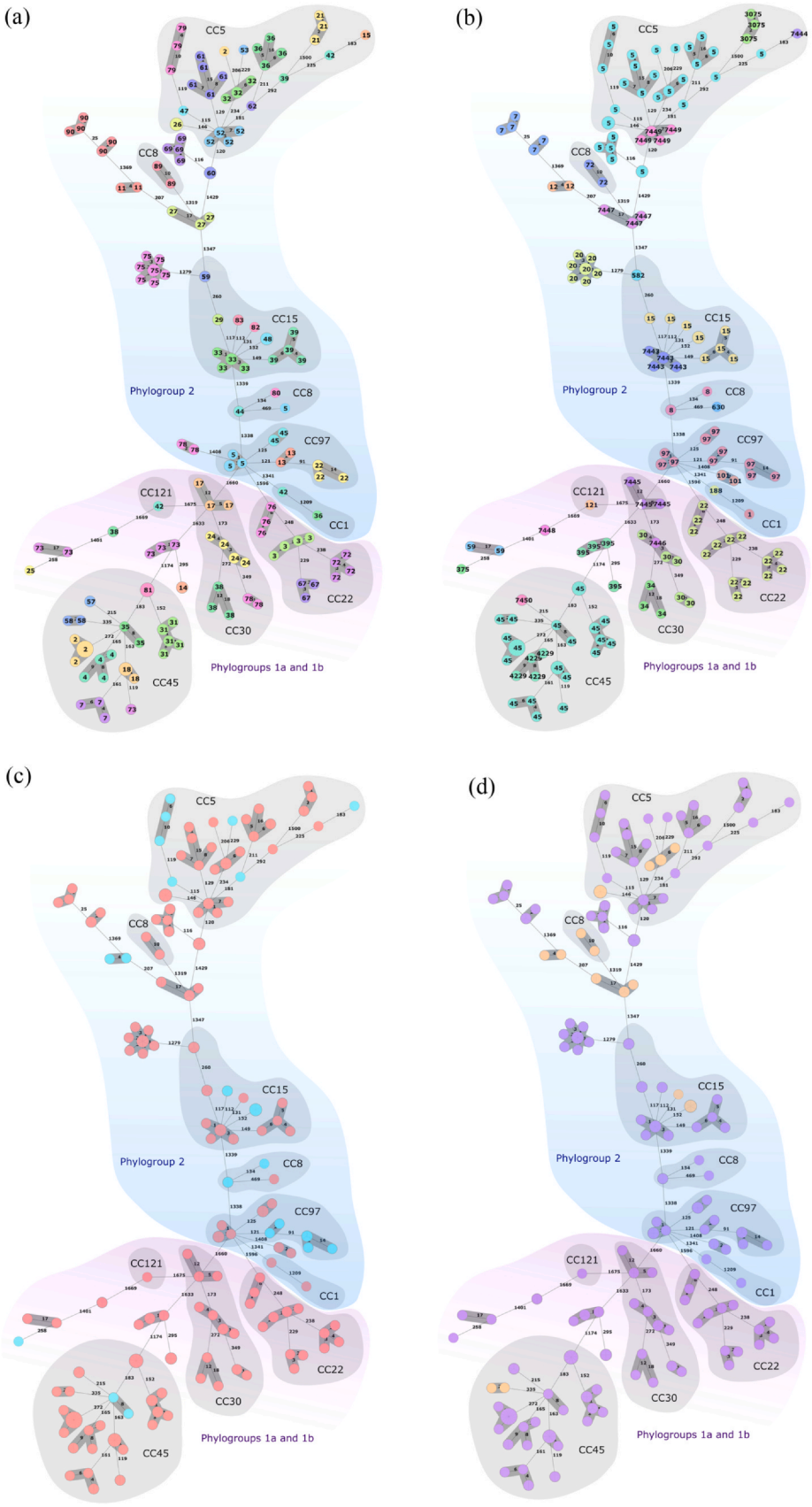

Fig. 5. Minimum spanning tree (MST) 255 *S. aureus* isolates based on cgMLST with 1861 allele positions, pairwise ignoring missing values. The MST cluster distance threshold was set to 24, and the tree is scaled logarithmically. Circles were colored by patient (a); sequence types (b), Colonized at admission (red) or not colonized at admission (blue) (c); deceased (orange) or survived (pink) (d).

aureus CC5 from America, Europe and Asia, the lineage was shown to be highly diverse and three major clades with different predominance of antibiotic and toxin targets could be distinguished.[25] Three out of five bloodstream isolates belonged to CC5 and CC15, which were associated with invasive infections rather than nasal carriage in a study by Rasmussen *et al.*[26] Distinction between colonization flora and infection when growing *S. aureus* from different sample sites in burn-injured patients is challenging and sometimes impossible. In the study, sample sites including nares, throat and perineum resembled colonization, however, concomitant cultured isolates from wounds and catheters could not be surely discriminated as colonized or infected. Although bloodstream isolates are often regarded as invasive pathogens, burn injuries cause significant barrier damage, making transient bacteraemia from colonized wounds possible. While colonization of *S. aureus* in the present study has not been associated to mortality, it contributed to prolonged hospitalization, likely due to prolonged wound healing and infectious complications.

It has been proposed that pre-colonization of burn eschar with biofilm producing *S. aureus* support secondary colonization with gram-negative rods that are more prone to cause invasive infection and systemic immune response.[6,8,27] In *S. aureus*, the formation of biofilm induces the production of leucocidins like Panton-Valentine leucocidin and HlgAB that trigger the NET-associated neutrophil death and thereby prevent the clearing of *S. aureus* biofilm by neutrophils.[28] From histological investigations of thermal injuries in a porcine model could be seen that *S. aureus* mono-infected wounds have a lower level of tissue necrosis and neutrophiles, compared to *P. aeruginosa* infected wounds.[29] Also, the distribution of *S. aureus* aggregates in the mature wound biofilm on the surface and *P. aeruginosa* aggregates in deeper levels, may contribute to inhibit wound healing and preserve the inflammatory stage of co-infected wounds.[30] When burn injuries in rodents were co-infected with both *S. aureus* and *P. aeruginosa*, the pathogen most frequently detected in the bloodstream was *P. aeruginosa*, while *S. aureus* was rather sporadically detected.[9]

In *S. aureus*, production of biofilm showed inter-clonal variability, with CC5, CC15 and CC45 being effective biofilm producers; but also intra-clonal variability in biofilm production was seen for CC22 and CC30.[31,32] Furthermore, regulation of biofilm production is complex and differs with concomitant bacterial flora and environmental factors.[33] Biofilm formation in *S. aureus* has previously been linked to the *agr* locus that is important for the species' quorum sensing system, and *agr* genotype I and III were related to medium biofilm producers, *agr* genotype II to strong producers and *agr* IV to weak biofilm production.[34] The study showed distinct differences in the timing of antibiotic treatment initiation based on the lineages of *S. aureus* isolates. Patients with isolates belonging to phylogroup 2 received antibiotics approximately one day later, and specifically, patients with CC5 and CC15 isolates received antibiotics nearly three days later compared to patients with CC22 and CC45 isolates. It is intriguing to speculate whether these findings could find a reasonable explanation with the above-described associations: All but one isolate that belonged to CC5 and CC15 had *agr* genotype II, which has been linked to strong biofilm production and invasiveness.[26] The colonization and establishment of the biofilm by endogenous *S. aureus* in the burn eschar could have been recognized with delay due to associated lower levels of inflammation, and thus leading to a delay in antibiotic treatment. Correspondently, study isolates belonging to CC22 and CC45 had *agr* genotype I that was previously associated to medium level biofilm production, and especially in CC22 bacterial toxicity was most important in prediction of mortality.[31,34]

Established biofilm infections in wounds are a recognized therapeutic challenge and have been associated with increased mortality, delayed wound healing and scarring. Therapeutic modalities include primarily aggressive debridement and topical antiseptics. Systemic antibiotic treatment is regularly insufficient in curing the local wound status, as it is rather controlling the systemic infection.[35] It has long been recognized that measures to impede wound infection have a central role in burn care and include intensive treatment rooms (laminar down-flow with positive air pressure, high ventilation rate, equipped with patient bound sterile coats, glove and gowns, etc), accompanied by thorough hygiene measures and cleaning routines implemented by the staff, and regular screening for wound colonization by wound pathogens in order to early initiate appropriate wound care. Intriguingly, while there is evidence that reduction of *S. aureus* carriage with nasal mupirocin application seem to reduce risk of infections in total joint arthroplasty, no evidence for reduction of burn wound colonization was seen by Jaspers.[36,37] Potentially, it could be too late to apply eradication strategies on nasal carriage in order to prevent the spread of *S. aureus* to the wounds after the burn injury has occurred. Therefore, alternative preventative strategies, such as vaccination, immune- or phage therapy, may hold greater potential in benefiting burn-injured patients.[11]

Conclusions

The present study substantiates that *S. aureus* colonization contributes to the course of burn treatment, particularly in patients with severe burn injuries requiring prolonged stays in intensive care. Remarkably, different lineages of *S. aureus* were associated with a varying delay in initiating antibiotic treatment, potentially reflecting differences in strain virulence. While *S. aureus* screening is standard in some settings, such as UK burns centers, our findings highlight the potential benefits of incorporating phylogenetic grouping into protocols. This approach could optimize antibiotic use by targeting specific lineages rather than applying uniform strategies. Although it remains unclear whether earlier or later initiation of antibiotics is more beneficial for patients, further investigation into the clonal properties of *S. aureus*, particularly in relation to co-colonization with Gram-negative bacteria, invasive infections, and scarring, is warranted. Tailored screening practices could increase awareness of infection risks and potentially intensify wound treatment.

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Author contributions

The authors contributed to the study as follows: study conception and design: FH, TS, FF, JIF; sample collection and culture: ML, ET; processing of experimental analysis: ET, SS, SF; calculations and visualization: SS, SF; SS and ML took lead in writing the manuscript; all authors provided critical feedback and helped shape the research throughout all study phases and contributed significantly to the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

Table 1. Summary of descriptive data from 80 patients with burn injuries treated at the Uppsala Burn Centre, Sweden. Data are presented as median (range) or rate in percent, respectively.

Patient characteristic		Patients (n=80)
Age (years)		51 (19–93)
Sex		Male 53/80 (66%)
		Female 27/80 (34%)
BMI (kg/m^2)		27 (15–47)
Concomitant disease at admission		54/80 (68%)
Concomitant medication at admission		35/80 (44%)
Mortality		13/80 (16%)
LOS at Uppsala burn centre (days)		13 (1–94)
Antibiotic-free days from admission (days)		2 (0–11)
Total Body Surface Area burned (TBSA)		20% (4–92)
Revised Baux score (rBaux)		77 (28–150)
Inhalation injury		20/80 (25%)
Type of injury:		
	Flame	66/80 (82%)
	Scald	10/80 (12%)
	Contact	2/80 (3%)
	Electric	2/80 (3%)

Table 2. Summary of sequence typing of whole-genome sequenced isolates. The isolates were assigned to phylogenetic lineages group 1a and 1b and 12 according to Feng *et al.*, Monecke *et al.* and Cooper *et al.*[13–15] Numbers in parenthesis indicate number of patients carrying at least one isolate with respective sequence type.

Clonal cluster	Sequence types	STs for blood stream isolates	STs for deceased patients
Group 1a			
CC22 (5)	ST22 (5)		
CC30 (4)	ST30 (2), ST34 (1), unknown ST (1), ST7445 (1), ST7446 (1),		
CC45 (10)	ST45 (8), ST4229 (1), ST7450 (1)	ST45 (1)	ST45 (1)
Group 1b			
CC121 (1)	ST121 (1)		
No assigned CC	ST59 (1)		
Group 1a or 1b			
No assigned CC	ST375 (1), ST395 (2), ST7448 (1)		
Group 2			
CC1 (2)	ST1 (1), ST188 (1)		
CC5 (15)	ST5(13), ST7444 (1), ST7449 (1),	ST5 (1)	ST5 (2)
CC8 (4)	ST8 (2), ST72 (1), ST630 (1)		ST72 (1)
CC15 (7)	ST15 (5), ST582 (1), ST7443 (1)	ST15 (1), ST7443 (1)	ST15 (2)
CC97 (4)	ST97 (4)	ST97 (1)	
No assigned CC	ST7 (1), ST12 (1), ST20 (1), ST101 (1), ST3075 (1), ST7447 (1),		ST12 (1), ST7447 (1)

Table 3. Summary of *agr* types in *S. aureus* colonized patients and results of outcome variables.

	agr I (n = 26)	*agr* II (n = 19)	*agr* III (n = 3)	More than one *agr* type (n = 4)
Length of stay at burn centre (days)	17 (1–94)	15 (1–84)	30 (23–40)	16 (8–68)
Antibiotic-free days (days)	2 (0–11)	4 (1–10)	1 (0–2)	2 (1–8)
Mortality	3/26 (12%)	5/19 (26%)	0	0

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