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colonization, carriage and transmission

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General introduction

GENERAL INTRODUCTION

Staphylococcus aureus colonization and carriage

Scottish surgeon Sir Alexander Ogston discovered staphylococcus in 1881 as a cause of wound infection. The nomenclature was derived from his microscopic observation of grape-like clusters (in Greek: *staphyle*) (1). In 1884, *Staphylococcus aureus* (*S. aureus*), synonymous with 'golden staph' because of its golden appearance on agar plates, was first isolated from human pleural fluid by Anton Rosenbach (2). *S. aureus* is capable of colonizing human skin and mucosa (3). The predominant niche of *S. aureus* is the anterior nares, and 20-30% of humans are colonized with this bacterium (4). Generally, the nasal microbiome is unique per individual and it may be influenced by health status. It is composed of different bacterial species, of which the *Staphylococcus* genus is the most abundant, besides the genera *Corynebacterium* and *Propionibacterium* (5). The chance of becoming colonized with *S. aureus* could also depend on the composition of the local microbiome. Frank et al. studied the influence of different bacterial species on nasal *S. aureus* colonization and they found that *Staphylococcus epidermidis* (*S. epidermidis*) has a negative influence on *S. aureus* nasal colonization (5, 6). *S. aureus* colonization extends beyond only the nose; other carriage locations are the pharynx, axilla, inguinal area, vagina and perineum (7). Traditionally, there are three patterns of nasal *S. aureus* carriage described: persistent-, intermittent- or non-carriage (4). Yet, during the years there have been many debates on how to classify the different *S. aureus* carriage patterns, as intermittent carriage was considered equal to non-carriage, in view of the shared characteristics (8). Additionally, even several definitions for persistent carriage are described. Nouwen et al. proposed the definition for persistent carriage as carriage with 10^3 or more colony forming units (CFU) of *S. aureus* in two consecutive cultures with a one-week interval (9). Other interpretations for persistent carriage were: all swabs in one individual needed to be cultured positive for *S. aureus,* independent of the number (10), or the use of a cut-off value of the number of positive swabs per total number of swabs was used (4). Finally, an interesting phenomenon is observed in the group of persistent nasal *S. aureus* carriers. In a human inoculation experiment, in which a mixture of different *S. aureus* strains including the individuals' endogenous strain was used, was observed that persistent carriers select their own strain back and harbor it over years (11).

The literature describes an association between *S. aureus* carriage and risks of developing an infection with the bacterium in populations that are prone to carriage. It was shown that persistent *S. aureus* carriers have a higher risk of developing infections than other carriers (12-14). In addition, some patient populations are because of their underlying disease more at risk for nasal *S. aureus* colonization. For instance, both dialysis-dependent diabetic patients and human immunodeficiency virus (HIV)-infected patients have a higher prevalence. Patients with atopic dermatitis and furunculosis also show increased carriage rates. Regarding autoimmune diseases, there is a relation between increased *S. aureus* carriage in

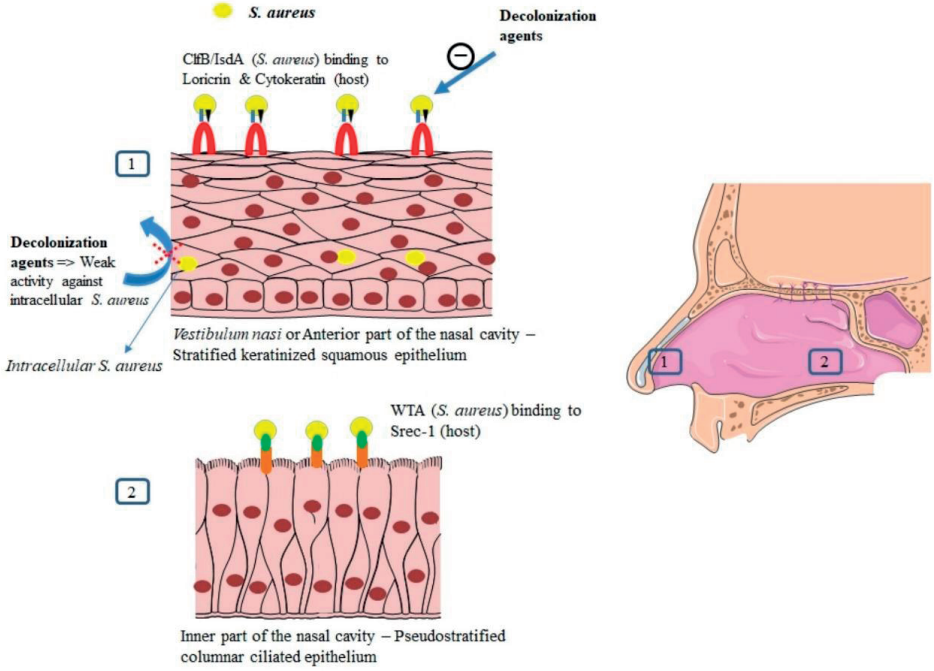
patients suffering from rheumatoid arthritis and granulomatosis with polyangiitis (5). Until now, there is no clear explanation why these populations are at risk for carriage. As far as we know, genetic factors do not play a role in whether or not humans become *S. aureus* carriers (15, 16).

So why is a subgroup of healthy and patient populations susceptible to *S. aureus* colonization, while others are non-receptive? A lot of research has been carried out in humans and several animal models, to understand *S. aureus* (de)colonization/carriage, which has certainly resolved some questions. First, the anterior nares or the *vestibulum nasi* is built up out of stratified, keratinized, non-ciliated squamous epithelium and ciliated columnar epithelium (17, 18). *S. aureus* recognizes both as habitats, after which colonization follows in susceptible individuals (19, 20). Multiple mechanisms are described that play a role in bacterial binding to the nasal tissue. The keratinocytes in the cornified layer of the epidermidis produce and express several proteins, e.g. loricrin and cytokeratin 10 (K10), to which some staphylococcal proteins are known to adhere. Examples are the surface proteins iron-regulated surface determinant A (isdA) and clumping factor B (clfB) (20-25). Recently, it has been shown that loricrin is the most important target for clfB to favor nasal colonization in mice. In a murine model, mice were inoculated with a *S. aureus* strain harboring clfB, whereupon in loricrin-deficient mice colonization mostly failed (20).

To help making colonization more successful, the bacterium needs to express locally many adhesive molecules that strengthen the pathogen-host interplay (26). In a human nasal inoculation experiment, Wertheim et al. showed that clfB plays an important role in colonization, as the inoculated clfB$^+$ *S. aureus* strain survived longer in comparison to the mutant strain. The study also showed the *in vitro* interaction of clfB with cytokeratin 10, which was mentioned earlier (22). Furthermore, wall teichoic acid (wta), a cell surface glycopolymer, was considered essential in colonization in a cotton rat model, as wta mutants were unable to adhere to nasal cells (27). In addition, the interaction between wta and SREC-1 (a member of the F-type scavenger receptor), a receptor that is expressed on epithelial cells in the nasal cavity of humans and cotton rats, positively influenced colonization (19). In a recent review on *S. aureus* colonization, a few other potential *S. aureus*-host ligands are mentioned, e.g. isdA-fibrinogen/fibronectin and sdrD-desmoglein 1 (5). Finally, Hanssen et al. described the intracellular localization of *S. aureus* in nasal tissue after biopsy in healthy individuals (28), of which we might speculate that the bacterium is capable of hiding from the immune system but also from antibiotic therapy. In Figure 1, a schematic overview is presented with the different mechanisms. It depicts the most important bacterial components involved in the process of nasal colonization.

As a counterpart of colonization studies, multiple decolonization strategies are studied in different animal models with mice and cotton rats. These experiments are often laborious because effects of decolonization cannot be monitored longitudinally, since separate groups of animals are needed at the different measuring points and animals have to be sacrificed

Figure 1. Interactions in *S. aureus* nasal colonization. Figure adapted from Sakr et al. (5)



at pre-specified time intervals to study the bacterial load (29-31). Decolonization studies are as important as colonization studies, because they enhance knowledge on how to eradicate carriage to lower infection rates, especially in populations at risk. In spite of all the research that has been performed on nasal (de)colonization in animals, probably the best way to study this is by using the human inoculation model (8, 11, 22, 32). Although, for ethical reasons, we are frequently dependent on the use of alternative animal models that could be helpful, a properly human-like animal model is lacking. As rhesus macaques have recently been added to the list of natural hosts of *S. aureus* (33), possibly these animals could be the answer.

Besides the possibility of using animal or human models, less invasive *in vitro* experiments may yield information on which bacterial components are involved in colonization and/or infection. Several studies have been published on induction of the humoral immune response by *S. aureus* colonization, infection, and on whether these antibodies were protective. In general, the *S. aureus* humoral immunoglobulin IgG antibody responses are studied extensively. In persistent *S. aureus* carriers, the serum levels of IgA and IgG directed to many staphylococcal proteins have been reported to be higher than they are in non-carriers (8). In mice models, different IgG responses indicate that there are numerous *S. aureus* antigens that are of importance and could be targeted, for example, in future vaccine studies (34, 35). In addition, in human *S. aureus* carriers versus non-carriers, IgG responses to toxic shock

syndrome toxin 1 (tsst-1), staphylococcal enterotoxin A (sea), clumping factor A (clfA) and clfB may predict the risk and outcome of *S. aureus* infections (36, 37).

S. aureus: the emerging pathogen causing (nosocomial) infections

The asymptomatically presence of *S. aureus* and its behavior of mostly acting as a commensal bacterium, does not imply that it is not pathogenic. *S. aureus* causes infections that vary from skin and soft tissue infections like impetigo and furunculosis, to more severe infections, such as pneumonia and osteomyelitis (38). *S. aureus* is an important causative microorganism in surgical site infections, especially in orthopedic and cardiac procedures (5). Furthermore, *S.aureus* is common in bloodstream infections (39) and they are associated with endocarditis and prosthetic device infections (40, 41).

S. aureus bacteremia is also common in very low birth weight (VLBW) infants, which makes this bacterial species one of the most important pathogens in neonatal intensive care units (NICU) (42-44). A significant risk factor for *S. aureus* bacteremia in VLBW infants is the presence of intravascular catheters, which are frequently required (45-47). All-cause mortality among neonates suffering from *S. aureus* bacteremia varies between 10 and 20% (46, 48). Yet, *S. aureus* is a well-established nosocomial pathogen that also causes multiple other types of neonatal infections (49, 50).

S. aureus treatment and outbreaks

Traditionally penicillins, especially methicillin, was the first-choice antibiotic for infections with this bacterium. Unfortunately, the first detection of methicillin-resistant *S. aureus* (MRSA) occurred rapidly after the introduction of methicillin (51). Since the 1960s, MRSA-related infections have been a problem in hospitals worldwide. Nowadays, in some communities, the emergence of (new) clones of MRSA has also occurred in the community among individuals who were not in contact with healthcare (52). The group of glycopeptides is one of the few groups of antibiotics that are left to treat MRSA-related infections (53).

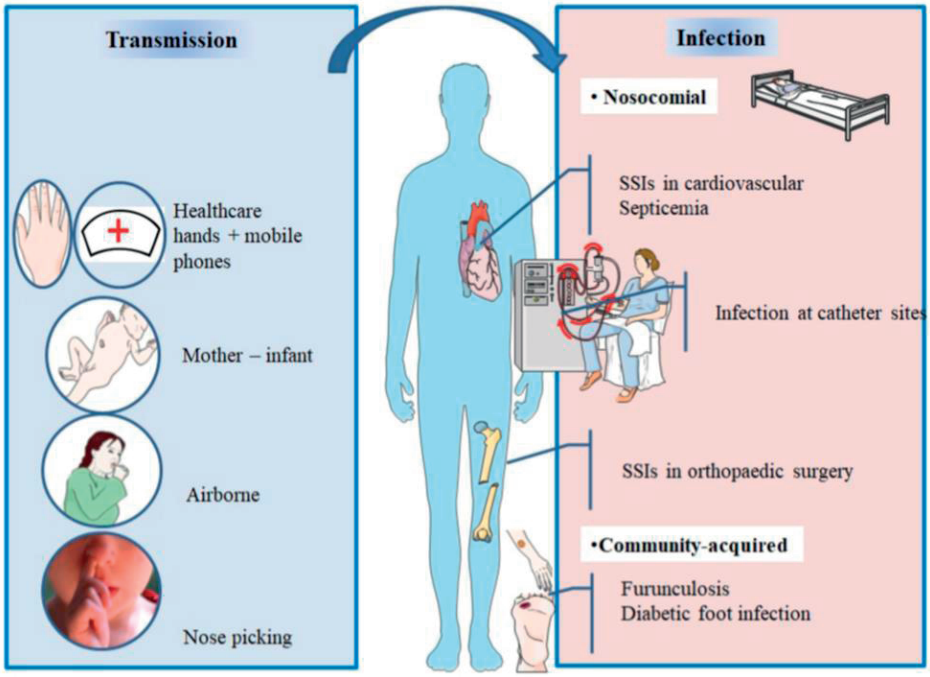
In the Netherlands, the prevalence of MRSA at hospital admission is very low, namely 0.13% between 2010-2017 (54). Due to a Search and Destroy policy (S&D), we are able to keep these numbers low. In Europe, including the Netherlands, for years there has been an increased incidence of carriage of a livestock-associated (LA)-MRSA. People in direct contact with livestock, such as farmers and veterinarians, are at risk of becoming a LA-MRSA carrier. The majority of these LA-MRSA cases is caused by ST398 (55). It was reported that humans, who temporarily are in close contact with livestock, easily acquire LA-MRSA ST398, but also shed the strain in less than one day (56). Furthermore, the nosocomial transmission of LA-MRSA ST398 was 72% less likely than with non-ST398 MRSA strains (57). At this moment, there is no data concerning the intrinsic capacity of ST398 to colonize the human nose.

Data on outbreaks with methicillin-susceptible *S. aureus* (MSSA) in adults is missing, as these outbreaks remain undetected. Yet, Price et al. showed that patients on an adult

intensive care unit got colonized and infected with genetically identical *S. aureus* strains transmitted via patients, the environment and healthcare workers (HCWs). Whole-genome sequencing (WGS), the most discriminatory typing method, was used to prove *S. aureus* transmission (58).

Neonates, with their immature microbiome, low gestational age and birth weight, and the immaturity of their organ systems, are prone to developing healthcare-associated infections (HAIs) (59). NICU outbreaks of MRSA and MSSA are frequently described and different typing techniques are used to show the genetic relatedness of the strains (60-67). As there is no direct patient-to-patient contact on a NICU, transmission via the hands of HCWs seems questionable. Studies are published in which HCWs are the source of *S. aureus* infections in neonates, resulting in outbreaks (65, 66). Risk factors for nosocomial transmission on the NICU are, besides the environment, overcrowding of patients and understaffing (58, 68, 69). Figure 2 shows the transmission of *S. aureus* causing (nosocomial) infections.

Figure 2. Transmission of *S. aureus* causing (nosocomial) infections. Figure adapted from Sakr et al. (5)



AIM AND OUTLINE OF THE THESIS

The primary aim of this thesis was to gain more insights in the colonizing capacity of *S. aureus*. For this purpose, we developed a novel experimental decolonization and carriage

model in rhesus macaques (*Macaca mulatta*), and we performed an artificial human inoculation study.

The secondary aim of this thesis was to investigate, by using whole-genome sequencing, whether nosocomial acquisition of *S. aureus* via healthcare workers occurred in neonates admitted to a neonatal intensive care unit. By determining the genetic makeup of neonatal bloodstream isolates, transmission could be detected and presence of specific virulence genes might possibly explain the invasiveness.

In this thesis we explored the possibility of developing an experimental decolonization and inoculation procedure in rhesus macaques, as a human-like animal model is still lacking (**Chapter 2.1)**. As data concerning the intrinsic capacity of *S. aureus* ST398 to colonize the human nose are not available, an artificial human inoculation experiment was performed with a mixture of a bovine methicillin-susceptible *S. aureus* ST398 (CC398) strain and a methicillin-susceptible *S. aureus* ST931 (CC8) of human origin. Over a period of 21 days, we determined their ability to survive in the anterior nares of healthy volunteers (**Chapter 2.2)**. In **Chapter 3.1**, we explored whether healthcare workers could possibly be involved in the *S. aureus* transmission to neonates on a neonatal intensive care unit by using whole-genome sequencing. Finally, we studied the transmission and genetic makeup of neonatal bloodstream isolates by using whole-genome sequencing in **Chapter 3.2**, to explore whether these data support the frequent occurrence of neonatal *S. aureus* bacteremia.

REFERENCES

1. Ogston A. Micrococcus Poisoning. J Anat Physiol. 1882;17(Pt 1):24-58.
2. Cowan ST, Shaw C, Williams RE. Type strain for Staphylococcus aureus Rosenbach. J Gen Microbiol. 1954;10(1):174-6.
3. Williams RE. Healthy carriage of Staphylococcus aureus: its prevalence and importance. Bacteriol Rev. 1963;27:56-71.
4. Eriksen NH, Espersen F, Rosdahl VT, Jensen K. Carriage of Staphylococcus aureus among 104 healthy persons during a 19-month period. Epidemiol Infect. 1995;115(1):51-60.
5. Sakr A, Bregeon F, Mege JL, Rolain JM, Blin O. Staphylococcus aureus Nasal Colonization: An Update on Mechanisms, Epidemiology, Risk Factors, and Subsequent Infections. Front Microbiol. 2018;9:2419.
6. Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, Pace NR. The human nasal microbiota and Staphylococcus aureus carriage. PLoS One. 2010;5(5):e10598.
7. 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(12):751-62.
8. 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(12):1820-6.
9. Nouwen JL, Ott A, Kluytmans-Vandenbergh MF, Boelens HA, Hofman A, van Belkum A, et al. Predicting the Staphylococcus aureus nasal carrier state: derivation and validation of a “culture rule”. Clin Infect Dis. 2004;39(6):806-11.
10. Muthukrishnan G, Lamers RP, Ellis A, Paramanandam V, Persaud AB, Tafur S, et al. Longitudinal genetic analyses of Staphylococcus aureus nasal carriage dynamics in a diverse population. BMC Infect Dis. 2013;13:221.
11. Nouwen J, Boelens H, van Belkum A, Verbrugh H. Human factor in Staphylococcus aureus nasal carriage. Infect Immun. 2004;72(11):6685-8.
12. Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, Roosendaal R, et al. Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. N Engl J Med. 2010;362(1):9-17.
13. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev. 1997;10(3):505-20.
14. Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, et al. Risk and outcome of nosocomial Staphylococcus aureus bacteraemia in nasal carriers versus non-carriers. Lancet. 2004;364(9435):703-5.
15. Andersen PS, Pedersen JK, Fode P, Skov RL, Fowler VG, Jr., Stegger M, et al. Influence of host genetics and environment on nasal carriage of staphylococcus aureus in danish middle-aged and elderly twins. J Infect Dis. 2012;206(8):1178-84.
16. Roghmann MC, Johnson JK, Stine OC, Lydecker AD, Ryan KA, Mitchell BD, et al. Persistent Staphylococcus aureus colonization is not a strongly heritable trait in Amish families. PLoS One. 2011;6(2):e17368.
17. Peacock SJ, de Silva I, Lowy FD. What determines nasal carriage of Staphylococcus aureus? Trends Microbiol. 2001;9(12):605-10.
18. Weidenmaier C, Goerke C, Wolz C. Staphylococcus aureus determinants for nasal colonization. Trends Microbiol. 2012;20(5):243-50.
19. Baur S, Rautenberg M, Faulstich M, Grau T, Severin Y, Unger C, et al. A nasal epithelial receptor for Staphylococcus aureus WTA governs adhesion to epithelial cells and modulates nasal colonization. PLoS Pathog. 2014;10(5):e1004089.

20. Mulcahy ME, Geoghegan JA, Monk IR, O'Keeffe KM, Walsh EJ, Foster TJ, et al. Nasal colonisation by Staphylococcus aureus depends upon clumping factor B binding to the squamous epithelial cell envelope protein loricrin. PLoS Pathog. 2012;8(12):e1003092.
21. Schaffer AC, Solinga RM, Cocchiaro J, Portoles M, Kiser KB, Risley A, et al. Immunization with Staphylococcus aureus clumping factor B, a major determinant in nasal carriage, reduces nasal colonization in a murine model. Infect Immun. 2006;74(4):2145-53.
22. Wertheim HF, Walsh E, Choudhurry R, Melles DC, Boelens HA, Miajlovic H, et al. Key role for clumping factor B in Staphylococcus aureus nasal colonization of humans. PLoS Med. 2008;5(1):e17.
23. Clarke SR, Brummell KJ, Horsburgh MJ, McDowell PW, Mohamad SA, Stapleton MR, et al. Identification of in vivo-expressed antigens of Staphylococcus aureus and their use in vaccinations for protection against nasal carriage. J Infect Dis. 2006;193(8):1098-108.
24. Clarke SR, Wiltshire MD, Foster SJ. IsdA of Staphylococcus aureus is a broad spectrum, iron-regulated adhesin. Mol Microbiol. 2004;51(5):1509-19.
25. Eckhart L, Lippens S, Tschachler E, Declercq W. Cell death by cornification. Biochim Biophys Acta. 2013;1833(12):3471-80.
26. Burian M, Wolz C, Goerke C. Regulatory adaptation of Staphylococcus aureus during nasal colonization of humans. PLoS One. 2010;5(4):e10040.
27. Weidenmaier C, Kokai-Kun JF, Kristian SA, Chanturiya T, Kalbacher H, Gross M, et al. Role of teichoic acids in Staphylococcus aureus nasal colonization, a major risk factor in nosocomial infections. Nat Med. 2004;10(3):243-5.
28. Hanssen AM, Kindlund B, Stenklev NC, Furberg AS, Fismen S, Olsen RS, et al. Localization of Staphylococcus aureus in tissue from the nasal vestibule in healthy carriers. BMC Microbiol. 2017;17(1):89.
29. Daniel A, Euler C, Collin M, Chahales P, Gorelick KJ, Fischetti VA. Synergism between a novel chimeric lysin and oxacillin protects against infection by methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2010;54(4):1603-12.
30. Desbois AP, Sattar A, Graham S, Warn PA, Coote PJ. MRSA decolonization of cotton rat nares by a combination treatment comprising lysostaphin and the antimicrobial peptide ranalexin. J Antimicrob Chemother. 2013;68(11):2569-75.
31. Kokai-Kun JF, Walsh SM, Chanturiya T, Mond JJ. Lysostaphin cream eradicates Staphylococcus aureus nasal colonization in a cotton rat model. Antimicrob Agents Chemother. 2003;47(5):1589-97.
32. Verkaik NJ, Benard M, Boelens HA, de Vogel CP, Nouwen JL, Verbrugh HA, et al. Immune evasion cluster-positive bacteriophages are highly prevalent among human Staphylococcus aureus strains, but they are not essential in the first stages of nasal colonization. Clin Microbiol Infect. 2011;17(3):343-8.
33. van den Berg S, van Wamel WJ, Snijders SV, Ouwerling B, de Vogel CP, Boelens HA, et al. Rhesus macaques (Macaca mulatta) are natural hosts of specific Staphylococcus aureus lineages. PLoS One. 2011;6(10):e26170.
34. Schulz D, Grumann D, Trube P, Pritchett-Corning K, Johnson S, Reppschlager K, et al. Laboratory Mice Are Frequently Colonized with Staphylococcus aureus and Mount a Systemic Immune Response-Note of Caution for In vivo Infection Experiments. Front Cell Infect Microbiol. 2017;7:152.
35. Zhao F, Cheng BL, Boyle-Vavra S, Alegre ML, Daum RS, Chong AS, et al. Proteomic Identification of saeRS-Dependent Targets Critical for Protective Humoral Immunity against Staphylococcus aureus Skin Infection. Infect Immun. 2015;83(9):3712-21.
36. Verkaik NJ, de Vogel CP, Boelens HA, Grumann D, Hoogenboezem T, Vink C, et al. Anti-staphylococcal humoral immune response in persistent nasal carriers and noncarriers of Staphylococcus aureus. J Infect Dis. 2009;199(5):625-32.

37. Ghasemzadeh-Moghaddam H, van Wamel W, van Belkum A, Hamat RA, Neela VK. Differences in humoral immune response between patients with or without nasal carriage of Staphylococcus aureus. Eur J Clin Microbiol Infect Dis. 2017;36(3):451-8.
38. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998;339(8):520-32.
39. Pittet D, Wenzel RP. Nosocomial bloodstream infections. Secular trends in rates, mortality, and contribution to total hospital deaths. Arch Intern Med. 1995;155(11):1177-84.
40. Fowler VG, Jr., Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB, et al. Clinical identifiers of complicated Staphylococcus aureus bacteremia. Arch Intern Med. 2003;163(17):2066-72.
41. Wang A, Athan E, Pappas PA, Fowler VG, Jr., Olaison L, Pare C, et al. Contemporary clinical profile and outcome of prosthetic valve endocarditis. JAMA. 2007;297(12):1354-61.
42. Carey AJ, Duchon J, Della-Latta P, Saiman L. The epidemiology of methicillin-susceptible and methicillin-resistant Staphylococcus aureus in a neonatal intensive care unit, 2000-2007. J Perinatol. 2010;30(2):135-9.
43. Dolapo O, Dhanireddy R, Talati AJ. Trends of Staphylococcus aureus bloodstream infections in a neonatal intensive care unit from 2000-2009. BMC Pediatr. 2014;14:121.
44. Ericson JE, Popoola VO, Smith PB, Benjamin DK, Fowler VG, Benjamin DK, Jr., et al. Burden of Invasive Staphylococcus aureus Infections in Hospitalized Infants. JAMA Pediatr. 2015;169(12):1105-11.
45. Ekkelenkamp MB, van der Bruggen T, van de Vijver DA, Wolfs TF, Bonten MJ. Bacteremic complications of intravascular catheters colonized with Staphylococcus aureus. Clin Infect Dis. 2008;46(1):114-8.
46. Hakim H, Mylotte JM, Faden H. Morbidity and mortality of Staphylococcal bacteremia in children. Am J Infect Control. 2007;35(2):102-5.
47. Murdoch F, Danial J, Morris AK, Czarniak E, Bishop JL, Glass E, et al. The Scottish enhanced Staphylococcus aureus bacteraemia surveillance programme: the first 18 months of data in children. J Hosp Infect. 2017;97(2):127-32.
48. Kaufman D, Fairchild KD. Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. Clin Microbiol Rev. 2004;17(3):638-80, table of contents.
49. Jeong IS, Jeong JS, Choi EO. Nosocomial infection in a newborn intensive care unit (NICU), South Korea. BMC Infect Dis. 2006;6:103.
50. Reichert F, Piening B, Geffers C, Gastmeier P, Buhrer C, Schwab F. Pathogen-Specific Clustering of Nosocomial Blood Stream Infections in Very Preterm Infants. Pediatrics. 2016;137(4).
51. Jevons MP, Coe AW, Parker MT. Methicillin resistance in staphylococci. Lancet. 1963;1(7287):904-7.
52. Deurenberg RH, Stobberingh EE. The evolution of Staphylococcus aureus. Infect Genet Evol. 2008;8(6):747-63.
53. Tenover FC, Biddle JW, Lancaster MV. Increasing resistance to vancomycin and other glycopeptides in Staphylococcus aureus. Emerg Infect Dis. 2001;7(2):327-32.
54. Weterings V, Veenemans J, van Rijen M, Kluytmans J. Prevalence of nasal carriage of methicillin-resistant Staphylococcus aureus in patients at hospital admission in The Netherlands, 2010-2017: an observational study. Clin Microbiol Infect. 2019;25(11):1428 e1- e5.
55. Wulf MW, Sorum M, van Nes A, Skov R, Melchers WJ, Klaassen CH, et al. Prevalence of methicillin-resistant Staphylococcus aureus among veterinarians: an international study. Clin Microbiol Infect. 2008;14(1):29-34.
56. van Cleef BA, Graveland H, Haenen AP, van de Giessen AW, Heederik D, Wagenaar JA, et al. Persistence of livestock-associated methicillin-resistant Staphylococcus aureus in field workers after short-term occupational exposure to pigs and veal calves. J Clin Microbiol. 2011;49(3):1030-3.

57. Wassenberg MW, Bootsma MC, Troelstra A, Kluytmans JA, Bonten MJ. Transmissibility of livestock-associated methicillin-resistant Staphylococcus aureus (ST398) in Dutch hospitals. Clin Microbiol Infect. 2011;17(2):316-9.
58. Price JR, Cole K, Bexley A, Kostiou V, Eyre DW, Golubchik T, et al. Transmission of Staphylococcus aureus between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. Lancet Infect Dis. 2017;17(2):207-14.
59. Phillips P, Cortina-Borja M, Millar M, Gilbert R. Risk-adjusted surveillance of hospital-acquired infections in neonatal intensive care units: a systematic review. J Hosp Infect. 2008;70(3):203-11.
60. Huang YC, Lien RI, Su LH, Chou YH, Lin TY. Successful control of methicillin-resistant Staphylococcus aureus in endemic neonatal intensive care units--a 7-year campaign. PLoS One. 2011;6(8):e23001.
61. Regev-Yochay G, Rubinstein E, Barzilai A, Carmeli Y, Kuint J, Etienne J, et al. Methicillin-resistant Staphylococcus aureus in neonatal intensive care unit. Emerg Infect Dis. 2005;11(3):453-6.
62. Reich PJ, Boyle MG, Hogan PG, Johnson AJ, Wallace MA, Elward AM, et al. Emergence of community-associated methicillin-resistant Staphylococcus aureus strains in the neonatal intensive care unit: an infection prevention and patient safety challenge. Clin Microbiol Infect. 2016;22(7):645 e1-8.
63. Achermann Y, Seidl K, Kuster SP, Leimer N, Durisch N, Ajdler-Schaffler E, et al. Epidemiology of Methicillin-Susceptible Staphylococcus aureus in a Neonatology Ward. Infect Control Hosp Epidemiol. 2015;36(11):1305-12.
64. Ramsing BG, Arpi M, Andersen EA, Knabe N, Mogensen D, Buhl D, et al. First outbreak with MRSA in a Danish neonatal intensive care unit: risk factors and control procedures. PLoS One. 2013;8(6):e66904.
65. Gomez-Gonzalez C, Alba C, Otero JR, Sanz F, Chaves F. Long persistence of methicillin-susceptible strains of Staphylococcus aureus causing sepsis in a neonatal intensive care unit. J Clin Microbiol. 2007;45(7):2301-4.
66. Koningstein M, Groen L, Geraats-Peters K, Lutgens S, Rietveld A, Jira P, et al. The use of typing methods and infection prevention measures to control a bullous impetigo outbreak on a neonatal ward. Antimicrob Resist Infect Control. 2012;1(1):37.
67. Lin MF, Huang ML, Lai SH. Investigation of a pyoderma outbreak caused by methicillin-susceptible Staphylococcus aureus in a nursery for newborns. J Hosp Infect. 2004;57(1):38-43.
68. Andersen BM, Lindemann R, Bergh K, Nesheim BI, Syversen G, Solheim N, et al. Spread of methicillin-resistant Staphylococcus aureus in a neonatal intensive unit associated with understaffing, overcrowding and mixing of patients. J Hosp Infect. 2002;50(1):18-24.
69. Popoola VO, Budd A, Wittig SM, Ross T, Aucott SW, Perl TM, et al. Methicillin-resistant Staphylococcus aureus transmission and infections in a neonatal intensive care unit despite active surveillance cultures and decolonization: challenges for infection prevention. Infect Control Hosp Epidemiol. 2014;35(4):412-8.

2

Staphylococcus aureus colonization and carriage

2.1

An experimental *Staphylococcus aureus* carriage and decolonization model in Rhesus Macaques (*Macaca mulatta*)

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ABSTRACT

Our human model of nasal colonization and eradication of *S. aureus* is limited by safety issues. As rhesus macaques are closely related to humans and natural hosts for *S. aureus*, we developed an experimental decolonization and inoculation protocol in these animals. Animals were screened for nasal carriage of *S. aureus* and 20 carriers were selected. Decolonization was attempted using nasal mupirocin (10 animals) or mupirocin plus trimethoprim/sulfadiazine intramuscularly (10 animals) both once daily for 5 days, and checked by follow-up cultures for 10 weeks. Intranasal inoculation was performed with *S. aureus* strain 8325-4 in culture-negative animals. 11/20 animals, of which 5 received mupirocin and 6 the combination treatment, became culture-negative for *S. aureus* for 10 weeks and these 11 animals were subsequently inoculated. Swabs were taken once a week for 5 weeks to test for the presence of the inoculated strain. In 3 animals, strain 8325-4 was cultured from the nose 1 week after inoculation, indicating short-term survival of this strain only, a finding similar to that previously found in our human model. These data demonstrate that rhesus macaques may constitute a relevant animal model to perform *S. aureus* eradication and inoculation studies with relatively limited invasive handling of the animals.

INTRODUCTION

It is well established that *Staphylococcus aureus* (*S. aureus*) carriage increases the risk of endogenous infections with this bacterium [1, 2]. Bode et al. showed that eradication of *S. aureus* from the nose and skin significantly decreases the risk of endogenous infections in a hospital setting [3]. Many different (methicillin-resistant and susceptible) *S. aureus* eradication and decolonization strategies have been described. Variations in these treatments are made based on the presence of clinically evident infection [4]. To further improve our understanding of human *S. aureus* nasal colonization and to support the development of new decolonization strategies additional well-designed experiments are needed. Although we have performed human nasal inoculation studies in our group [5-9], for ethical and safety reasons these experiments are restricted to a conformational setup while more exploratory designs are often desired. Therefore, animal models of *S. aureus* carriage are needed and some have been presented in the literature. Ideally, an animal model closely mimics the human condition, in this case requiring the animal to be a natural host of *S. aureus* and to be evolutionary closely related to humans. Furthermore, (de) colonization is best studied over a period of time in which at different time points of follow-up samples can be taken from the same animal. In one model the cotton rat requires relatively high inocula ($1*10^7$ to $1*10^9$ CFU) in order to establish nasal colonization [10-12]. In addition, animals need to be sacrificed at pre-specified intervals in order to determine bacterial colonization. Thus, bacterial and host factors interfering with colonization cannot be studied longitudinally as for each time-point separate groups of animals are included [10-12]. The same limitations apply for mouse models [13, 14]. Successful *S. aureus* colonization has been realized in pigs, but only in piglets delivered by Caesarean section and deprived of colostrum [15, 16]. In addition to research regarding determinants of colonization, *S. aureus* decolonization strategies have also been studied in mice and cotton rats, but we are confronted with the same limitations [17-19]. Recently, rhesus macaques (*Macaca mulatta*) were added to the list of natural hosts of *S. aureus* [20] making them potentially an ideal human-like model to study *S. aureus* (de) colonization.

Therefore, in this study we explored the possibility to develop an experimental decolonization and inoculation procedure in rhesus macaques. To do so, we applied two decolonization strategies; both used in humans, and investigated their potential to decolonize the nares of rhesus macaques carrying *S. aureus* in their nares. In addition, we studied the possibility to colonize rhesus macaques with a human *S. aureus* strain.

MATERIALS AND METHODS

Study setup

Rhesus macaques were screened for *S. aureus* nasal carriage and carriers were included (definition below). The study design had two phases: in phase 1 *S. aureus* decolonization with two different treatment strategies was performed, followed by phase 2 in which nasal inoculation with a human *S. aureus* strain was attempted.

Animal Study Population

Animals were selected from the macaque-breeding colony of the Biomedical Primate Research Centre Rijswijk, the Netherlands. They were born and raised at the institute and remained in their natal groups for at least 4 years before being selected for research protocols. All animals received a complete physical, haematological and biochemical examination before inclusion and were social-housed (pair or trio) for at least 3 weeks before the onset in 3 animal rooms. Physical contact with other rhesus macaques from the same experimental group in neighboring cages was possible. In each room the cages were located in two rows opposite to each other. Because of welfare some of the animals were placed with their buddy rhesus macaque during phase 1 and 2. A buddy rhesus macaque was not screened for *S. aureus* nasal carriage but it did receive the same decolonization treatment as its *S. aureus* carrier cage mate. Their culture results were not included in the study.

Ethical Considerations

All animals were socially housed in cages measuring at least 3.6 m^3 (2x1.8x1 m) in line with the size requirements of EU Directive 2010/63. Cages were provided with bedding and food and non-food enrichment items. Water was provided *ad libitum*. The animals were fed commercial monkey pellets (Ssniff, Soest, Germany) supplemented with a variation of bread, vegetables and fruit. All experimental procedures with the animals were performed under sedation with 10-mg/kg ketamine. The study protocol was approved by the Animal Ethics Committee of the Biomedical Primate Research Centre (BPRC) Rijswijk, The Netherlands (DEC #706).

Sampling procedures

The nose was sampled by swabbing both the left and right anterior nares with a single swab (Amies charcoal transport swabs orange, Copan, CA, USA). Throat and rectal swabs (Amies charcoal transport swabs black, Copan, CA, USA) were taken respectively by swabbing the back of the pharynx and by revolving a swab 2 cm into the rectum. The swabs were plated on Colombia 5% sheep blood agar (OXOID, Hampshire, UK) within a few hours. Plates were screened for *S. aureus* suspected colonies based on morphology after 24 and 48 hours of incubation at 35˚C. Final identification of *S. aureus* was performed by a latex slide agglutina-

tion test that detects clumping factor and Protein A (Staphytect Plus, OXOID, Hampshire, UK).

Susceptibility of the *S. aureus* strains isolated from the rhesus macaques

The VITEK 2 system (bioMérieux) was used for susceptibility testing. The *S. aureus* strains were picked randomly and had to originate from the nose from day 0 of the decolonization phase. *S. aureus* colonies were suspended in 0.9% saline (NaCl) to make a 0.5 McFarland (McF) bacterial suspension. Susceptibility card AST-P633 was used for staphylococci. This card contained benzylpenicillin, oxacillin, gentamicin, kanamycin, tobramycin, ciprofloxacin translated to levofloxacin, erythromycin, clindamycin, linezolid, teicoplanin, vancomycin, tetracycline, fosfomycin, fusidic acid, mupirocin, chloramphenicol, rifampicin and trimethoprim/sulfamethoxazole.

***S. aureus* strain for inoculation**

For the inoculation procedure we selected human strain 8325-4 (*spa* type 211; ST8) [21] which was previously used in human nasal inoculation studies [5, 8]. Bacteria grown overnight on Colombia 5% sheep blood agar were used to inoculate Brain Heart Infusion broth (BHI). After a few hours of growth at 37°C an OD of 0.5 McF was achieved. The pellet of bacterial culture was suspended in Phosphate Buffered Saline (PBS) to get the inoculum of $2*10^8$ CFU/ml. 50 microliters of this amount of bacteria in PBS contained $1*10^7$ CFU of bacterial cells.

Screening and Decolonization protocol

Taking 4-5 nasal swabs with intervals of one week, determined the nasal carriage state for *S. aureus*. Nasal carriers of *S. aureus* were defined as animals with four swabs (≥80%) culture-positive for *S. aureus.* Only carriers were included. Two different treatment strategies were used for decolonization. One treatment consisted of mupirocin nasal ointment (2%; Glaxo- SmithKline, Waltham, MA, USA) once daily for a total period of 5 days (treatment A). The other treatment consisted of a combination of mupirocin nasal ointment once daily with 18 mg/kg trimethoprim/sulfadiazine (40 mg/ml/200 mg/ml; Duphatroxim, Zoetis, NL) intramuscular injections once daily for a total period of 5 days (treatment B). The mupirocin nasal ointment was applied in the vestibulum nasi with a swab (Heinz Herenz sterile cotton swab). Trimethoprim/sulfadiazine was injected alternately into the left and right vastus lateralis. The study was performed in two different time periods in which the animals were equally divided over the two treatment regimens. In the follow-up period of 9 weeks a total of 9 (including t = 0, before start of the treatment) nasal, throat and rectal swabs were taken to investigate recolonization with *S. aureus*. More specifically, on day 0, 3 or 4, 7, 9, 14, 21, 35, 49 or 50 and 63 swabs were taken from the three culture sites.

Inoculation protocol

Nasal inoculation was only performed in animals successfully decolonized. Noses of animals were inoculated 7 days after the last follow-up culture moment from phase 1. As *S. aureus* nasal carriage was an exclusion criterion for further follow-up after inoculation, a control swab was taken from all culture sites on the day of inoculation. 50 microliters of the inoculum containing $1*10^7$ CFU of bacterial cells was pipetted separately into the left and right nostril while the head was put for two minutes in such a position that the inoculum could be absorbed by the mucosa. In the follow-up period the nose, throat and rectum were cultured in week 1, 2, 3 and 5. For identification of strain 8325-4 after inoculation, all cultured *S. aureus* strains obtained in the follow-up period from the inoculation phase, were analyzed by PCR analysis of the *spa* gene [22].

Hygiene protocol

To prevent any *S. aureus* acquisition from environmental, human or animal origin during both phases of the study, a hygiene protocol was implemented. Before entering the animal room personnel had to wear non-sterile gloves (Kimtech) and a new gown including a hood (Tyvek). After entering the room gloves were immediately disinfected with alcohol 70%. Care was taken to handle first the animals included in the study to prevent possible spread of *S. aureus* from animals that were not included. During the five consecutive days of sedation in the decolonization phase, the animals received 100 ml Nutrison Concentrated Food 2.0 kcal/ml through a gastric tube to prevent malnutrition. Non-food enrichment was cleaned with alcohol before rotation and bedding was replaced twice a week. For activities like contact with every separate cage animal caretakers disinfected their hands. Finally, keys were put in a bath with alcohol 70% to limit contamination.

STATISTICAL ANALYSIS

Recurrence, defined as the first occasion when *S. aureus* was isolated again after decolonization, was analyzed starting from day 7 after start of the decolonization. Recurrence was determined in either nose only, other sites than the nose or any site, i.e. nose, throat and/or rectum. Results were analyzed using Kaplan-Meier curves.

RESULTS

Screening and Decolonization

A total of 97 rhesus macaques were selected from the BPRC colony and their *S. aureus* carriage status was determined. 20 out of 73 rhesus macaques that were at least once

positive for *S. aureus* in the nose were found to be nasal *S. aureus* carriers. In total 9 buddies participated. Divided over 13 cages, 3 animals were triple-housed and the others were pair-housed. 10 rhesus macaques received treatment A (3 females, 7 males) and the others received treatment B (3 females, 7 males). Follow-up cultures were taken from the nose, throat and rectum.

Individual culture results from the start of both treatments including the follow-up period are shown in S1 Table. In the group that received treatment A, 2 animals (R10024 and R10122) were negative in the nose at day 0 and they were therefore excluded from analysis. For completeness, culture results from the buddy rhesus macaques were analyzed and added in this table. The results show in most cases variable carriage rates on the three culture sites in the different animals during follow-up. No specific carriage patterns were seen in the individual rhesus macaques except for some animals in which throat carriage was more persistent over time than in the other animals. Furthermore, cage mates were seldom carrier at the same culture site at the same culture moment (S1 Table).

In animals that received mupirocin only (treatment A) no *S. aureus* was cultured from the nose in any of the animals from day 7-21. *S. aureus* was observed in one animal after 21 days but all animals were once more negative in the following culture moment at day 35. From day 49 after the treatment 2 animals were again colonized with *S. aureus* in the nose (S1A Fig). In this treatment group a rapid decline in *S. aureus* throat and rectum carriage to 0% was observed within the first nine days. In some animals recolonization occurred from day 14 until the end of the follow-up period (S1 Table, S1B Fig).

S. aureus was eradicated from the noses of all animals that received mupirocin and trimethoprim/sulfadiazine (treatment B) from day 7-21 after the start of treatment. On day 35 and 49, 2 animals were positive in the nose for *S. aureus* and this number increased to 4 at the end of the follow-up period (S1A Fig). Throat carriage rates after treatment B declined from 7 animals to 1 in the first 9 days. In the remaining follow-up period *S. aureus* throat carriage varied between 3 and 4 animals. Finally, at the start of treatment B, 1 animal was positive in the rectum for *S. aureus* and during the follow-up carriage on this site was at most found in 1 animal (S1 Table, S1B Fig).

There was no difference between the treatment groups when the portion of animals positive for *S. aureus* was determined for nose only, other sites than the nose or any site. In addition, the first occasion when *S. aureus* was cultured after decolonization was analyzed using Kaplan-Meier curves for either nose only (Fig 1A), other sites than the nose (Fig 1B) or any site, i.e. nose, throat and/or rectum (Fig 1C). The data demonstrate that there is no difference between the two treatments with respect to the time that in some animals the nose became positive. Even after 63 days 60% of all animals from both treatment groups were negative in the nose (Fig 1A). However, when throat or rectum were analyzed separately or any site, more animals became positive again and a trend was seen suggesting that in the animals that received treatment B the time to become positive was longer than in animals

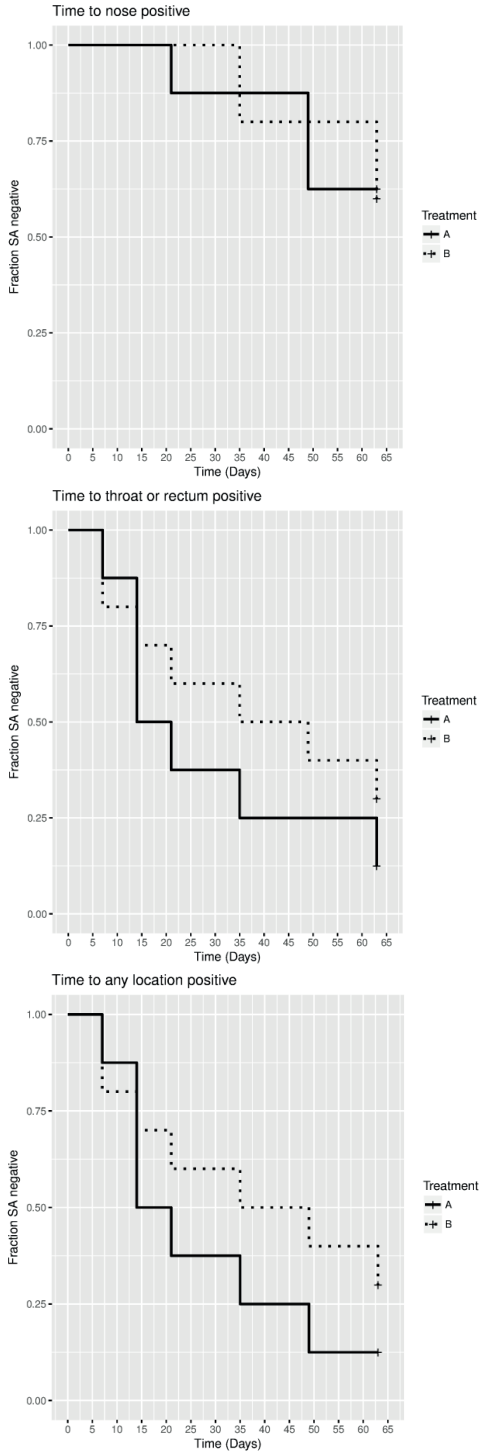

Figure 1. Kaplan-Meier curves. The proportion of *S. aureus* positive animals is represented by a continuous (treatment A) or dotted (treatment B) line. Fig 1A, 1B and 1C show respectively results from nose only, other sites than nose and any site.

that received treatment A (Fig 1B and 1C). This trend is not statistically significant because this study concerned a proof-of-principle study for decolonization and it was not powered for the statistical detection of this difference.

Inoculation

A total of 11 animals never became positive for *S. aureus* in the nose during the follow-up period of 63 days (S1 Table). Of these 11 animals, 5 had received treatment A and 6 had received treatment B. Animals were inoculated with human *S. aureus* strain 8325-4. 2 rhesus macaques were found to be positive for *S. aureus* in the nose on the day of inoculation and they were excluded from further analyses. Follow-up cultures were taken from nose, throat and rectum in week 1, 2, 3 and 5. In 3/9 animals remaining in the experiment, the inoculated *S. aureus* strain 8325-4, was found in the nose one week after inoculation. 2 of these animals had received treatment B and the other animal treatment A. The successive follow-up cultures on all culture sites became negative in these 3 animals. In the other 6 animals strain 8325-4 was never cultured but in 3 of them *S. aureus* other than 8325-4 was cultured in the throat and in 1 also in the rectum.

Susceptibility of the *S. aureus* strains isolated from the rhesus macaques

For susceptibility testing 10 *S. aureus* strains were used. These strains were derived from the nasal cavities of 10 different animals. All strains were susceptible to all the antimicrobials that were tested, including mupirocin and trimethoprim/sulfamethoxazole.

DISCUSSION

To our knowledge this is the first study in which a controlled experimental *S. aureus* nasal decolonization and inoculation model in rhesus macaques is presented. With the standardized protocols the noses of carriers were successfully decolonized for a period of 10 weeks or more. We tested 2 *S. aureus* decolonization strategies, a topical treatment with mupirocin nasal ointment and a combination treatment of mupirocin nasal ointment with systemic trimethoprim/sulfadiazine. There was no significant difference between the two treatments as almost all animals became negative for *S. aureus* in the nose, throat and rectum and in both groups comparable numbers of animals became positive again. Also with respect to time of first recurrence of *S. aureus*, there was no difference between the two treatment groups when only the nose was analyzed. However, analysis of recurrence of *S. aureus* at any site showed a trend suggesting that treatment B was more effective than treatment A.

The two different treatment strategies for decolonization of nasal carriers were based on the eradication strategies for complicated and uncomplicated MRSA carriage in humans. Uncomplicated carriage implies nasal colonization only and complicated carriage is defined

as nasal colonization in combination with one or more of the following criteria: extra-nasal carriage sites, or a clinically active infection, or the presence of skin lesions, foreign-body material, or the finding of mupirocin resistance in the colonizing strain. For uncomplicated carriage mupirocin nasal ointment and chlorhexidine-containing body wash is recommended for humans. In complicated *S. aureus* carriers this topical treatment regimen is augmented with systemic antimicrobials [4]. In both protocols used in this study we did not add chlorhexidine-containing body washes because of two practical reasons: the large amount of body hair of these animals was thought to limit the effectiveness of skin decolonization and body washes would require additional periods of anesthesia which would increase the risk of complications. A systemic intramuscularly injectable antimicrobial trimethoprim/sulfadiazine was chosen because it is registered in veterinary medicine in the Netherlands whereas cotrimoxazole (trimethoprim/sulfamethoxazole), commonly used in human medicine, does not guarantee optimal oral uptake in animals. Furthermore, Ammerlaan *et al.* recommended to always using a combination of two systemic antimicrobials to eradicate *S. aureus*, and to choose an antimicrobial combination that included either fusidic acid or rifampicin [4]. However, these latter two antimicrobials are not registered in veterinary medicine and were therefore, not considered for this series of experiments. In order to limit the number of sedations we administered both treatments once daily. Our data indicate that (un) complicated *S. aureus* carriage in rhesus macaques can well be eradicated with topical applied mupirocin alone, the addition of systemic trimethoprim/sulfadiazine did not enhance decolonization.

Although we were able to inoculate the noses of the rhesus macaques with human *S. aureus* strain 8325-4, we did not observe stable long-term colonization with this strain. Though, in previous studies, *S. aureus* 8325-4 was shown to persist for at least 4 weeks in the human nose despite the presence of a defect in the sigma factor (SigB) locus [5, 8, 23]. By choosing this strain we had the benefit to be able to use our previous experience in human inoculation experiments as a starting point. Furthermore, 8325-4 is suitable for genetic modification and can be applied for both knockout [5, 8] and knock-in studies. On the contrary, by selecting 8325-4 we took the risk on poor colonization, yet we wanted to investigate if we could use a human strain to inoculate a rhesus macaque. Our data show that 8325-4 presumably is not the ideal strain to work with in the rhesus macaque model. In a preceding study we have shown that rhesus macaques are naturally colonized with *S. aureus* strains that have genetically different backgrounds from human strains of this species [20]. Therefore, in future experiments, selecting a strain from the collection of *S. aureus* cultured from rhesus macaque (persistent) carriers might show to be a better take.

In addition to natural variation in nasal microflora, an alternative explanation for our limited success in the colonization phase of the study could be the method we used to apply the inoculum. In earlier human inoculation experiments $1*10^7$ CFU were applied in the nose with a swab [5-9] but the (smaller) nasal anatomy of rhesus macaques does not

allow to use the same swab as used in humans. Therefore, we had to adapt the inoculation procedure, which might have resulted in loss of part of the bacterial inoculum due to swallowing. Although, in the cotton rat model the same inocula and method of instillation are used for nasal inoculation experiments, in the rat it seems more successful than in our rhesus macaque model in comparable periods of follow-up. Possibly, this is due to the fact that, after sacrificing the rats, noses are completely removed and then homogenized which might result in better recovery of the bacteria from the nose [10-12]. A third possible explanation could be that mupirocin was still active at the time of inoculation. Fernandez et al. studied the efficacy of mupirocin on *S. aureus* nasal colonization in which recolonization after 2-4 months was seen in 56% of the study population [24]. On the contrary, our human model [5-9] showed successful inoculation 6 weeks after decolonization with mupirocin and chlorhexidine-containing body wash. Nevertheless, we showed that we were able to inoculate the noses of some of the rhesus macaques for at least one week.

Concerning nasal *S. aureus* carriage, animals were swabbed 4-5 times with intervals of one week. We defined carriage as animals in which at least 80% of the nasal cultures had to be *S. aureus* positive. Our definition is in line with the definition of (persistent) carriage used in several publications of human studies. However, in some publications additional criteria are defined which are related to the number of consecutive swabs that have to be taken, the number of CFU/swab but also the added value to test for genetic background [9, 25-28]. While many variations in definition exist, for this study we elected to stick to one that is practical and commonly used in literature.

During the development of this model we were confronted with a couple of issues. Two animals were culture-negative for *S. aureus* in the nose on the day of start of the treatments. These animals were already classified as nasal carriers, so this result required exclusion. It is apparent that in macaques, as in humans, carriage rate and densities, although we did not culture quantitatively, are prone to some variation over time, which was also seen in the follow-up period after treatment A and B, especially for the extra-nasal culture sites. We observed very low carriage rates in the rectum, which could be a result of our culture conditions. *S. aureus* could have been overgrown by gram negative bacteria from the gut so maybe a selective agar would have been a better choice. Another, the throat in particular, was alternately positive in most of the animals. Recognition of this phenomenon should be acknowledged when designing, implementing and interpreting studies on decolonization.

Another finding, albeit not a focus in this study, concerned the transmission of *S. aureus*. Mollema et al. showed that (human) household members have a risk of 47% to transmit *S. aureus* when one of them is positive [29]. In our study we showed that some monkeys including the buddies never acquired any *S. aureus* in the follow-up period while *S. aureus* was present in their cage mate. Also, some animals were positive for *S. aureus* at a specific carrier site while the cage mate was a carrier at another site. Since we did not type these strains it is unknown whether transmission of *S. aureus* had occurred or not. Finally, we used

spa typing, which is less delicate than for example whole genome sequencing but it served its purpose of identifying strain 8325-4.

In conclusion, this model can be used for *S. aureus* decolonization and inoculation studies in a properly controlled fashion. In contrast to small animal models, this model is attractive since this species of animals allows for long-term follow-up of the carrier state in individual subjects, and does not require animals to be sacrificed as part of the experimental protocol. Moreover, macaques are natural carriers of *S. aureus* and are closely related to humans, which makes them also attractive to study host-pathogen interaction.

SUPPORTING INFORMATION

S1 Table. *S. aureus* carriage in nose, throat and rectum in the individual rhesus macaque and their cage buddies in the follow-up period of the decolonization phase.

S1 Fig. *S. aureus* decolonization of rhesus macaques. Each red or blue dot represents the fraction of cultures positive for *S. aureus* before and after treatment A (mupirocin; red) and B (mupirocin and trimethoprim/sulfadiazine; blue). Nasal carriage and carriage at any site are shown in S1A and S1B Fig respectively.

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Writing – review & editing: Boudewijn Ouwerling, Mehri Tavakol, Susan V. Snijders, Henri A. Verbrugh, Margreet C. Vos, Edmond J. Remarque.

REFERENCES

1. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev. 1997;10(3):505-20. PubMed PMID: 9227864; PubMed Central PMCID: PMCPMC172932.
2. Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, et al. Risk and outcome of nosocomial Staphylococcus aureus bacteraemia in nasal carriers versus non-carriers. Lancet. 2004;364(9435):703-5. doi: 10.1016/S0140-6736(04)16897-9. PubMed PMID: 15325835.
3. Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, Roosendaal R, et al. Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. N Engl J Med. 2010;362(1):9-17. Epub 2010/01/08. doi: 362/1/9 [pii] 10.1056/NEJMoa0808939. PubMed PMID: 20054045.
4. Ammerlaan HS, Kluytmans JA, Berkhout H, Buiting A, de Brauwer EI, van den Broek PJ, et al. Eradication of carriage with methicillin-resistant Staphylococcus aureus: determinants of treatment failure. J Antimicrob Chemother. 2011;66(10):2418-24. doi: 10.1093/jac/dkr250. PubMed PMID: 21719471.
5. Wertheim HF, Walsh E, Choudhurry R, Melles DC, Boelens HA, Miajlovic H, et al. Key role for clumping factor B in Staphylococcus aureus nasal colonization of humans. PLoS Med. 2008;5(1):e17. Epub 2008/01/18. doi: 07-PLME-RA-0084 [pii] 10.1371/journal.pmed.0050017. PubMed PMID: 18198942; PubMed Central PMCID: PMC2194749.
6. Slingerland BC, Tavakol M, McCarthy AJ, Lindsay JA, Snijders SV, Wagenaar JA, et al. Survival of Staphylococcus aureus ST398 in the human nose after artificial inoculation. PLoS One. 2012;7(11):e48896. doi: 10.1371/journal.pone.0048896. PubMed PMID: 23155425; PubMed Central PMCID: PMCPMC3498341.
7. Nouwen J, Boelens H, van Belkum A, Verbrugh H. Human factor in Staphylococcus aureus nasal carriage. Infect Immun. 2004;72(11):6685-8. doi: 10.1128/IAI.72.11.6685-6688.2004. PubMed PMID: 15501803; PubMed Central PMCID: PMCPMC523019.
8. Verkaik NJ, Benard M, Boelens HA, de Vogel CP, Nouwen JL, Verbrugh HA, et al. Immune evasion cluster-positive bacteriophages are highly prevalent among human Staphylococcus aureus strains, but they are not essential in the first stages of nasal colonization. Clin Microbiol Infect. 2011;17(3):343-8. Epub 2010/04/08. doi: CLM3227 [pii] 10.1111/j.1469-0691.2010.03227.x. PubMed PMID: 20370801.
9. 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(12):1820-6. Epub 2009/05/08. doi: 10.1086/599119. PubMed PMID: 19419332.
10. Winstel V, Kuhner P, Salomon F, Larsen J, Skov R, Hoffmann W, et al. Wall Teichoic Acid Glycosylation Governs Staphylococcus aureus Nasal Colonization. MBio. 2015;6(4):e00632. doi: 10.1128/mBio.00632-15. PubMed PMID: 26126851; PubMed Central PMCID: PMCPMC4488942.
11. Baur S, Rautenberg M, Faulstich M, Grau T, Severin Y, Unger C, et al. A nasal epithelial receptor for Staphylococcus aureus WTA governs adhesion to epithelial cells and modulates nasal colonization. PLoS Pathog. 2014;10(5):e1004089. doi: 10.1371/journal.ppat.1004089. PubMed PMID: 24788600; PubMed Central PMCID: PMCPMC4006915.
12. Burian M, Rautenberg M, Kohler T, Fritz M, Krismer B, Unger C, et al. Temporal expression of adhesion factors and activity of global regulators during establishment of Staphylococcus aureus nasal colonization. J Infect Dis. 2010;201(9):1414-21. doi: 10.1086/651619. PubMed PMID: 20307206.
13. Park B, Iwase T, Liu GY. Intranasal application of S. epidermidis prevents colonization by methicillin-resistant Staphylococcus aureus in mice. PLoS One. 2011;6(10):e25880. doi: 10.1371/journal.pone.0025880. PubMed PMID: 21998712; PubMed Central PMCID: PMCPMC3187813.

14. Xu SX, Kasper KJ, Zeppa JJ, McCormick JK. Superantigens Modulate Bacterial Density during Staphylococcus aureus Nasal Colonization. Toxins (Basel). 2015;7(5):1821-36. doi: 10.3390/toxins7051821. PubMed PMID: 26008236; PubMed Central PMCID: PMCPMC4448176.
15. Moodley A, Latronico F, Guardabassi L. Experimental colonization of pigs with methicillin-resistant Staphylococcus aureus (MRSA): insights into the colonization and transmission of livestock-associated MRSA. Epidemiol Infect. 2011;139(10):1594-600. doi: 10.1017/S0950268810002888. PubMed PMID: 21156101.
16. Verstappen KM, Duim B, van Nes A, Snijders S, van Wamel WJ, Wagenaar JA. Experimental nasal colonization of piglets with methicillin-susceptible and methicillin-resistant Staphylococcus aureus. Vet Microbiol. 2014;174(3-4):483-8. doi: 10.1016/j.vetmic.2014.09.019. PubMed PMID: 25448448.
17. Desbois AP, Sattar A, Graham S, Warn PA, Coote PJ. MRSA decolonization of cotton rat nares by a combination treatment comprising lysostaphin and the antimicrobial peptide ranalexin. J Antimicrob Chemother. 2013;68(11):2569-75. doi: 10.1093/jac/dkt243. PubMed PMID: 23800902.
18. Daniel A, Euler C, Collin M, Chahales P, Gorelick KJ, Fischetti VA. Synergism between a novel chimeric lysin and oxacillin protects against infection by methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2010;54(4):1603-12. doi: 10.1128/AAC.01625-09. PubMed PMID: 20086153; PubMed Central PMCID: PMCPMC2849374.
19. Kokai-Kun JF, Walsh SM, Chanturiya T, Mond JJ. Lysostaphin cream eradicates Staphylococcus aureus nasal colonization in a cotton rat model. Antimicrob Agents Chemother. 2003;47(5):1589-97. PubMed PMID: 12709327; PubMed Central PMCID: PMCPMC153340.
20. van den Berg S, van Wamel WJ, Snijders SV, Ouwerling B, de Vogel CP, Boelens HA, et al. Rhesus macaques (Macaca mulatta) are natural hosts of specific Staphylococcus aureus lineages. PLoS One. 2011;6(10):e26170. Epub 2011/10/27. doi: 10.1371/journal.pone.0026170 PONE-D-11-04904 [pii]. PubMed PMID: 22028827; PubMed Central PMCID: PMC3197613.
21. Novick RP, Richmond MH. Nature and Interactions of the Genetic Elements Governing Penicillinase Synthesis in Staphylococcus Aureus. J Bacteriol. 1965;90:467-80. PubMed PMID: 14329463; PubMed Central PMCID: PMCPMC315668.
22. Harmsen D, Claus H, Witte W, Rothganger J, Turnwald D, Vogel U. Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol. 2003;41(12):5442-8. Epub 2003/12/10. PubMed PMID: 14662923; PubMed Central PMCID: PMC309029.
23. Giachino P, Engelmann S, Bischoff M. Sigma(B) activity depends on RsbU in Staphylococcus aureus. J Bacteriol. 2001;183(6):1843-52. Epub 2001/02/27. doi: 10.1128/JB.183.6.1843-1852.2001. PubMed PMID: 11222581; PubMed Central PMCID: PMC95078.
24. Fernandez C, Gaspar C, Torrellas A, Vindel A, Saez-Nieto JA, Cruzet F, et al. A double-blind, randomized, placebo-controlled clinical trial to evaluate the safety and efficacy of mupirocin calcium ointment for eliminating nasal carriage of Staphylococcus aureus among hospital personnel. J Antimicrob Chemother. 1995;35(3):399-408. PubMed PMID: 7782256.
25. Nouwen JL, Ott A, Kluytmans-Vandenbergh MF, Boelens HA, Hofman A, van Belkum A, et al. Predicting the Staphylococcus aureus nasal carrier state: derivation and validation of a “culture rule”. Clin Infect Dis. 2004;39(6):806-11. doi: 10.1086/423376. PubMed PMID: 15472812.
26. Nouwen J, Schouten J, Schneebergen P, Snijders S, Maaskant J, Koolen M, et al. Staphylococcus aureus carriage patterns and the risk of infections associated with continuous peritoneal dialysis. J Clin Microbiol. 2006;44(6):2233-6. doi: 10.1128/JCM.02083-05. PubMed PMID: 16757626; PubMed Central PMCID: PMCPMC1489388.

27. Ritchie SR, Isdale E, Priest P, Rainey PB, Thomas MG. The turnover of strains in intermittent and persistent nasal carriers of Staphylococcus aureus. J Infect. 2016;72(3):295-301. doi: 10.1016/j.jinf.2015.12.010. PubMed PMID: 26724770.
28. Verhoeven PO, Grattard F, Carricajo A, Lucht F, Cazorla C, Garraud O, et al. An algorithm based on one or two nasal samples is accurate to identify persistent nasal carriers of Staphylococcus aureus. Clin Microbiol Infect. 2012;18(6):551-7. doi: 10.1111/j.1469-0691.2011.03611.x. PubMed PMID: 21851484.
29. Mollema FP, Richardus JH, Behrendt M, Vaessen N, Lodder W, Hendriks W, et al. Transmission of methicillin-resistant Staphylococcus aureus to household contacts. J Clin Microbiol. 2010;48(1):202-7. doi: 10.1128/JCM.01499-09. PubMed PMID: 19923490; PubMed Central PMCID: PMCPMC2812253.

Supporting Information

S1 Table. *S. aureus* carriage in nose, throat and rectum in the individual rhesus macaque and their cage buddies in the follow-up period of the decolonization phase.

Room	Cage	Animal	Treatment	Day 0	Day 3/4	Day 7	Day 9	Day 14	Day 21	Day 35	Day 49/50	Day 63
43	3	R09147	A	N/T +	-	-	-	-	T +	-	-	-
		R11080	A	N +	-	-	-	-	-	-	-	-
43	5	R10034	A	N/T/R+	N +	-	-	-	-	T +	T +	-
		R11098	A	T/R +	-	-	-	T +	T +	-	-	-
45	1	R10024	A									
		R04062	A	N/T +	-	-	-	T +	-	-	T +	-
45	3	R05070	A	N/T +	-	-	-	R +	-	-	-	-
	*	R07022	A	N +	-	-	-					
45	6	R07076	A	N/T/R+	T +	T +	-	-	T +	-	N/T +	N +
		R08133	A	N/T +	-	-	-	-	-	-	-	-
46	1	R10122	A									
		R02038	A	-	-	-	-	-	T +	-	-	-
46	2	R07026	A	N/T +	-	-	-	-	-	-	N +	T +
		R08134	A	-	-	-	-	-	-	-	-	-
46	3	R10030	A	N/T/R+	T +	-	-	T +	N/R +	T/R +	R +	N/T +
		R11057	A	N/R +	T +	-	-	T +	-	N +	N/T/R+	N/T +
43	2	R10053	B	N/T +	T +	T +	T +	T +	T +	T +	T +	T +
		R11093	B	N +	T +	-	-	-	N/T +	-	T +	-
43	4	R08055	B	N/T +	T +	T +	-	T +	T +	N/T +	N/T +	N/T +
		R11119	B	N +	-	-	-	-	-	-	-	-
45	4	98007	B	N +	N +	-	-	-	-	-	-	T +
		98024	B	N +	-	-	-	-	-	-	-	-
		98036	B	N +	-	-	-	-	-	-	-	-
46	4	R00035	B	N/T +	-	-	-	-	-	-	T +	N +
		R01079	B	N/T +	R +	-	-	-	-	N/T/R+	N/T/R+	N +
46	5	R10069	B	N/T/R+	T +	-	-	-	T +	-	-	-
		R09041	B	N/T +	-	-	-	-	-	-	-	-
46	6	R07124	B	N/T +	-	-	-	T +	T/R +	-	-	N +
		R07123	B	-	-	-	-	-	-	-	-	N +

[a.] N = nose; T = throat; R = rectum

[b.] + = positive for *S. aureus*; - = negative for *S. aureus*

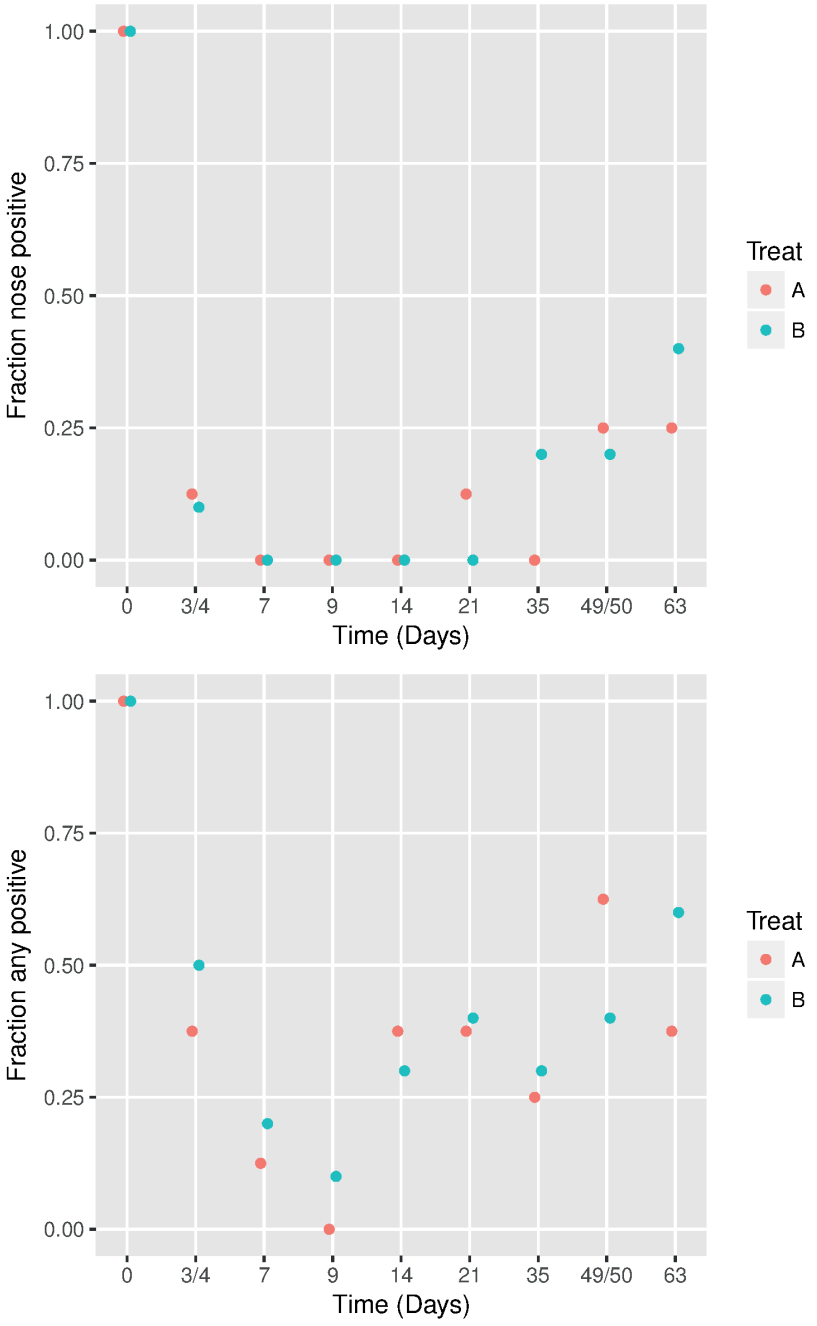
[c.] Treatment A = mupirocin; Treatment B = mupirocin and trimethoprim/sulfadiazine

[d.] Grey background = buddy

[e.] Yellow background = excluded animals from the decolonization phase

[f.] * = Buddy R07022 was euthanized because of severe complications of diverticulosis.

S1 Fig. *S. aureus* decolonization of rhesus macaques.



Each red or blue dot represents the fraction of cultures positive for *S. aureus* before and after treatment A (mupirocin; red) and B (mupirocin and trimethoprim/sulfadiazine; blue). Nasal carriage and carriage at any site are shown in S1A and S1B Fig respectively.

2.2

Survival of *Staphylococcus aureus* ST398 in the Human Nose after Artificial Inoculation

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ABSTRACT

There is evidence that MRSA ST398 of animal origin is only capable of temporarily occupying the human nose, and it is therefore, often considered a poor human colonizer.

We inoculated 16 healthy human volunteers with a mixture of the human MSSA strain 1036 (ST931, CC8) and the bovine MSSA strain 5062 (ST398, CC398), 7 weeks after a treatment with mupirocin and chlorhexidine-containing soap. Bacterial survival was studied by follow-up cultures over 21 days. The human strain 1036 was eliminated faster (median 14 days; range 2 - 21 days) than the bovine strain 5062 (median 21 days; range 7 - 21 days) but this difference was not significant (p = 0.065). The bacterial loads were significantly higher for the bovine strain on day 7 and day 21. 4/14 volunteers (28.6%) showed elimination of both strains within 21 days. Of the 10 remaining volunteers, 5 showed no differences in bacterial counts between both strains, and in the other 5 the ST398 strain far outnumbered the human *S. aureus* strain. Within the 21 days of follow-up, neither human strain 1036 nor bovine strain 5062 appeared to acquire or lose any mobile genetic elements.

In conclusion, *S. aureus* ST398 strain 5062 is capable of adequately competing for a niche with a human strain and survives in the human nose for at least 21 days.

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a well known pathogen and is capable of colonizing the skin and mucosa of humans with the anterior nares being the most common carriage site [1]. Three human nasal carriage patterns can be distinguished: the persistent (30%), intermittent (40%) and non-carriage (30%) pattern [2]. This was recently reduced to two major phenotypes: persistent and non-carriage only [3]. Importantly, nasal carriage of *S. aureus* increases the risk for infection with this bacterial species [4]. The control of methicillin-resistant *S. aureus* (MRSA) reservoirs and infections is often problematic because these populations are resistant to almost al β-lactam antibiotics, the treatment of choice for Staphylococcal infections, and are often resistant to other commonly prescribed antibiotics [5]. Recently there has been a worldwide change in the epidemiology of MRSA. MRSA populations have been a problem in hospitals worldwide since the 1960s, but the emergence of new clones of MRSA has occurred in the community among individuals who lacked contact with healthcare [6]. In the US nearly all MRSA are associated with the community-associated (CA)-MRSA USA300 clone [7]. Nowadays in many European countries, Northern Americas, Australia and Asia there has also been an increased incidence of carriage of a livestock-associated (LA)-MRSA, especially in people with direct contact with livestock, such as farmers and veterinarians [8]. The majority of these LA-MRSA cases are caused by MRSA multi-locus sequence type (ST) 398, a lineage that can be detected by the fact that strains are Pulsed Field Gel Electrophoresis (PFGE) non-typeable, by restriction-modification (RM) testing and PCR testing [9,10].

Currently ST398 MRSA/MSSA is reported in hospitals where it caused a broad spectrum of relatively mild infections including soft skin and tissue infections (SSTI) [11,12], abscesses, urinary tract infections (UTI) and wound infections [13,14]. In rare cases severe infections such as endocarditis [15] and bacteraemia [13] have been observed, although these occurred in older patients with underlying diseases. Often these cases were livestock-associated but occasionally infections occurred in people lacking contact with livestock.

The level of intensity and the duration of direct contact with livestock are important factors in proving positive for MRSA ST398. Prevalence of MRSA ST398 in farm-workers decreases substantially during holidays and in periods of less intense contact with livestock [16]. Van Cleef et al. showed that humans, who are temporarily in close contact with livestock, easily acquire MRSA ST398 but also shed the strain in less than 24 hours [17]. Whilst, in the hospital environment, Wassenberg et al. showed that nosocomial transmission of ST398 is 72% less likely to occur compared to non-ST398 strains [18]. It remains unclear whether these low rates of transmission and persistence are pathogen or patient-related.

S. aureus adapt to different host environments by acquiring mobile gene elements (MGEs) carrying genes encoding host-specific immune evasion strategies. The best characterised human-specific factor is the ϕ3 bacteriophage that carries the immune evasion cluster (IEC) genes *chp*, *sak* and *scn* [19]. This bacteriophage is commonly found in human *S. aureus* but

is rare amongst animal *S. aureus* [20,21]. Recently it was shown that many ST398 isolates of human origin do not carry the ϕ3 bacteriophage or any of the IEC genes suggesting that ST398 can survive and even cause infection in the human host without acquisition of the ϕ3 bacteriophage [22].

At the moment data concerning the intrinsic capacity of ST398 to colonize the human nose are lacking. In this study, we undertook an artificial human inoculation experiment with a mixed inoculum of a bovine MSSA ST398 (CC398) strain and human MSSA ST931 (CC8) strain, 7 weeks after a treatment with mupirocin and chlorhexidine-containing soap, and determined their ability to survive in the anterior nares. In addition, we used microarray analysis to compare the genomes of parental strains and strains that survived in the nose.

MATERIALS AND METHODS

Study population

Twenty-two healthy volunteers were included in this study (seven males and fifteen females, median age of 27 years, range 19-57 years). An infectious disease physician was on call for the entire study period and all volunteers provided their written informed consent. The study protocol was approved by the local Medical Ethical Committee of the Erasmus University Medical Centre Rotterdam, The Netherlands (MEC-2011-131).

S. aureus strains

The human *S. aureus* strains (502A, 274, 1036, P1, P2 and I) were all used in earlier inoculation experiments [3,23]. The bovine MSSA ST398 strains used in this study were obtained in 2008 from healthy calves in The Netherlands as part of a MRSA prevalence study [24]. Of the MSSA ST398 strains we also obtained MRSA counterparts, which were isolated from the same calf. For determination of the genetic background MLST analyses [25] and *spa*-typing were performed [26]. The *agr* locus was amplified to determine the *agr*-type (1 - 4) [27]. The detection of *sea* – *seu*, *tst* [28], *eta*, *etb* and *lukS/lukF* [29] was performed by PCR. PCR analysis with ST398-specific primer set A07 [10] was performed. The VITEK (bioMérieux, Marcy l'Etoile, France) was used to determine the antibiotic susceptibility of the strains. For a second opinion, the strains were sent to the National Institute for Public Health and the Environment (RIVM, Bilthoven, The Netherlands), and were analyzed for toxin production. Bacterial growth rates of the strains were determined in Brain Heart Infusion (BHI) and Tryptic Soy Broth (TSB). Bacteria were grown for 7 hours at 37° C.

Artificial inoculation protocol

The artificial inoculation protocol was as described previously [3,20,30]. In brief, before inoculation the carriage state for *S. aureus* was determined by taking two nasal swabs with

an interval of one week. We defined carriers as persons with two consecutive nasal swabs culture-positive for *S. aureus.* A non-carrier had one or no positive nasal cultures. Blood was drawn in week 1 to determine C-reactive protein (CRP) levels (mg/L) and the leukocyte number (*10E9/L). After determining their carriage state, all volunteers were instructed to use mupirocin nasal ointment (2%; GlaxoSmithKline, Waltham, MA, USA) twice daily and chlorhexidine-containing soap once daily for five days as eradication treatment for *S. aureus*. The volunteers received hygienic advice and medical check-ups. These medical check-ups included questions about signs of infection and when indicated physical examination. Volunteers remaining positive for *S. aureus* after eradication treatment were excluded from the inoculation phase. Therefore, the anterior nares were again cultured six weeks after the treatment to determine if the treatment had been successful. Artificial inoculation was performed in successfully decolonized volunteers one week later by applying 10*7 CFU per strain in the left and right nostril using a 1:1 mixture of human strain 1036 and bovine strain 5062. In the follow-up period the anterior nares were cultured on day 1, 2, 4, 7, 10, 14 and 21 after inoculation. At the end of the study, pharyngeal and perineal swabs were cultured as well. CRP and the leukocyte number were again determined on day 21. Eradication treatment was given to volunteers still carrying the inoculated *S. aureus* strain(s) at the end of follow-up.

Nasal swab cultures

The nose was sampled by revolving a single swab around both the left and right anterior nares four times. Swabs were submerged in Stuart's transport medium and vortexed (15 s). Swab eluates were cultured quantitatively at 37°C via plating of serial dilutions of the eluates on phenol red mannitol salt agar plates (PHMA) (2 days). The eluted swabs were submerged and incubated in phenol red mannitol salt broth (PHMB) for 7 days at 37°C. The PHMA culture plates were incubated at room temperature for 5 further days. Both strain 1036 and 5062 are morphologically distinguishable by colony colour which was used to determine the total density of each strain per swab. Furthermore, strain 5062 ferments lactose while strain 1036 does not. Therefore, twenty-five colonies of each morphotype were selected by colour and placed on lactose agar plates for verification. A latex agglutination test (Slidex Staph Plus, bioMérieux, Marcy-l'Etoile, France) was performed for suspected colonies (yellow colour and/or haemolytic zone). For final identification, ten out of the twenty-five isolates of each morphotype were analyzed by PCR-analysis of the *spa*-gene.

Microarray analysis

Microarray experiments were performed using a 62-strain *S. aureus* microarray (SAM-62), as previously described (McCarthy et al. 2011). SAM-62 contains 29,739 60-mer oligo probes representing 6,520 genes, and an additional 579 gene variants, from the first 62 sequenced *S. aureus* genomes and from 153 sequenced plasmid genomes. The array design is available

in BμG@Sbase (Accesion No. A-BUGS-38; http://bugs.sgul.ac.uk/A-BUGS-38) and ArrayExpress (Accession No. A-BUGS-38). All data analysis was performed in GeneSpring GX v11.01 (Agilent Technologies). Fully annotated microarray data have been deposited in BμG@Sbase (accession number E-BUGS-131; http://bugs.sgul.ac.uk/E-BUGS-131) and also ArrayExpress (accession number E-BUGS-131).

Statistical analysis

Statistical analyses were performed with SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA). The primary outcome after artificial inoculation was the survival time of *S. aureus* in the nose. We defined survival time as the time in days until the final positive nasal culture for each of the inoculated *S. aureus* strains. A Kaplan-Meier survival analysis (log-rank test) was used to compare survival between the human and bovine strain. The Mann-Whitney *U*-test was used to compare median number of CFUs. $P < 0.05$ was considered statistically significant.

RESULTS

S. aureus strains

All *S. aureus* strains were extensively analysed for genetic background, toxin gene content, *agr*-type and antibiotic resistance profile. For obvious ethical reasons, MSSA deficient in toxin genes, sensitive to as many antibiotics possible, sharing the same *agr*-type and growth characteristics were selected for the inoculation experiment. Therefore, out of the initially 6 human isolates, we selected strain 1036 which belonged to ST931 (CC8). The strain was susceptible to all common antimicrobials (e.g. mupirocin, flucloxacillin and vancomycin), had *agr*-type 1 and lacked superantigens (*sea – seu, tst*), enterotoxins (*eta, etb*) and *lukS/lukF*.

The selected bovine strain was 5062 and belonged to ST398 (CC398). This strain was resistant to tetracycline and trimethoprim, but was susceptible to other common antimicrobials and was devoid of the staphylococcal toxins mentioned above. This strain had *agr*-type 1 and *spa*-type t034. PCR analysis with primer set A07 confirmed that strain 5062 was of the ST398 lineage. The MRSA ST398 counterpart that was isolated from the same calf shared the same *spa*-type, PFGE-type and other characteristics (e.g. gene content, antibiotic susceptibility) except for the presence of the *mecA*-gene. The RIVM confirmed that both the human and bovine strain was not producing the staphylococcal toxins mentioned above. Growth kinetics in Brain-Heart Infusion (BHI) were comparable for strain 1036 and 5062 (data not shown).

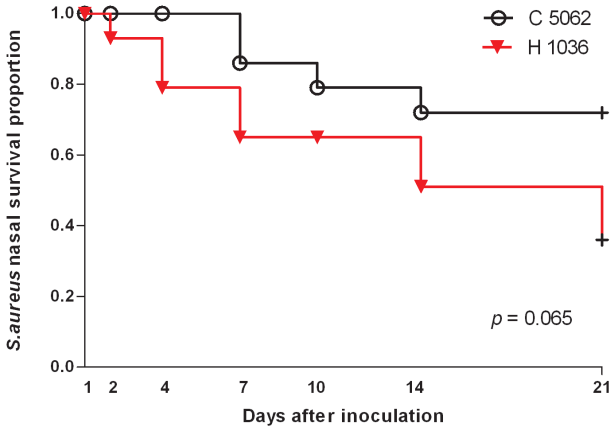
Artificial nasal inoculation with human strain 1036 and bovine strain 5062

A total of 22 volunteers were included in the study and their carriage state was determined. Six volunteers (27%) were classified as carriers and sixteen volunteers (73%) were classified as noncarriers. All volunteers used the eradication treatment as described and six weeks af-

ter the treatment five carriers were positive for *S. aureus* and they were therefore excluded from further intervention and follow-up. One participant was excluded because of private reasons. The remaining sixteen volunteers (1 carrier; 15 non-carriers) were inoculated with a mixture of the two strains seven weeks after the treatment. Two volunteers were excluded from further analyses because of the use of antibiotics during the study period. One started antibiotics for UTI on the day of inoculation; the other participant developed an intranasal furuncle which was treated. All volunteers adhered to the study protocol.

S. aureus survival was determined by quantitative cultures of 7 consecutive nasal swabs (day 1, 2, 4, 7, 10, 14, 21) in a period of 21 days. The Kaplan-Meier curves in Figure 1 show the proportion of positive cultures during follow-up. Overall the human strain 1036 was eliminated at a faster pace (median 14 days; range 2 – 21 days) than the bovine strain 5062 (median 21 days; range 7 – 21 days) but this difference was not significant ($p = 0.065$).

Figure 1. Survival of the inoculated strains Kaplan-Meier survival curves showing the proportion of volunteers who are *S. aureus* culture-positive after the artificial inoculation with human strain 1036 and bovine strain 5062.

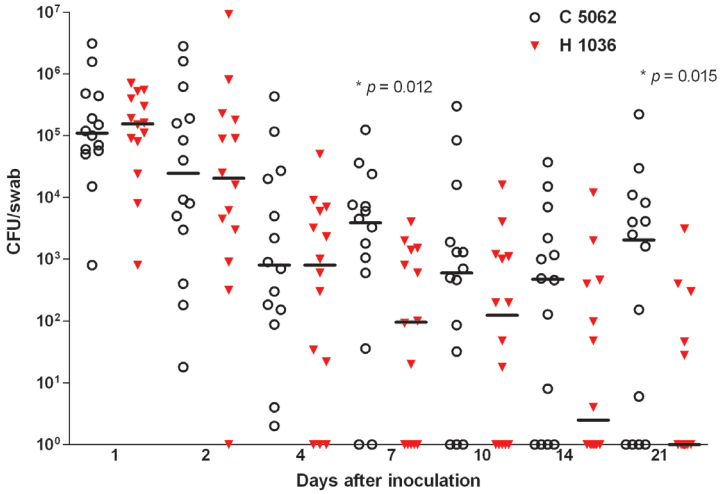


The densities of the two strains in the anterior nares rapidly decreased during the first days after inoculation. When the differences in bacterial counts between the human and bovine strain were studied on day 7 and 21, significantly higher densities were found for the bovine strain in comparison to the human strain (respectively $p = 0.012$ and $p = 0.015$) (Fig. 2).

Two patterns of elimination of the inoculated strains were observed. One group of volunteers (28.6%, $n = 4/14$; 4 non-carriers) showed elimination of both strains within 21 days (Fig. 3A), while in the remaining 10 volunteers (71.4%; 1 carrier, 9 non-carriers) *S. aureus* was culture-positive up to day 21 until the end of the study. Interestingly, this last group of 10 volunteers could be further sub-divided. In 5 volunteers (1 carrier, 4 non-carriers) no differences in bacterial counts between both strains were observed during the 21 days (Fig.

3B). In contrast, in the remaining 5 volunteers (all non-carriers) strain 5062 far outnumbered strain 1036 (Fig. 3C).

Figure 2. Bacterial loads in the nares of volunteers after inoculation.



Each dot represents the number of CFUs per swab at day 1, 2, 4, 7, 10, 14 and 21 after the inoculation with the mixture of *S. aureus* strain 1036 of human origin and strain 5062 of bovine origin. The horizontal bars represent the median number of CFUs at indicated sampling times.

An increase in bacterial load of the bovine strain at the end of the follow-up period could indicate adaptation to the host or waning of the host immune response. In order to study if acquisition of MGEs by (one of) the inoculated strains could have occurred, microarray analysis was performed on both parent strains as well as on every last positive culture isolate for strain 1036 and 5062 from the 10 volunteers who showed no elimination of both strains within 21 days. The parent human strain 1036 carried a *S. aureus* pathogenicity island (SaPI)2, plasmids with *rep* genes rep_5, rep_{21}, rep_{23} and rep_{30}, and genes *cadDX* and *qacA* conferring resistance to cadmium and antiseptics (Figure S1). This strain did not carry the ϕ3 bacteriophage or any of the IEC genes. The parent bovine strain 5062 carried the ϕ6 bacteriophage and the *tetM* and *dfrG* genes encoding resistance to tetracycline and trimethoprim.

After 21 days of follow-up, the bovine 5062 strains did not acquire any MGE from the human strains in any of the 10 volunteers. In addition, no bovine strain lost an MGE that was present in the parental strain, although microheterogeneity was detected in some of these bovine strains. Likewise, the human 1036 strains did not acquire or lose any MGEs. In these human strains microheterogeneity was also seen, but this was not due to acquisition of MGEs. We note that isolate H1036 from volunteer 23 has the ϕ6 integrase gene, but as it does not possess any other bacteriophage genes we are confident this is a false positive.

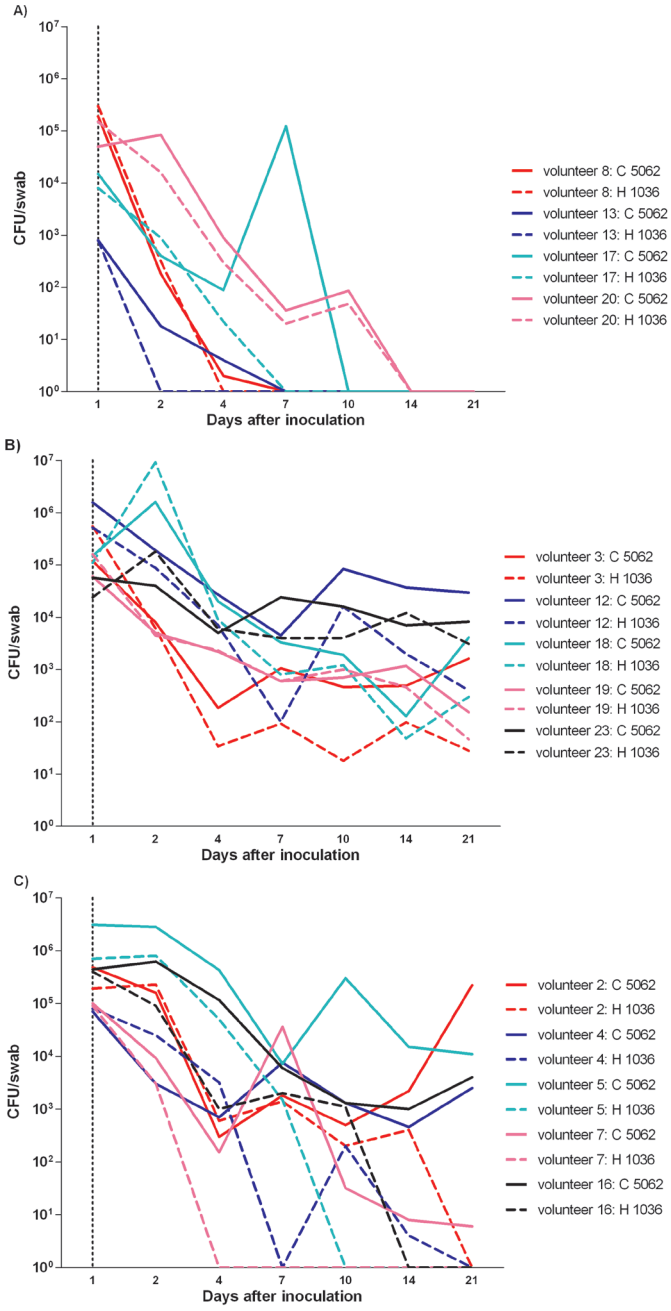


Figure 3. Three elimination patterns Each line represents the number of CFUs of each volunteer at day 1, 2, 4, 7, 10, 14 and 21 after the inoculation with the mixture of *S. aureus* strain 1036 of human origin and strain 5062 of bovine origin. Fig. 3A shows the data of those volunteers who eliminated both strains within 21 days. Fig. 3B shows those volunteers in whom both strains showed similar elimination rates and Fig. 3C those where the bovine strain survived more successfully than the human strain.

At the end of the study, all volunteers were in healthy condition. Laboratory values indicated no signs of infection. In five volunteers, all nasal swabs and two pharyngeal swabs were still positive for the inoculated bovine strain 5062. In five other volunteers, all nasal swabs and four pharyngeal swabs were positive for both of the inoculated strains. Eradication treatment was given to all these volunteers. Nasal and pharyngeal swabs following eradication treatment were all negative.

DISCUSSION

We demonstrate in an artificial human nasal inoculation model, that *S. aureus* ST398 of bovine origin is capable of surviving in the nose in 10 healthy volunteers for at least 21 days when inoculated 7 weeks after an eradication treatment with mupirocin and chlorhexidine-containing soap. We found no evidence that survival of ST398 in the human host was due to the acquisition of MGEs. There is evidence that MRSA ST398 of animal origin is only capable of temporarily occupying the human nose. It is, therefore, often considered as a poor human colonizer [17,31]. Our study shows this loss of colonization in livestock workers is not due to an intrinsic inability of ST398 to survive in the human nose.

Van Cleef et al. showed that MRSA ST398 can easily be acquired but is also lost within 24 hours by those who are temporarily in close contact with livestock [17]. An explanation for the discrepancy between our data and that of van Cleef et al. could be the inoculum size and or immunological effect. In our inoculation experiment we used an inoculum of 10*7 bacteria per strain per nostril, but currently it is not known what the level of bacterial exposure is during an average day of farming. It could very well be that this is a much lower number of bacteria than the inoculum we used. Another difference between our study and exposure to ST398 on farms is that we pretreated all our volunteers with mupirocin, an intervention that may eradicate other elements of the nasal microflora, coagulase-negative staphylococci in particular, that play a role in the resistance of the nose against *S. aureus* colonization [32].

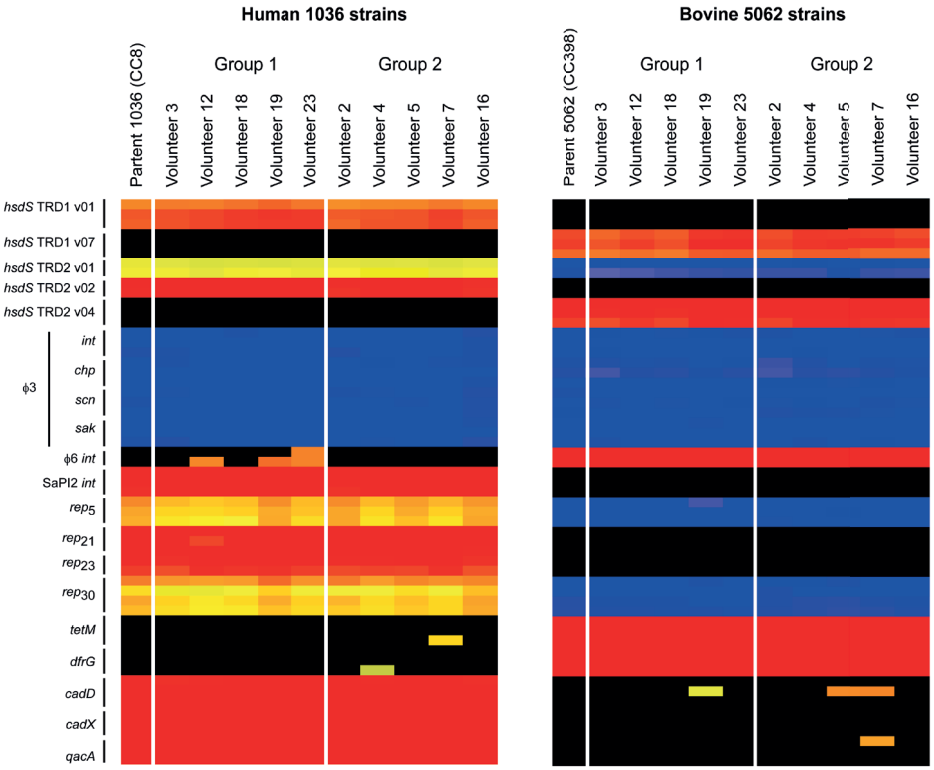
In the first days after inoculation we observed a rapid decrease in bacterial load of both strains in the nares of all volunteers, resulting in the elimination of both strains within 21 days in four volunteers. Interestingly, in the remaining 10 volunteers ST398 could still be detected after 21 days. In half of this latter group we observed, after a decrease in bacterial loads of both strains, that the loads stabilized after 21 days. In the remaining five individuals, cell counts for strain ST398 increased at the end of follow-up where in most of these cases the human strain was eliminated. Our data clearly indicate that in 28.6% of the volunteers *S. aureus* is rapidly eradicated even when exposed to significant numbers of bacteria. Yet, 71.4% of the volunteers were not able to eradicate either or one of the inoculated *S. aureus* strains.

We found no evidence that persistence of ST398 in the human host was due to the acquisition of MGEs. This suggests that animal ST398 is able to survive for several weeks in the human nares without gaining or losing MGEs. This agrees with a previous study that showed that human-specific ϕ3 bacteriophage and the IEC genes encoding *chp, sak* and *scn* are absent in the majority of ST398 isolates from humans [22]. How does ST398 colonize different host species? *S. aureus* encode multiple surface proteins that interact with host ligands, and many of these proteins often have overlapping functions and can function in multiple hosts [33].

In conclusion, MSSA strain 5062 of bovine origin (ST398, *spa*-type t034) is capable of surviving in the human nose for at least 21 days where it appears to successfully compete with human strain 1036.

SUPPORTING INFORMATION

Figure S1. SAM-62 microarray analysis of parent strains and colonizing isolates in 10 human volunteers Isolates are represented by vertical lines and information about the origin of each isolate is given at the top of the figure. Group 1 are the volunteers in whom no difference in bacterial load between both strains was observed (Fig. 3B) and Group 2 are those who did show a difference in bacterial load between both strains at the end of follow-up (Fig. 3C). Horizontal lines represent 57 different 60-mer oligo probes specific to 5 *hsdS* variants, 4 bacteriophage genes, 1 SaPI gene, 4 plasmid *rep* families, and 5 different antimicrobial, biocide and heavy metal resistance genes. The colour depicts if the gene is present or absent in the respective isolate; red or yellow = present, blue or black = absent. After 21 days of follow-up, neither human strains 1036, nor bovine strains 5062 acquired or lost any MGEs.



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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: BCGCS MT SVS AB WJBW HAV MCV AJM JAL. Performed the experiments: BCGCS MT SVS AJM. Analyzed the data: BCGCS MT SVS AJM JAL WJBW HAV MCV JAW. Contributed reagents/materials/analysis tools: BCGCS MT WJBW AJM JAL JAW. Wrote the paper: BCGCS WJBW AJM.

REFERENCES

1. Williams RE (1963) Healthy carriage of Staphylococcus aureus: its prevalence and importance. Bacteriol Rev 27: 56-71.
2. Eriksen NH, Espersen F, Rosdahl VT, Jensen K (1995) Carriage of Staphylococcus aureus among 104 healthy persons during a 19-month period. Epidemiol Infect 115: 51-60.
3. van Belkum A, Verkaik NJ, de Vogel CP, Boelens HA, Verveer J, et al. (2009) Reclassification of Staphylococcus aureus nasal carriage types. J Infect Dis 199: 1820-1826.
4. Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, et al. (2010) Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. N Engl J Med 362: 9-17.
5. Jevons MP, Coe AW, Parker MT (1963) Methicillin resistance in staphylococci. Lancet 1: 904-907.
6. Deurenberg RH, Stobberingh EE (2008) The evolution of Staphylococcus aureus. Infect Genet Evol 8: 747-763.
7. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, et al. (2006) Methicillin-resistant S. aureus infections among patients in the emergency department. N Engl J Med 355: 666-674.
8. Wulf MW, Sorum M, van Nes A, Skov R, Melchers WJ, et al. (2008) Prevalence of methicillin-resistant Staphylococcus aureus among veterinarians: an international study. Clin Microbiol Infect 14: 29-34.
9. Stegger M, Lindsay JA, Moodley A, Skov R, Broens EM, et al. (2011) Rapid PCR detection of Staphylococcus aureus clonal complex 398 by targeting the restriction-modification system carrying sau1-hsdS1. J Clin Microbiol 49: 732-734.
10. van Wamel WJ, Hansenova Manaskova S, Fluit AC, Verbrugh H, de Neeling AJ, et al. (2010) Short term micro-evolution and PCR-detection of methicillin-resistant and -susceptible Staphylococcus aureus sequence type 398. Eur J Clin Microbiol Infect Dis 29: 119-122.
11. Wu D, Wang Q, Yang Y, Geng W, Yu S, et al. (2010) Epidemiology and molecular characteristics of community-associated methicillin-resistant and methicillin-susceptible Staphylococcus aureus from skin/soft tissue infections in a children's hospital in Beijing, China. Diagn Microbiol Infect Dis 67: 1-8.
12. Grisold AJ, Zarfel G, Stoeger A, Feierl G, Raggam RB, et al. (2009) Emergence of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) in Southeast Austria. J Infect 58: 168-170.
13. van Belkum A, Melles DC, Peeters JK, van Leeuwen WB, van Duijkeren E, et al. (2008) Methicillin-resistant and -susceptible Staphylococcus aureus sequence type 398 in pigs and humans. Emerg Infect Dis 14: 479-483.
14. Welinder-Olsson C, Floren-Johansson K, Larsson L, Oberg S, Karlsson L, et al. (2008) Infection with Panton-Valentine leukocidin-positive methicillin-resistant Staphylococcus aureus t034. Emerg Infect Dis 14: 1271-1272.
15. Schijffelen MJ, Boel CH, van Strijp JA, Fluit AC (2010) Whole genome analysis of a livestock-associated methicillin-resistant Staphylococcus aureus ST398 isolate from a case of human endocarditis. BMC Genomics 11: 376.
16. Graveland H, Wagenaar JA, Bergs K, Heesterbeek H, Heederik D (2011) Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. PLoS One 6: e16830.
17. van Cleef BA, Graveland H, Haenen AP, van de Giessen AW, Heederik D, et al. (2011) Persistence of livestock-associated methicillin-resistant Staphylococcus aureus in field workers after short-term occupational exposure to pigs and veal calves. J Clin Microbiol 49: 1030-1033.
18. Wassenberg MW, Bootsma MC, Troelstra A, Kluytmans JA, Bonten MJ (2011) Transmissibility of livestock-associated methicillin-resistant Staphylococcus aureus (ST398) in Dutch hospitals. Clin Microbiol Infect 17: 316-319.

19. van Wamel WJ, Rooijakkers SH, Ruyken M, van Kessel KP, van Strijp JA (2006) The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of Staphylococcus aureus are located on beta-hemolysin-converting bacteriophages. J Bacteriol 188: 1310-1315.
20. Verkaik NJ, Benard M, Boelens HA, de Vogel CP, Nouwen JL, et al. (2011) Immune evasion cluster-positive bacteriophages are highly prevalent among human Staphylococcus aureus strains, but they are not essential in the first stages of nasal colonization. Clin Microbiol Infect 17: 343-348.
21. Sung JM, Lloyd DH, Lindsay JA (2008) Staphylococcus aureus host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. Microbiology 154: 1949-1959.
22. McCarthy AJ, Witney AA, Gould KA, Moodley A, Guardabassi L, et al. (2011) The Distribution of Mobile Genetic Elements (MGEs) in MRSA CC398 Is Associated with Both Host and Country. Genome Biol Evol 3: 1164-1174.
23. Nouwen J, Boelens H, van Belkum A, Verbrugh H (2004) Human factor in Staphylococcus aureus nasal carriage. Infect Immun 72: 6685-6688.
24. Graveland H, Wagenaar JA, Heesterbeek H, Mevius D, van Duijkeren E, et al. (2010) Methicillin resistant Staphylococcus aureus ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. PLoS One 5: e10990.
25. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol 38: 1008-1015.
26. Harmsen D, Claus H, Witte W, Rothganger J, Turnwald D, et al. (2003) Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol 41: 5442-5448.
27. Gilot P, Lina G, Cochard T, Poutrel B (2002) Analysis of the genetic variability of genes encoding the RNA III-activating components Agr and TRAP in a population of Staphylococcus aureus strains isolated from cows with mastitis. J Clin Microbiol 40: 4060-4067.
28. Hwang SY, Kim SH, Jang EJ, Kwon NH, Park YK, et al. (2007) Novel multiplex PCR for the detection of the Staphylococcus aureus superantigen and its application to raw meat isolates in Korea. Int J Food Microbiol 117: 99-105.
29. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, et al. (1999) Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis 29: 1128-1132.
30. Wertheim HF, Walsh E, Choudhurry R, Melles DC, Boelens HA, et al. (2008) Key role for clumping factor B in Staphylococcus aureus nasal colonization of humans. PLoS Med 5: e17.
31. Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA (2011) Livestock-associated methicillin-resistant Staphylococcus aureus in animals and humans. Int J Med Microbiol 301: 630-634.
32. Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, et al. (2010) Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. Nature 465: 346-349.
33. McCarthy AJ, Lindsay JA (2010) Genetic variation in Staphylococcus aureus surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. BMC Microbiol 10: 173.

3

Staphylococcus aureus acquisition in neonates

3.1

Neonatal *Staphylococcus aureus* acquisition at a tertiary intensive care unit

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ABSTRACT

Background

In this study, we explored the role of colonization in health care workers (HCWs) in transmission of methicillin-susceptible *Staphylococcus aureus* (MSSA) to neonates at a level IV neonatal intensive care unit (NICU).

Methods

All available screening and clinical MSSA isolates from the period March 2015 through April 2016, isolated from HCWs and neonates at the level IV NICU, were included. MSSA isolates were initially genotyped using *spa* typing and for the most prevalent *spa* types, whole-genome sequencing (WGS) was performed.

Results

From March 2015 until April 2016, 159 neonates and 115 HCWs were found positive for MSSA, and all isolates were typed by means of *spa* typing. Twenty-three *spa* types were found in both HCWs and neonates. Within the most prevalent *spa* types (t002, t015 and t2787), 4 WGS clusters of genetically indistinguishable MSSA isolates were found in which 4 HCWs and 35 neonates were involved. A total of 10 neonates included in the 4 WGS clusters suffered from bacteremia.

Conclusion

We showed that HCWs carried the same MSSA isolates as those found in neonates, and that HCWs might serve as a reservoir for transmission of MSSA to neonates. Ten neonates suffered from a bacteremia caused by a MSSA previously detected in a HCW.

INTRODUCTION

Neonates admitted to an intensive care unit (NICU) are with their low gestational age, low birth weight, a microbiome under construction, and with immaturity of organ systems prone to develop health care-associated infections (HAIs), in particular bloodstream infections [1]. In neonates, *Staphylococcus aureus* (*S. aureus*) is one of the three most commonly found bacteria in the blood [2-4]. In adults, it is known that the majority of *S. aureus* bacteremias are caused by the endogenous strain [5], however, in neonates, with their immature microbiome, the cause and effect are unknown. There are many studies published that focus on transmission of methicillin-resistant *S. aureus* (MRSA) on NICUs that resulted in colonization or infection with MRSA [6-12]. However, there is less data available on transmission of methicillin-susceptible *S. aureus* (MSSA) on NICUs, whereas this could at least have a similar impact given the number of infections [13-17]. In this study, we explored the possibility of health care workers (HCWs) being a reservoir of transmission of MSSA to neonates at a level IV NICU in a 14-month period of time. Whole-genome sequencing (WGS) was used as a typing method to detect genetic relatedness of the isolates.

METHODS

Population

The NICU of Erasmus MC-Sophia, Rotterdam, The Netherlands, is a level IV 27-beds facility. It is divided into 4 units with 6-8 beds each. About 750 neonates are admitted annually, and nearly 40% of those are below 32 weeks of gestational age, with the majority being born in the hospital.

Ethics statement

Because this was a retrospective observational study using anonymized patient data collected during routine clinical practice, informed consent was not mandatory, according to the Dutch Medical Research Involving Human Subjects Act (WMO). The Institutional Ethics Review Board of the Erasmus MC reviewed the study protocol and provided an exemption from formal ethical assessment (MEC-2015-306) based on the non-interventional design. The study was carried out in accordance with the current ethical guidelines for epidemiological research.

Screening

At the level IV NICU in the Erasmus MC-Sophia, Rotterdam, The Netherlands, all neonates are screened weekly by swabbing the throat and the rectum. Additional screening cultures for *S. aureus*, from neonates as well as HCWs, were taken in the period from March 2015

through April 2016, because of 3 separate MSSA outbreaks at the NICU, not described in the present study. HCWs who took care of neonates involved in the outbreaks and neonates who shared the same unit, in the same time period, with neonates involved in the outbreaks, were considered contacts. For HCWs who were contacts of neonates involved in the outbreaks, the nasal and the throat swabs were collected. For neonates who were contacts, additional rectal, nasal or perineal swabs were collected. In case of suspicion of infection, clinical samples were collected as part of routine diagnostics. Clinical samples included blood cultures, sputum, skin cultures, wound fluid or cultures from specific body sites (eg, eye or ear). All *S. aureus* isolates derived from neonates and HCWs, obtained from routine diagnostics as well as from contact investigations, are used in this study. Contact investigation is defined as an active screening to detect transmission between HCWs and neonates.

Culture, identification, and susceptibility of *S. aureus* isolates

Swabs and clinical samples were cultured on Trypticase Soy Agar II with 5% Sheep Blood (BD, Heidelberg, Germany). After 24-48 hours of incubation at 35°C, plates were screened for *S. aureus* based on colony morphology and MALDI-TOF analysis (Maldi-tof MS system, Bruker). *S. aureus* isolates were stored at – 20 °C or – 80 °C until use. The VITEK 2 system (bioMérieux, Marcy l'Etoile, France) was used for antibiotic-susceptibility testing.

Spa typing of MSSA isolates

DNA was isolated from pure colonies using the automated MagNA Pure 96 platform in combination with the MagNA Pure DNA and Viral Nucleic Acid Small Volume Kit (Roche diagnostics, Almere, the Netherlands). PCR reactions were performed in 25-μl reactions using 1 μL of isolated DNA in 1x FastStart PCR Master (Roche) and 0.5 μM of both forward primer (5'-AACAACGTAACGGCTTCATCC-3') and reverse primer (5'- GCTTTTGCAATGTCATTTACTG-3'). Thermal cycling consisted of an initial denaturation step for 10 min at 95°C, followed by 35 cycles of 30s at 95°C, 30s at 60°C and 1 min at 72°C. After a final extension step of 10 min at 72°C, reactions were cooled to room temperature. Five μl of PCR product was analyzed on agarose gel to confirm amplification. The remainder of the PCR product was treated with 2 μL of ExoSAP-IT (Isogen Life Science, De Meern, the Netherlands) for 15 min at 37°C, following inactivation for 15 min at 80°C. Amplicon sequencing was performed by BaseClear (Leiden, the Netherlands) using the forward amplification primer as sequencing primer. Electropherograms were analyzed and interpreted using the *spa* typing plugin in BioNumerics v7.6 software (Applied Maths, Sint-Martens-Latem, Belgium).

WGS

The selection of MSSA isolates for WGS was based on the results of *spa* typing. MSSA isolates were subjected to next-generation sequencing (NGS) by generating PE125 reads on an Illumina HiSeq 2500 (BaseClear, Leiden, the Netherlands). Sequence data was assembled

using CLC Genomics Workbench v11 software (Qiagen, Hilden, Germany) and analyzed using the available *S. aureus* schemes in SeqSphere v 4.1.9 software (Ridom, Münster, Germany).

Definitions

Possible transmission: if the MSSA isolate from the HCW-to-neonate or neonate-to-neonate are genetically indistinguishable determined by WGS.

WGS cluster: a cluster of indistinguishable MSSA isolates defined as a difference of less than 12 alleles on the core genome [18].

RESULTS

In the period from March 2015 until April 2016, 159 neonates and 115 HCWs were found positive for MSSA and all isolates were typed by means of *spa* typing. In the 159 neonates, a total number of 60 different *spa* types were found. In total 255 positive MSSA cultures were taken from the 159 neonates of which 59% were screening cultures (31% rectum, 17% nose, 8% throat and 3% perineal cultures). The other positive MSSA cultures originated from blood (11%), skin (7%), sputum (7%), wound fluid (6%), eye (4%), ear (1%) or origin unknown (4%). In 115 HCWs, 71 different *spa* types were detected. Cultures from HCWs originated from nose or throat. Fourteen neonates and 7 HCWs were colonized, with 2 morphologically different MSSA with different *spa* types. Twenty-three *spa* types were found in both HCWs and neonates. In these 23 shared *spa* types, 117 of 159 (74%) neonates and 67 of 115 (58%) HCWs were included. Table 1 shows the number of cultures, the number of positive blood cultures in neonates, and the number of HCWs and neonates in the 23 shared *spa* types.

Genetic relatedness based on WGS

As *spa* typing has less discriminatory power than WGS, WGS was performed on MSSA isolates of the 3 most prevalent *spa* types. *Spa* types t002, t015 and t2787 were selected to perform WGS. These 3 *spa* types involved 29.3% (n = 54; 40 neonates and 14 HCWs) of all neonates and HCWs (Table 1), and represented 11 out of the total number of 20 (55%) positive blood cultures in neonates (Table 1). One MSSA isolate per individual was used for WGS. Figure 1 shows that 4 different WGS clusters were identified that considered transmission possible. A total of 4 HCWs and 35 neonates, of whom 10 neonates suffered from a neonatal bacteremia, were involved in 4 different WGS clusters. Consequently, the MSSA isolates from 10 of 14 HCWs and 5 of 40 neonates could not be clustered by WGS. Figure 2 shows the epicurves of WGS clusters A-D. WGS clusters A, B and D included neonates as well as HCWs. WGS cluster A (*spa* type t2787) was the largest cluster and included only 1 HCW who was cultured positive in April 2015. A total of 19 out of 20 neonates included in this WGS cluster with indistinguishable MSSA were cultured positive in the period April 2015 through

Table 1. The 23 shared *spa* types with size of each *spa* type cluster and the number of cultures and positive blood cultures in neonates.

spa type	No. of neonates	No. of cultures	No. of pos. blood cultures	No. of HCWs	No. of cultures
t002 ◊	9	13	4	8	9
t005	1	1	0	1	1
t008	4	4	0	3	4
t012	3	3	0	4	5
t015 ◊	10	14	3	5	8
t026	2	2	0	1	1
t065	1	1	0	4	5
t084	5	19	1	7	7
t091	8	12	0	3	3
t127	8	15	1	2	3
t189	6	9	1	5	12
t223	12	17	2	7	10
t230	3	3	1	3	6
t250	5	5	0	2	2
t491	3	6	0	1	1
t571	5	6	1	3	6
t659	5	13	0	3	5
t864	3	4	0	1	4
t16721	1	1	1	1	2
t16723	2	3	0	1	2
t16731	1	2	0	1	1
t16779	6	6	1	1	3
t2787 ◊	21	25	4	1	2
Total	124*	184	20	68**	102

HCWs, health care workers; pos., positive

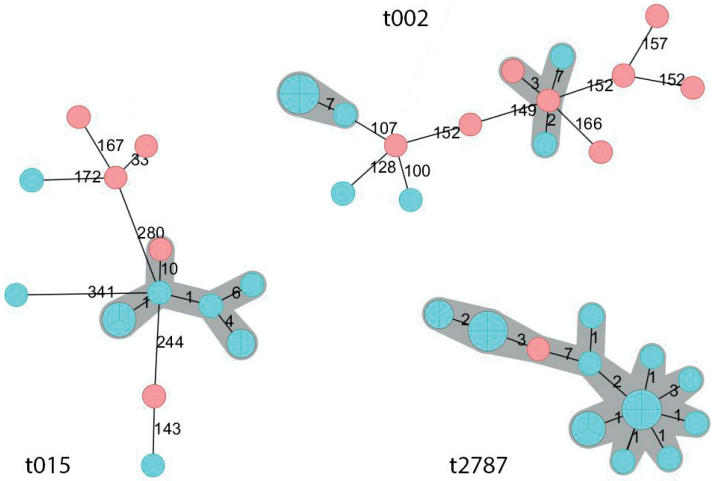
* A total of 7 neonates were positive for 2 MSSA isolates with different *spa* types.

** One HCW was positive for 2 MSSA isolates with different *spa* types.

◊ *spa* type cluster used for whole-genome sequencing.

November 2015. Four neonates suffered from a bacteremia with a MSSA isolate from WGS cluster A. In WGS cluster B (*spa* type t002), 2 HCWs were cultured positive in April 2015, and 2 neonates were cultured positive. In 1 of these neonates, the MSSA isolate responsible for WGS cluster B caused a bacteremia. WGS cluster C (*spa* type t002) included neonates only. Three of the total 5 neonates had a positive blood culture for the MSSA isolate responsible for WGS cluster C. WGS cluster D (*spa* type t015) included 1 HCW cultured positive in April 2015, and 8 neonates of whom 2 suffered from a bacteremia.

Figure 1. Whole-genome sequencing of 3 clusters of *spa* types revealed 4 new WGS clusters, with each WGS cluster representing a genetically different MSSA isolate.



Minimum spanning tree representing cgMLST analysis of 54 selected MSSA isolates based on *spa* typing (as indicated). Each circle represents a different genotype. Numbers correspond to the number of allelic differences between the genotypes. Segments within circles represent the number of isolates of that genotype. Clusters of genotypes with <12 differences are indicated with a grey background. Colors indicate the origin of the isolates: patient (blue) and health care worker (red). WGS, whole-genome sequencing.

DISCUSSION

By using WGS we showed that HCWs might serve as a reservoir for transmission of MSSA to neonates on a level IV NICU. Of the 4 HCWs and 35 neonates, 10 neonates suffered from a neonatal bacteremia and were involved in 4 different WGS clusters. By analyzing WGS data, we conclude that 10 of 20 episodes of MSSA bacteremia, of which the MSSA isolates belonged to a *spa* type found in both neonates and HCWs, were due to the presence of isolates that were already present in HCWs or neonates admitted earlier and possibly also from the environment. Data concerning MSSA transmission from HCW to neonate are scarce since normally no contact investigation is performed when a neonate is positive for MSSA, in contrast with MRSA findings. In three studies MSSA transmission from HCW to patient was described. Gomez-Gonzalez et al. showed, albeit by use of Pulse Field Gel Electrophoresis (PFGE), a technique with less discriminatory power, that HCWs acted as a reservoir for neonates that later on developed a MSSA sepsis [17]. In another study, a HCW proved to be the source of an outbreak in neonates with MSSA bullous impetigo by Raman spectroscopy typing [19]. In addition, Price et al. showed that patients on an adult intensive care unit got colonized and infected with genetically identical *S. aureus* strains transmitted via patients, the environment and health care workers. Whole-genome sequencing was used to prove transmission [20].

Figure 2. Epicurves of WGS clusters A – D.

Cluster A

HCW	---------	□	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------
neonate	○	○○○○○○○○	○○○○	---------	◊ ○○	---------	---------	◊ ○	◊◊	---------	---------	---------	---------	---------
month	M	A	M	J	J	A	S	O	N	D	J	F	M	A

◊ neonate - positive blood culture for MSSA
○ neonate - other culture positive for MSSA
□ HCW – nose or throat positive for MSSA

time (March 2015 – April 2016)

Cluster B

HCW	---------	□□	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------
neonate	---------	---------	---------	---------	◊	---------	---------	---------	---------	---------	---------	---------	---------	○
month	M	A	M	J	J	A	S	O	N	D	J	F	M	A

◊ neonate - positive blood culture for MSSA
○ neonate - other culture positive for MSSA
□ HCW – nose or throat positive for MSSA

time (March 2015 – April 2016)

Cluster C

HCW	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------
neonate	---------	○	---------	---------	---------	---------	---------	---------	---------	---------	---------	◊	◊ ○	◊
month	M	A	M	J	J	A	S	O	N	D	J	F	M	A

◊ neonate - positive blood culture for MSSA
○ neonate - other culture positive for MSSA

time (March 2015 – April 2016)

Cluster D

HCW	---------	□	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------
neonate	---------	---------	○○	◊ ○	◊	---------	---------	---------	---------	○	○	○	---------	---------
month	M	A	M	J	J	A	S	O	N	D	J	F	M	A

◊ neonate - positive blood culture for MSSA
○ neonate - other culture positive for MSSA
□ HCW – nose or throat positive for MSSA

time (March 2015 – April 2016)

As there is obviously no patient-to-patient contact at the NICU, we hypothesize that transmission was by direct contact of contaminated hands of the HCWs (eg, by touching the nose or nose picking) or, that the MSSA isolates of the HCWs survived in the environment and then were transmitted by hand after touching the environment. This could also explain why WGS cluster C prolonged for 12 months whereas no HCW was cultured positive in that period for this specific MSSA isolate. Other reported risk factors for transmission are, besides environment, overcrowding of patients and understaffing [10,20,21].

Our study has several limitations. This study is retrospective and performed in a single center. HCWs were not routinely cultured and seldom more than once. As the culture moments were not defined in advance no conclusions can be drawn on whether or not the HCWs were actually *S. aureus* persistent or intermittent carriers. No other carriage sites, other than nose and throat, from HCWs were cultured therefore the number of HCWs carrying *S. aureus* could have been an underestimation. Neither the environment nor the parents were cultured for MSSA; this could have given more clues about other reservoirs and route(s) of transmission. In addition, if MSSA was also detected in the environment, we could have used disinfection measures to stop ongoing transmission. If parents proved to be a reservoir of MSSA, probably transmission from the neonate to the HCW may have occurred, instead of vice versa, as we assume now. However, this would not explain persistence and transmission of the same MSSA isolate over a period of time and to more than one neonate. Demographic data are not included because, regardless of the medical history of the patient, transmission of MSSA will probably still occur. Lastly, *spa* typing proved to have less discriminatory power to show genetic relatedness compared to WGS. The initial 3 most prevalent clusters of *spa* types revealed 4 new clusters based on WGS, of which 2 WGS clusters were even included in just 1 of the *spa* type clusters (t002). Therefore, regarding future transmission or epidemiology studies, we would not advise the use *spa* typing as a typing method. Finally, our data could be an underestimation of the problem as we performed WGS for only 3 clusters of *spa* types.

CONCLUSIONS

In conclusion, we showed that HCWs might be an important reservoir and link in the transmission of MSSA to neonates. Ten neonates developed a MSSA bacteremia with a nosocomial acquired MSSA isolate that was previously detected in a HCW. Although the route of transmission remains to be elucidated, these data clarify that additional infection prevention measures are urgently needed. Although hand hygiene is well performed at the NICU when observed (directly unobtrusively) [22], one might still consider additional training programs on hand hygiene. Other options are more intensive daily cleaning of the environ-

ment or wearing a protective mask by the HCW in order to prevent touching the nose, the niche of *S. aureus*, and to prevent spreading of droplets.

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REFERENCES

1. Phillips P, Cortina-Borja M, Millar M, Gilbert R. Risk-adjusted surveillance of hospital-acquired infections in neonatal intensive care units: a systematic review. *J Hosp Infect.* 2008;70(3):203-211.
2. Mitt P, Metsvaht T, Adamson V, et al. Five-year prospective surveillance of nosocomial bloodstream infections in an Estonian paediatric intensive care unit. *J Hosp Infect.* 2014;86(2):95-99.
3. Jeong IS, Jeong JS, Choi EO. Nosocomial infection in a newborn intensive care unit (NICU), South Korea. *BMC Infect Dis.* 2006;6:103.
4. Piening BC, Geffers C, Gastmeier P, Schwab F. Pathogen-specific mortality in very low birth weight infants with primary bloodstream infection. *PLoS One.* 2017;12(6):e0180134.
5. Wertheim HF, Vos MC, Ott A, et al. Risk and outcome of nosocomial Staphylococcus aureus bacteraemia in nasal carriers versus non-carriers. *Lancet.* 2004;364(9435):703-705.
6. Hitomi S, Kubota M, Mori N, et al. Control of a methicillin-resistant Staphylococcus aureus outbreak in a neonatal intensive care unit by unselective use of nasal mupirocin ointment. *J Hosp Infect.* 2000;46(2):123-129.
7. Regev-Yochay G, Rubinstein E, Barzilai A, et al. Methicillin-resistant Staphylococcus aureus in neonatal intensive care unit. *Emerg Infect Dis.* 2005;11(3):453-456.
8. Reich PJ, Boyle MG, Hogan PG, et al. Emergence of community-associated methicillin-resistant Staphylococcus aureus strains in the neonatal intensive care unit: an infection prevention and patient safety challenge. *Clin Microbiol Infect.* 2016;22(7):645 e641-648.
9. Huang YC, Lien RI, Su LH, Chou YH, Lin TY. Successful control of methicillin-resistant Staphylococcus aureus in endemic neonatal intensive care units--a 7-year campaign. *PLoS One.* 2011;6(8):e23001.
10. Ramsing BG, Arpi M, Andersen EA, et al. First outbreak with MRSA in a Danish neonatal intensive care unit: risk factors and control procedures. *PLoS One.* 2013;8(6):e66904.
11. Popoola VO, Budd A, Wittig SM, et al. Methicillin-resistant Staphylococcus aureus transmission and infections in a neonatal intensive care unit despite active surveillance cultures and decolonization: challenges for infection prevention. *Infect Control Hosp Epidemiol.* 2014;35(4):412-418.
12. Koser CU, Holden MT, Ellington MJ, et al. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. *N Engl J Med.* 2012;366(24):2267-2275.
13. Achermann Y, Seidl K, Kuster SP, et al. Epidemiology of Methicillin-Susceptible Staphylococcus aureus in a Neonatology Ward. *Infect Control Hosp Epidemiol.* 2015;36(11):1305-1312.
14. Bertini G, Nicoletti P, Scopetti F, Manoocher P, Dani C, Orefici G. Staphylococcus aureus epidemic in a neonatal nursery: a strategy of infection control. *Eur J Pediatr.* 2006;165(8):530-535.
15. Otter JA, Davies B, Menson E, et al. Identification and control of a gentamicin resistant, meticillin susceptible Staphylococcus aureus outbreak on a neonatal unit. *J Infect Prev.* 2014;15(3):104-109.
16. Lin MF, Huang ML, Lai SH. Investigation of a pyoderma outbreak caused by methicillin-susceptible Staphylococcus aureus in a nursery for newborns. *J Hosp Infect.* 2004;57(1):38-43.
17. Gomez-Gonzalez C, Alba C, Otero JR, Sanz F, Chaves F. Long persistence of methicillin-susceptible strains of Staphylococcus aureus causing sepsis in a neonatal intensive care unit. *J Clin Microbiol.* 2007;45(7):2301-2304.
18. Leopold SR, Goering RV, Witten A, Harmsen D, Mellmann A. Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes. *J Clin Microbiol.* 2014;52(7):2365-2370.
19. Koningstein M, Groen L, Geraats-Peters K, et al. The use of typing methods and infection prevention measures to control a bullous impetigo outbreak on a neonatal ward. *Antimicrob Resist Infect Control.* 2012;1(1):37.

20. Price JR, Cole K, Bexley A, et al. Transmission of Staphylococcus aureus between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. *Lancet Infect Dis.* 2017;17(2):207-214.
21. Andersen BM, Lindemann R, Bergh K, et al. Spread of methicillin-resistant Staphylococcus aureus in a neonatal intensive unit associated with understaffing, overcrowding and mixing of patients. *J Hosp Infect.* 2002;50(1):18-24.
22. van Dijk MD, Mulder SA, Erasmus V, et al. A multimodal regional intervention strategy framed as friendly competition to improve hand hygiene compliance. *Infect Control Hosp Epidemiol.* 2019;40(2):187-193.

3.2

Whole-genome sequencing to explore nosocomial transmission and virulence in neonatal methicillin-susceptible *Staphylococcus aureus* bacteremia

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ABSTRACT

Background

Neonatal *Staphylococcus aureus* (*S. aureus*) bacteremia is an important cause of morbidity and mortality. In this study, we examined whether methicillin-susceptible *S. aureus* (MSSA) transmission and genetic makeup contribute to the occurrence of neonatal *S. aureus* bacteremia.

Methods

A retrospective, single-centre study was performed. All patients were included who suffered from *S. aureus* bacteremia in the neonatal intensive care unit (NICU), Erasmus MC-Sophia, Rotterdam, the Netherlands, between January 2011 and November 2017. Whole-genome sequencing (WGS) was used to characterize the *S. aureus* isolates, as was also done in comparison to reference genomes. Transmission was considered likely in case of genetically indistinguishable *S. aureus* isolates.

Results

Excluding coagulase-negative staphylococci (CoNS), *S. aureus* was the most common cause of neonatal bacteremia. Twelve percent (*n* = 112) of all 926 positive blood cultures from neonates grew *S. aureus*. Based on core genome multilocus sequence typing (cgMLST), 12 clusters of genetically indistinguishable MSSA isolates were found, containing 33 isolates in total (2-4 isolates per cluster). In seven of these clusters, at least two of the identified MSSA isolates were collected within a time period of one month. Six virulence genes were present in 98-100% of all MSSA isolates. In comparison to *S. aureus* reference genomes, toxin genes encoding staphylococcal enterotoxin A (*sea*) and toxic shock syndrome toxin 1 (*tsst-1*) were present more often in the genomes of bacteremia isolates.

Conclusion

Transmission of MSSA is a contributing factor to the occurrence of *S. aureus* bacteremia in neonates. *Sea* and *tsst-1* might play a role in neonatal S*. aureus* bacteremia.

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a well-established nosocomial pathogen that causes multiple types of neonatal infections [1,2]. Invasive *S. aureus* infections in neonates (e.g. bacteremia) are common in very low birth weight (VLBW) infants, which makes this bacterial species one of the most important pathogens in neonatal intensive care units (NICU) [3-5]. A significant risk factor for *S. aureus* bacteremia in VLBW infants is the presence of intravascular catheters, which are frequently required [6-8]. In addition, *S. aureus* bacteremia can result in severe complications such as endocarditis and osteomyelitis [5,9,10]. All-cause mortality among neonates suffering from *S. aureus* bacteremia varies between 10 and 20% [7,11]. So there is an urgent need to prevent this infection. To prevent *S. aureus* bacteremia in neonates, it is important to know the factors contributing to the high frequency and severity of this infection.

Previously, the virulence factors *tsst-1* and *sea* were implicated to play a role in *S. aureus* bacteremia [12-14]. Furthermore, transmission of *S. aureus* might contribute to the high frequency of bacteremia. Outbreaks of methicillin-resistant *S. aureus* (MRSA) at the NICU are described and relatively easy to detect [15-18]. Meanwhile, the detection of methicillin-sensitive *S. aureus* (MSSA) outbreaks seems to be more difficult, excluding outbreaks in patients who suffer from a skin infection [19-22]. In this study, whole-genome sequencing (WGS), the typing method with the highest discriminatory power, was used to determine whether MSSA transmission and genetic makeup, contribute to the occurrence of neonatal *S. aureus* bacteremia.

METHODS

Population

The NICU of Erasmus MC-Sophia, Rotterdam, the Netherlands, is a level IV, 27-beds facility. It is divided into four units with six to eight beds each. Per year, about 750 neonates are admitted. Nearly 40% of them are below 32 weeks of gestation and were in majority born in this hospital.

Screening

We included neonates with a presumed infection, of whom blood cultures were obtained between January 2011 and November 2017 that showed to be positive for *S. aureus*. Clinical data concerning gender, gestational age, birth weight and survival were obtained from patient records.

S. aureus isolates

Blood from neonates was cultured in BACTEC plus PEDS aerobic bottles and incubated in the Bactec FX (BD, Heidelberg, Germany). In case of positive blood cultures, plates were inoculated and, after 16-24 hours of incubation at 37°C, screened for *S. aureus* based on colony morphology. Identification was performed by means of a latex agglutination test (Slidex Staph Plus, bioMérieux, Marcy-l'Etoile, France) and/or via matrix-assisted laser desorption/ionisation, time-of-flight, mass spectrometry (MALDI-TOF MS system, Bruker). *S. aureus* isolates were stored at – 20 °C or – 80 °C until use. The VITEK 2 system (bioMérieux) was used for antimicrobial susceptibility testing (AST).

Whole-genome sequencing

Transmission

S. aureus isolates were processed according to the bioMérieux EpiSeq^{cs} V1 programme and sent to LGC Genomics GmbH (Berlin, Germany) for next-generation sequencing (NGS). We used Illumina chemistry, which generated paired end 2x150 bp reads. Sequences were assembled using the proprietary built-in assembler from CLC Genomics Workbench v11 software (Qiagen, Hilden, Germany) with default parameters. We analysed them by means of the available *S. aureus* core genome multilocus sequence typing scheme (cgMLST) [23] in BioNumerics 7.6.3 (bioMérieux, Sint-Martens-Latem, Belgium) which contains 1,861 loci. Allele calling was performed using two algorithms, one based on the assembly using a BLAST approach (assembly-based calling) and one based on the trimmed sequencing data using a kmer based approach (assembly-free calling). A consensus of both algorithms was used to assign final allele calls: when both algorithms were in agreement or when an allele call was made by only one of the algorithms, the allele call was considered in the consensus. However, when both algorithms were in disagreement, the allele call was not considered in the consensus. Both allele calling algorithms were executed using default parameters. Conventional MLST types were inferred *in silico* from the WGS data. To this end, the seven MLST loci were identified using the sequence extraction tool and the MLST plugin from BioNumerics 7.6.3 that is synchronized to the pubMLST.org public repository (accession date: April 5, 2019. For the visualisation of the genetic relatedness between the isolates, we used a minimum spanning tree for the cgMLST data. The MST was generated using default parameters, and no re-sampling was performed. Isolates containing less than 12 allelic differences in the *S. aureus* core genome were considered genetically indistinguishable [23]. We defined a cluster as more than two genetically indistinguishable isolates and, within a cluster, considered transmission of *S. aureus* likely. To further validate the results based on the cgMLST approach, as additional method, we evaluated transmission events using a SNP based approach (Additional file 1: Table S1).

Virulence

The presence of virulence genes was assessed, using the sequence extraction tool in BioNumerics 7.6.3. Extraction parameters (percentage coverage and identity) were individualised to accommodate for the different levels of sequence diversity within and between the virulence genes. Anticipating problems upon assembling virulence genes containing repetitive motifs (*sdrA*, *-B* and *-C*, *clfA* and *–B*, *cna*, *sasG*) using the short read sequence data, only the largest non-repetitive part of these genes was used for quering. In order to obtain data from a general *S. aureus* population, the prevalence of virulence genes was also assessed by means of the available genomic sequences in the Refseq Genome Database, using the BLAST interface (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This database contained 10,288 *S. aureus* genomes at the time of analysis. Virulence gene-specific search parameters were used as discussed above. Role and function of the *S. aureus* virulence genes were described in more detail earlier [12,24]. An overview of analysed virulence genes, their role, search parameters and query sequence are shown in Additional file 2: Table S2.

RESULTS

Patient characteristics

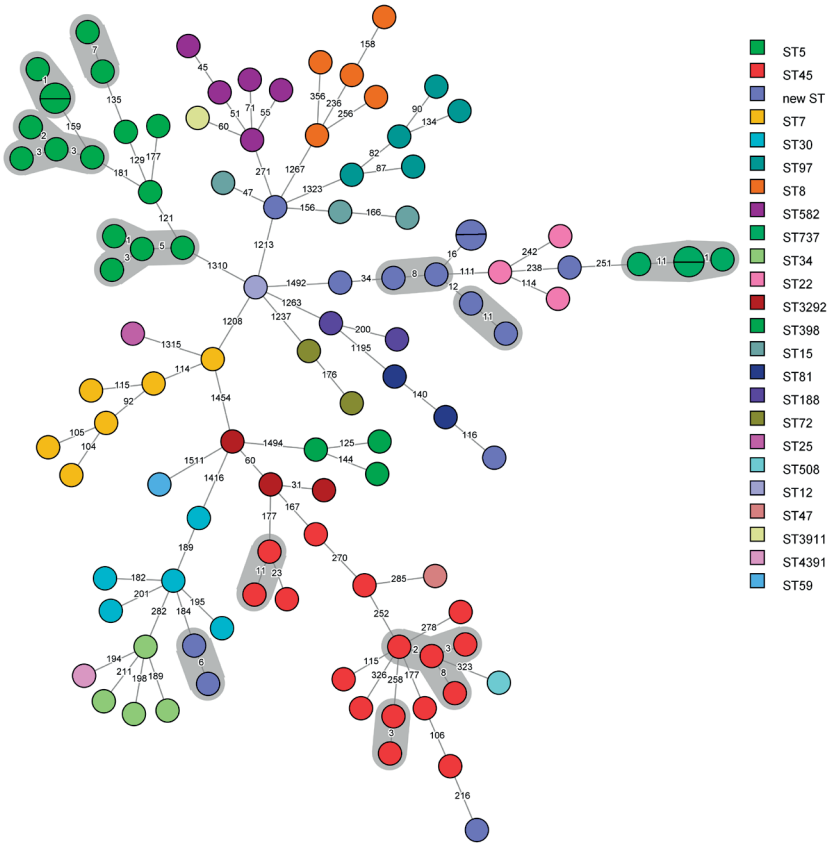
After coagulase-negative staphylococci (CoNS), MSSA was the most frequent causative pathogen of bacteremia in neonates. Several species of CoNS were isolated from neonatal blood, but they were considered to be one group. Twelve percent (*n* = 112) of 926 positive blood cultures from neonates (one blood culture per episode per patient), taken in the period January 2011 – November 2017, were positive for MSSA. Fifty-nine of the 112 neonates (52.7%) with MSSA bacteremia were male. The median (interquartile range) for gestational age and birth weight were 26 3/7 (25 1/7 – 30) weeks and 880 (680 – 1150) grams, respectively. The onset of all episodes of MSSA bacteremia occurred 72 hours after birth, at a median postnatal age of 10 (7 – 19) days. The overall mortality among the included 112 patients was 20.5% while 11 of these 23 neonates died of MSSA septicaemia.

Genetic relatedness

One hundred and four MSSA isolates from the total of 112 neonatal bloodstream isolates (93%) were available and therefore included for WGS (including only the first isolate per patient). Based on WGS, a total of 23 classical MLST types were identified. The most predominant MLST types were ST5 and ST45 (for both *n* = 16). For 11 MSSA isolates a novel MLST type was found. To assess the genetic relatedness between the 104 isolates based on the more discriminatory cgMLST scheme, we visualised the number of allelic differences of the isolates in Fig. 1. Twelve cgMLST clusters of genetically indistinguishable isolates were observed, containing a total of 33 isolates (2 - 4 isolates per cluster). In seven of these

cgMLST clusters, at least two of the identified MSSA isolates were collected within a time period of one month. In two cgMLST clusters, all MSSA isolates were found within a time period of one year, but the shortest time interval between isolates of two neonates was forty days. In the other three cgMLST clusters, there was a time interval of more than one year between culturing the MSSA bloodstream isolates of two neonates. The SNP approach confirmed our results based on the cgMLST approach (Additional file 1: Table S1).

Figure 1. Minimum spanning tree, based on the core genome of 104 *S. aureus* isolates.



Colours indicate the classical MLST sequence types (ST). Twelve cgMLST clusters containing at least two isolates with a maximum of eleven allelic differences are indicated with a grey background.

S. aureus virulence genes

An overview of virulence genes present in the 104 MSSA isolates is provided in Table 1. Of the immunomodulatory proteins, staphylococcal complement inhibitor (*scin*) was present in 100% of all bloodstream isolates. Alpha-hemolysin (*hla*) was present in 99% of the isolates.

Table 1. Presence of virulence genes in neonatal *S. aureus* isolates compared to reference genomes.

genes	neonatal isolates (%)	refseq (%)	genes	neonatal isolates (%)	refseq (%)
sea	24.0	9.4	***cna***	51.0	34.6
seb	4.8	5.9	***eap-map***	64.4	93.7
sec	18.3	10.8	***ebp***	95.2	95.2
sed	10.6	8.8	***fnbpA***	66.3	75.2
seg	51.9	55.3	***fnbpB***	65.4	75.0
seh	5.8	1.7	***sdrC***	91.3	96.3
sei	55.8	55.2	***sdrD***	50.0	74.9
sej	10.6	11.1	***sdrE***	89.4	85.0
sek	3.8	19.0	***efb***	77.9	94.7
sel	18.3	10.8	***icaA***	100.0	97.8
sem	55.8	55.6	***icaB***	89.4	98.5
sen	45.2	55.2	***icaC***	99.0	97.8
seo	58.7	49.1	***icaD***	100.0	98.0
sep	4.8	19.1	***icaR***	95.2	97.7
seq	4.8	19.1	***isaA***	100.0	98.3
ser	10.6	10.9	***isdA***	100.0	97.6
ses	0.0	0.3	***isdH***	100.0	97.5
set	0.0	0.3	***sasG***	54.8	56.7
seu	51.9	54.8	***edn***	1.0	1.4
sey	1.0	5.8	***etA***	1.0	1.0
aur	100.0	98.2	***etB***	0.0	0.2
coa	70.2	83.0	***etD***	1.0	0.8
geh	87.5	97.5	***hla***	99.0	97.1
hysA	98.1	96.5	***hlb***	100.0	95.1
sak	73.1	78.5	***hld***	90.4	98.0
sspA	99.0	98.1	***hlgA***	96.2	97.6
sspB	91.3	98.1	***hlgB***	100.0	97.8
sspC	100.0	98.3	***hlgC***	98.1	97.5
vWbp	78.8	98.1	***lukD***	49.0	66.5
adsA	96.2	98.1	***lukE***	49.0	65.9
chp	68.3	67.2	***lukF***	0.0	18.8
sbi	100.0	97.7	***lukM***	0.0	0.7
scn	98.1	98.2	***lukS***	0.0	19.0
clfA	98.1	97.4	***tsst-1***	18.3	5.8
clfB	100.0	97.8			

We also found a 98-100% presence of the MSCRAMMs clumping factors A and B (*clfA*, *clfB*), immunodominant surface antigen A (*isaA*) and iron-responsive surface determinants A and H (*isdA*, *isdH*). When compared to a reference population of *S. aureus* genomes, a few observations stand out. Remarkably, staphylococcal enterotoxin A (*sea*) and toxic shock syndrome toxin 1 (*tsst-1*) were, respectively, 2.6 and 3.2 times more prevalent among the 104 neonatal bloodstream isolates, relative to the reference genomes. Likewise, staphylococcal enterotoxin h (*seh*) was 3.4 times more prevalent although, in absolute numbers, this involved only a few isolates (6/104 versus 173/10288). For the other virulence genes, no such increases were detected (Table 1).

DISCUSSION

At our level IV neonatal intensive care unit, as in many centres [3-5], *S. aureus* is a frequent cause of neonatal bacteremia. In our study, we explored the role of MSSA transmission and the possible contribution of virulence genes. By using WGS, 12 different cgMLST clusters of MSSA isolates were found. Seven of these twelve cgMLST clusters included at least two MSSA isolates, cultured from blood of neonates within one month, indicative for transmission. Transmission should therefore be considered as a contributing factor for the frequent occurrence of neonatal *S. aureus* bacteremia, as was recently described by *Rouard* et al. [13]. Although it seems reasonable to assume that transmission, irrespective of the source, can only occur through the hands of healthcare workers (HCWs), we did not prove this, since we did not culture the environment, nor the HCWs or parents. Still, general measures such as improvement of the current (daily) cleaning, disinfection procedures as well as hand hygiene, will be likely to help. It was already proven that neonatal hospital-acquired infections could in part be prevented by strict infection control measures [8,25,26]. In addition, reinforcement of the implementation of central-line bundles has the potential to reduce the incidence of central line-associated bloodstream infections (CLABSIs); although these bundles are already implemented, compliance can still be improved and additional measures can be explored [27].

Besides transmission, it was determined whether the presence of certain virulence factors is associated with neonatal *S. aureus* bacteremia. Since it was difficult to define a suitable control population of neonates, we chose to compare neonatal *S. aureus* bacteremia isolates to all available *S. aureus* genomes from the Refseq Genome Database (*N* = 10,288 at the time of analysis). Remarkably, the genes *sea* and *tsst-1* were found a factor 2.6 and 3.2 times more often in the MSSA bloodstream isolates, compared to the reference genomes in the Refseq Genome Database. The overrepresentation of *tsst-1* could not be explained by the frequent presence of MLST ST5 and ST45 in our isolates collection, since *tsst-1* was not associated with these sequence types. On the other hand, 11 of the 25 isolates carrying *sea*

were found in ST5 isolates. Still, this cannot be the full explanation for finding an association between *sea* and neonatal MSSA bacteremia. Many studies have been executed on *S. aureus* toxins and their pathogenic roles, particularly on *sea* and *tsst-1*. Previously, it was described that antibody responses to these two specific toxins were higher in patients with *S. aureus* bacteremia, compared to control patients [12]. In addition, in a recent publication about a NICU MSSA outbreak, *tsst-1* and especially *sea* were found in bloodstream isolates, compared to colonisation isolates [13]. Another review article describes the association of these toxins with bacteremia [14]. Therefore, this may suggest that *sea* and *tsst-1* might play a role in the pathogenesis of *S. aureus* bacteremia. The other virulence genes were present in virtually all study isolates, but in virtually all reference genomes as well (Table 1).

Our study has its limitations. It was performed retrospectively, in a single centre. We considered less than 12 allelic differences in the *S. aureus* core genome as indistinguishable, as described by *Leopold* et al. for MRSA outbreaks [23]. Still, it is a matter of debate which cut-off should be used to define MSSA isolates as indistinguishable. If the cut-off would have been set at 20 alleles [28], this would have led to 10 larger instead of 12 indistinguishable MSSA clusters, which would not have changed the conclusion regarding transmission. Additional studies are needed to define a clear cut-off. Finally, we compared neonatal isolates to a large number of reference genomes, but these originate from several countries, several clinical sites and patients of all ages. It would have been ideal if the reference genomes had originated from colonized but not infected neonates, admitted to the same NICU, in the same time period.

CONCLUSIONS

In conclusion, transmission of MSSA seems a contributing factor to the occurrence of *S. aureus* bacteremia in neonates. The possibility of MSSA transmission in neonatal intensive care should be explored to prevent this invasive and serious infection. The exact role of *sea* and *tsst-1* warrants further investigation.

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AUTHOR'S CONTRIBUTIONS

BS contributed to the study design, collected, analysed and interpreted the data, and drafted the initial manuscript. NV conceptualised and designed the study, collected, analysed, interpreted and supervised the data collection and critically revised and reviewed the manuscript for intellectual content. MV contributed to the study design, while MV WB collected and interpreted the data, and critically revised and reviewed the manuscript for intellectual content. RK IR contributed to the study design, collected data, and critically revised and reviewed the manuscript for intellectual content. DDC CK contributed to the study design, collected, analysed and interpreted the data, and critically revised and reviewed the manuscript for intellectual content. AVB WG conceptualised and designed the study, interpreted the data and critically revised and reviewed the manuscript for intellectual content.

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AVAILABILITY OF DATA AND MATERIALS

The data generated and analysed in this study are included in the current article.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Because this was a retrospective observational study in which anonymised patient data were used, collected during routine clinical practice, informed consent was not mandatory according to the Dutch Medical Research Involving Human Subjects Act (WMO). The Institutional Ethics Review Board of the Erasmus MC reviewed the study protocol and provided an exemption from formal ethical assessment (MEC-2015-306), based on the non-interventional design. The study was carried out in accordance with the current ethical guidelines for epidemiological research.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

All other authors declare that there are no competing personal or institutional interests.

REFERENCES

1 Jeong IS, Jeong JS, Choi EO: Nosocomial infection in a newborn intensive care unit (NICU), South Korea. BMC Infect Dis 2006;6:103.

2 Reichert F, Piening B, Geffers C, Gastmeier P, Buhrer C, Schwab F: Pathogen-Specific Clustering of Nosocomial Blood Stream Infections in Very Preterm Infants. Pediatrics 2016;137

3 Ericson JE, Popoola VO, Smith PB, Benjamin DK, Fowler VG, Benjamin DK, Jr., Clark RH, Milstone AM: Burden of Invasive *Staphylococcus aureus* Infections in Hospitalized Infants. JAMA Pediatr 2015;169:1105-1111.

4 Carey AJ, Duchon J, Della-Latta P, Saiman L: The epidemiology of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit, 2000-2007. J Perinatol 2010;30:135-139.

5 Dolapo O, Dhanireddy R, Talati AJ: Trends of *Staphylococcus aureus* bloodstream infections in a neonatal intensive care unit from 2000-2009. BMC Pediatr 2014;14:121.

6 Ekkelenkamp MB, van der Bruggen T, van de Vijver DA, Wolfs TF, Bonten MJ: Bacteremic complications of intravascular catheters colonized with *Staphylococcus aureus*. Clin Infect Dis 2008;46:114-118.

7 Hakim H, Mylotte JM, Faden H: Morbidity and mortality of Staphylococcal bacteremia in children. Am J Infect Control 2007;35:102-105.

8 Murdoch F, Danial J, Morris AK, Czarniak E, Bishop JL, Glass E, Imrie LJ: The Scottish enhanced *Staphylococcus aureus* bacteraemia surveillance programme: the first 18 months of data in children. J Hosp Infect 2017;97:127-132.

9 Le J, Dam Q, Tran T, Nguyen A, Adler-Shohet FC, Kim S, Schmidt K, Lieberman JM, Bradley JS: Epidemiology and hospital readmission associated with complications of *Staphylococcus aureus* bacteremia in pediatrics over a 25-year period. Epidemiol Infect 2017;145:2631-2639.

10 Valente AM, Jain R, Scheurer M, Fowler VG, Jr., Corey GR, Bengur AR, Sanders S, Li JS: Frequency of infective endocarditis among infants and children with *Staphylococcus aureus* bacteremia. Pediatrics 2005;115:e15-19.

11 Kaufman D, Fairchild KD: Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. Clin Microbiol Rev 2004;17:638-680, table of contents.

12 Verkaik NJ, Dauwalder O, Antri K, Boubekri I, de Vogel CP, Badiou C, Bes M, Vandenesch F, Tazir M, Hooijkaas H, Verbrugh HA, van Belkum A, Etienne J, Lina G, Ramdani-Bouguessa N, van Wamel WJ: Immunogenicity of toxins during *Staphylococcus aureus* infection. Clin Infect Dis 2010;50:61-68.

13 Rouard C, Bourgeois-Nicolaos N, Rahajamanana L, Romain O, Pouga L, Derouin V, De Luca D, Doucet-Populaire F: Evaluation of an 'all-in-one' seven-day whole-genome sequencing solution in the investigation of a *Staphylococcus aureus* outbreak in a neonatal intensive care unit. J Hosp Infect 2019;102;297-303.

14 Xu S, McCormick J: Staphylococcal superantigens in colonization and disease. Front Cell Infect Microbiol 2012;2;52.

15 Regev-Yochay G, Rubinstein E, Barzilai A, Carmeli Y, Kuint J, Etienne J, Blech M, Smollen G, Maayan-Metzger A, Leavitt A, Rahav G, Keller N: Methicillin-resistant *Staphylococcus aureus* in neonatal intensive care unit. Emerg Infect Dis 2005;11:453-456.

16 Reich PJ, Boyle MG, Hogan PG, Johnson AJ, Wallace MA, Elward AM, Warner BB, Burnham CA, Fritz SA: Emergence of community-associated methicillin-resistant *Staphylococcus aureus* strains in the neonatal intensive care unit: an infection prevention and patient safety challenge. Clin Microbiol Infect 2016;22:645 e641-648.

17 Huang YC, Lien RI, Su LH, Chou YH, Lin TY: Successful control of methicillin-resistant *Staphylococcus aureus* in endemic neonatal intensive care units--a 7-year campaign. PLoS One 2011;6:e23001.

18 Ramsing BG, Arpi M, Andersen EA, Knabe N, Mogensen D, Buhl D, Westh H, Ostergaard C: First outbreak with MRSA in a Danish neonatal intensive care unit: risk factors and control procedures. PLoS One 2013;8:e66904.

19 Achermann Y, Seidl K, Kuster SP, Leimer N, Durisch N, Ajdler-Schaffler E, Karrer S, Senn G, Holzmann-Burgel A, Wolfensberger A, Leone A, Arlettaz R, Zinkernagel AS, Sax H: Epidemiology of Methicillin-Susceptible *Staphylococcus aureus* in a Neonatology Ward. Infect Control Hosp Epidemiol 2015;36:1305-1312.

20 Lin MF, Huang ML, Lai SH: Investigation of a pyoderma outbreak caused by methicillin-susceptible *Staphylococcus aureus* in a nursery for newborns. J Hosp Infect 2004;57:38-43.

21 Gomez-Gonzalez C, Alba C, Otero JR, Sanz F, Chaves F: Long persistence of methicillin-susceptible strains of *Staphylococcus aureus* causing sepsis in a neonatal intensive care unit. J Clin Microbiol 2007;45:2301-2304.

22 Koningstein M, Groen L, Geraats-Peters K, Lutgens S, Rietveld A, Jira P, Kluytmans J, de Greeff SC, Hermans M, Schneeberger PM: The use of typing methods and infection prevention measures to control a bullous impetigo outbreak on a neonatal ward. Antimicrob Resist Infect Control 2012;1:37.

23 Leopold SR, Goering RV, Witten A, Harmsen D, Mellmann A: Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes. J Clin Microbiol 2014;52:2365-2370.

24 Verkaik NJ, van Wamel WJ, van Belkum A: Immunotherapeutic approaches against *Staphylococcus aureus*. Immunotherapy 2011;3:1063-1073.

25 Borghesi A, Stronati M: Strategies for the prevention of hospital-acquired infections in the neonatal intensive care unit. J Hosp Infect 2008;68:293-300.

26 Trevisanuto D, Arnolda G, Chien TD, Xuan NM, Thu le TA, Kumara D, Lincetto O, Moccia L: Reducing neonatal infections in south and south central Vietnam: the views of healthcare providers. BMC Pediatr 2013;13:51.

27 Ista E, van der Hoven B, Kornelisse RF, van der Starre C, Vos MC, Boersma E, Helder OK: Effectiveness of insertion and maintenance bundles to prevent central-line-associated bloodstream infections in critically ill patients of all ages: a systematic review and meta-analysis. Lancet Infect Dis 2016;16:724-734.

28 Goyal M, Javerliat F, Palmieri M, Mirande C, van Wamel W, Tavakol M, Verkaik NJ, van Belkum A: Genomic Evolution of *Staphylococcus aureus* During Artificial and Natural Colonization of the Human Nose. Front Microbiol 2019;10:1525.

ADDITIONAL FILE 1

Methods

Whole-genome Single-Nucleotide Polymorphism analysis
A whole-genome Single-Nucleotide Polymorphism (wgSNP) analysis was performed on the samples within a potential transmission cluster identified by wgMLST analysis (BioNumerics 7.6.3 (Applied Maths, Sint-Martens-Latem, Belgium)). For each potential transmission cluster a reference genome for mapping with Bowtie 2 using default parameters was selected based on the N50 of the assembly and number of loci which were assigned an allele during wgMLST analysis. The strict SNP filtering template was applied.

Results

SNP matrices of the possible transmission clusters detected by cgMLST.
Cluster numbers correspond to the clusters mentioned in the manuscript and Figure 1 involving samples with a maximum of 11 alleles difference using the cgMLST approach.

Table S1

CLUSTER 1

isolate	1	2
1	0	32
2	32	0

CLUSTER 2

isolate	1	2	3
1	0	5	5
2	5	0	0
3	5	0	0

CLUSTER 3

isolate	1	2	3	4
1	0	10	11	7
2	10	0	12	8
3	11	12	0	10
4	7	8	10	0

CLUSTER 4

isolate	1	2	3	4
1	0	1	1	4
2	1	0	2	5
3	1	2	0	4
4	4	5	4	0

CLUSTER 5

isolate	1	2	3	4
1	0	2	2	37
2	2	0	0	37
3	2	0	0	37
4	37	37	37	0

CLUSTER 6

isolate	1	2
1	0	17
2	17	0

Table S1 (*continued*)

CLUSTER 7

isolate	1	2
1	0	21
2	21	0

CLUSTER 8

isolate	1	2
1	0	59
2	59	0

CLUSTER 9

isolate	1	2
1	0	62
2	62	0

CLUSTER 10

isolate	1	2
1	0	8
2	8	0

CLUSTER 11

isolate	1	2	3	4
1	0	1	4	16
2	1	0	3	17
3	4	3	0	20
4	16	17	20	0

CLUSTER 12

isolate	1	2
1	0	13
2	13	0

Table S2. *S. aureus* virulence genes

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
Enterotoxin	sea	staphylococcal enterotoxin A	95	619	atgaaaaaacagcatttatactacttttattcattgccctaacgtggacaacaagtccacttgtaaatggtagcgagaaaagcgaagaaataaatgaaaaagatttgcgaaaaaagtctgaattgcagggagcagctttaggcaatcttaaacaaatctattattacaatgaaaaagctaaaactgaaaataaagagagtcacgatcaatttttacagcatactatattgtttaaaggctttttacaaatcattcatggtataacgatttattagtagattttgattcaaaggatattgttgataaatataaagggaaaaaagtagacttatatggtgcttattatggttatcaatgtgcgggtggtacaccaaacaaaacagcttgcatgtatggtggtgtaacgttacatgataataatcgattgaccgaagagaaaaaagtgccgatcaatttatggctagacggtaaacaaaatacagtacctttggaaacggttaaaacgaataagaaaaatgtaactgttcaggagttggatcttcaagcaagacgttatttacaggaaaaatataatttatatatactctgatgtttttgatgggaaggttcagaggggattaatcgtgtttcatacttctacagaaccttcggttaattacgatttatttggtgctcaaggacagaattcaaatacactattaagaatatatagagataataaaacgattaactctgaaaacatgcatattgatatatatttatatacaagttaa
Enterotoxin	seb	staphylococcal enterotoxin B	95	641	atgtataagagattatttatttcacatgtaattttgatattcgcactgatattagttatttctacacccaacgttttagcagagagtcaaccagatcctaaaccagatgagttgcacaaatcgagtaaattcactggtttgatggaaaatatgaaagttttgtatgatgataatcatgtatcagcaataaacgttaaatctatagatcaatttctatactttgacttaatatattctattaaggacactaagttagggaattatgataatgttcgagtcgaatttaaaaacaaagatttagctgataaatacaaagatgaaatacgtagatgtgtttggagctaattattattatcaatgttatttttctaaaaaaacgaatgatattaattcgcatcaaactgacaaacgaaaacttgtatgtatggtggtgtaactgagcataatggaaaccaattagataaatatagaagtattactgttcgggtatttgaagatggtaaaaatttatatctttgacgtacaaactaataagaaaaaggtgactgctcaagaattagattacctaactcgtcactatttggtgaaaaataaaaaactctatgaatttaacaactcgccttatgaaacgggatatattaaatttatagaaaatgagaatagcttttggtatgacatgatgcctgcaccaggagataaatttgaccaatctaaatatttaatgatgtacaatgacaataaaatggttgattctaaagatgtgaagattgaagtttatcttacgacaaagaaaaagtga
Enterotoxin	sec	staphylococcal enterotoxin C	95	641	atgaataagagtcgatttatttcatgcgtaattttgatattcgcacttatactagttcttttacacccaacgtattagcagagagccaaccagaccctacgccagatgagttgcacaaagcgagtaaattcactggtttgatggaaaatatgaaagttttatatgatgatcattatgtatcagcaactaaagttaagtctgtagataaatttttggcacatgatttaatttataacattagtgataaaaaactgaaaaattatgacaaagtgaaaacagagttattaaatgaaggtttagcaaagaagtacaaagatgaagtagttgatgtgtatggatcaaattactatgtaaactgctatttttcatccaaagataatgtaggtaaagttacaggtggcaaaacttgtatgtatggaggaataacaaaacatgaaggaaaccacttttgataatgggaacttacaaaatgtacttataagagttttatgaaaataaaagaaacacaatttcttttgaagtgcaaactgataagaaaagtgtaacagctcaagaactagacataaaagctaggaatttttttaattaataaaaaaaaatttgtatgagtttaacagttcaccatatgaaacaggatatataaaatttattgaaaataacggcaatacttttttggtatgatatgatgcctgcaccaggcgataagtttgaccaatctaaatatttaatgatgtacaacgacaataaaacggttgattctaaaagtgtgaagatagaagtcgcaccttacaacaaagaatggataa
Enterotoxin	sed	staphylococcal enterotoxin D	95	622	atgaaaaaatttaacattcttattgcattactctttttactagtttggtaatatctccttttaaacgttaaagccaatgaaaacattgattcagtaaaagagaaagaattgcataaaaaatctgaattaagtagtaccgcgctaaataatatgaaacattcttatgcagataaaaatccaataataggagaaaataaaagtacaggagatcaatttttagaaaatacttttgctttacaaaaaatttttttactgaccttatcaatttttgaagatttattaataaacttcaattcaaaagaaatggctcaacatttcaaatctaaaaatgtagatgttttaccctataagatatagcattaattgttatggtggtgaaatagataggactgcttgtacatatggaggtgtcactccacacgaaggtaataaattaaaagaacgaaaaaaaatacccaatcaatttgtggataaatggtgtacaaaaagaagtttctttagataaagttcaaacagataaaaaaaatgttaccgtacaagaattagatgcacaagcaaggcgctatttgcaaaaggatttaaaattgtatataatgatactctcggaggaaaaatacagcgcggaaaaatagagtttgattcttctgatgggtctaaagtctcttatgatttatttgatgttaagggtgattttcccgaaaaacaattacgaatatacagtgataataaaacattatccacagagcaccttcatattgacatctatttatatgaaaagtag
Enterotoxin	seg	staphylococcal enterotoxin G	95	622	atgaagaaattatctactgtaattattatttttgattctagaaatagtttttcataatatgaattatgtgaatgctcaacccgatcctaaattagacgaactaaataaagtaagtgattataaaaataataagggaactatgggtaatgtaatgaatctttatacgtctccacctgttgaaggaagaggagttattaattctagacagttttttatctcatgatttaatttttccaattgagtataagagttataatgaggttaaaactgaattagaaaatacagaattagctaacaattatagatataaaaagtagacatttttggcgttccatatttttatacatgtataatacctaaatctgaaccggatataaaccaaaatttttggaggttgttgtatgtatggtggtcttacatttaatagttcagaaaatgaaagagataataattattactgtacaggtaacaatcgacaatagacaatcacttggatttacaataactacaaataagaatatggttactattcaggaactagattacaaagcaagacactggctcactaaagaaaaaaagctatacgagtttgatggttctgcatttgaatctggatatataaaatttactgaaaagaacaatacaagtttttggtttgacttatttcctaaaaaagaactagtacctttttgttccatataagttttttaaatatttacggagatataataagttgttgattctaagagtattaaaatggaagtatttcttaatactcactga
Enterotoxin	seh	staphylococcal enterotoxin H	95	581	atgattaataaaattaaaatattatttttcgtttttagcattattactttcattcacatcatatgcgaaagcagaagatttacacgataaaagtgagttaacagatttagctttagctaatgcatatggtcaatatatcacccattcattaaagaaaatataaagagtgatgaaataagtggagaaaaagatttaatatttagaaatcaaggtgatagtggcaatgatttgagagtaaagtttgcaactgctgatttagctcagaagtttaaaaataaaaatgtagatatatatggggcatctttttattataagtgtgaaaaaataagtgaaaatatttctgaatgtctatatggaggtacaacactaaatagtgaaaaattggcacagaaaagggtgattggtgctaatgtttgggtagatggtattcaaaaagaaacagaattaatacgaacaaatagaaaaatgtgacattgcaagaattagatataaagatcagaaaaatattgtccgataaatataaatttattatataaagacagcgaaataagtaaaggtctaattgaatttgatatgaaaactcctagagattactcattcgacatttatgatttaaaaggagaaaatgactatgagatagataaaattatgaagacaataaaactttaaaatctgatgatataagtcatattgatgtaaatctatatactaagaaaaaagtataa

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
Enterotoxin	sei	staphylococcal enterotoxin I	95	583	atgaaaaaatttaaatatagttttatattagtttttatattactttttaacattaaagatcttacgtatgctcaaggtgatattggtgtaggtaacttaagaaatttctatacaaaacatgattatatagatttaaaaggcgtcacagataaaaacctacctattgcaaatcaactcgaatttttcaacaggtaccaatgatttgatctcagaatctaataattgggacgaaataagtaaatttaaaggaaagaaactggatatttttggcattgattataatggtccttgtaaatctaaatacatgtatggaggggccactttatcaggacaatacttaaattctgctagaaaaatccccattaatctttgggttaatggcaaacataaaacaatttctactgacaaaatagcaactaataaaaaactagtaacagctcaagaaattgatgttaaattaagaagatatcttcaagaagaatacaatatatatggtcataataacactggtaaaggcaaagaatatggatataaatctaaatttattcaggttttaataatgggaaagttttatttcatttaaataatgaaaaatcattttcatatgatttgttttatacaggagatggactgcctgtaagttttttgaaaatttatgaagataataaaataataggaatctgaaaaatttcatcttgatgtcgaaatatcatatgtagatagtaactaa
Enterotoxin	sej	staphylococcal enterotoxin J	95	646	atgaaaaaaagaatatttatactgattttctccctgacgttaacactactaataaccccttttagtttacagcgatagcaaaaatgaaacaattaaagaaaagaatttgcacaaaaaatcagagttaagtagtattacttttaaataacctcagacatatatacttctttaacgaaaagggtatctctgaaaagataatgacagaagatcaatttttagattacacactattatttaaaagttttttcataagtcattcacagtataacgacttattggtacaattcgattcaaaagaaacagttaataagtttaaaggcaaacaagtagatctatacggatcttattatggttttcaatgttctggtggtaaaccaaataaaacagcatgtatgtatggtggagtaacactgcatgaaaacaatcaacttttatgatactaaaaaaatacctattaatttatggattgatagcatcagaactgttgttccgctagatatagttaaaacaaaataagaaaaaagtaaccattcaagaactagatcttcaggcaagatattatttacataaacaatatatatttatacaaccctagtacctttggtggtaaaattcagaaaggactaatcgtgtttcatacctcaaaagaaccgttggttagttatgatttatttaatgttatagtcaatatccagacaaattattaaaaatatacaagatataaaatcatagaatctgaaaacatgcatatcgatatatatttatatacaagcctaattgtattaataagtctacctttggttctgtag
Enterotoxin	sek	staphylococcal enterotoxin K	95	583	atgaaaaaattaataagcatcttattaataaatataataatttttaggtgtctctaataatgccagcgctcaaggcgatataggaattgataatctcaggaatttttatacaaaaaaagactttgtagatttaaaagatgtaaaagacaatgatactcctatagctaatcaactacaatttttcaaatgaatcttatgatttaatttcagaatcaaaagatttttaataaatttagtaatttcaagggaaaaaaacttgatgttttttggtattagttataatggccagtgtaacactaaatacatatatggtggagtcacagctactaacgaatatctagataaatctagaaatatacctataaatatatggatcaatggaaatcacaaaactatttctaccaataaagtttcgacaaataaaaaattagttaccgctcaagagattgatgtcaaattaagaaagtaccttcaagaagaatacaacatttatggacataacggcactaaaaaaggagaagaatatggtcataaatcaaaatttttattctggatttaatattggtaaagtaacgttccatttaaataataatgacacttttttcatatgatttattctacacaggagatgatgggttaccaaaaagttttttaaaaatttacgaagacaataaaactgtagagtctgagaaattccatttggatgtcgatatttcttataaagaaacgatataa
Enterotoxin	sel	staphylococcal enterotoxin L	95	578	atgaaaaaaagattattatttgtaattgttattactttatttatttttttcttctaatcatacagtcttatctaacggcgatgtaggtccaggaaatctaagaaatttttatactaaatatgaatatgtgaattttaaagaatgttaaagacaaaaattcaccagaatcacaccgcttagaatactcgtatataaaatgatacattgtatgctgaatttgacaatgaatatataactagtgatctaaagggaaaaaatgtcgatgttttggtataagctataaatatggttctaactctcgtactatatatggtggttactaaagcagaaaacaataaattagattcgccaagaatcatacctataaatttaattatcaatggcaagcatcaaacagttacaactaaaagtgtttctacagataaaaaaatggttaccgcacaagaaatagatgtcaaactaagaaaatacttgcaagatgaatttaatatttatggacacaatgatactggtaaaggtaaagaatacggcacttcttcaaaatttatagcggttttgataaggggagtgtagtattccatatgaatgatggttctaatttctcgtatgatttatttttacacaggatacggtcttccagaaagcttcttaaaaatttacaaagataataaaactgttgattcaacacaatttcatctagatgtcgaaatttcaaaaagatga
Enterotoxin	sem	staphylococcal enterotoxin M	90	576	atgaaaagaatacttatcattgttgttttattgttttgttattcacaaaatcatatcgcaaccgctgatgttggagttttgaatcttaggaactattatggtagctatccaatcgaagaccatcaaaatattaatcctgacaataatcgtctttcacatcaattagtttttttctaaggatgaattcgacagtaacagctgaatttaagaatgttgaagatgtaaaaaaattcaaaaatcgtgctgttgatgtatacggcctaagttatagtggatattgtctaaaaaataaatatatgtacggaggagttactttagcgggtgattatttagagaagtctagatgtattcctattaatctttgggttaatggcaaccttaaaacaatttctactgacaaagtatcaactaataaaaagatagtaacagctcaagaaattgatactaaattaagaagatatctacaagaagaatataacatttatggctttaatgatacaaataaaggaagaaattacggtacaaaatccaagttttttttctggatttaatactgggaaaatatcatttcatttgaatgacggtacatcattctcttatgacttatttgatacaggaacagggcaagctgaaagtttcctaaaaatatataatgacaacaaaactgtcgaaactgataaattccatttagatgtagaaatatcttatgaaggacgaaagttga
Enterotoxin	sen	staphylococcal enterotoxin N	95	605	atgagattgttctacatagctgtaattataataacttttattatgtcttataaataataaatatgttaatgctgaagtagacaaaaaagttttaaagaaaaaatctgatatagatagtagtaatttatttaatttaacaagctattatactgatataacgtggcaattagacgagtcaaataaaattagtacagatcaactactgaataatactataatattaaaaaacattgatatatccgtacttaaaacttctagtttgaaagttgagtttaactcagcagatttagcaaatcaatttaaagggaaaaatatagatatttattggactgtattatggaaataaatgtgtaggcttaactgaagaaaaaacatcgtgcttaaacggaggagttacgatacatgatggaaatcaattagatgaagagaaagttatagggcgttaatgtatttaaagatggtgtccaacaagaaggttttgttataaaaactaaaaaggctaaagtaacagtacaagaattagatactaaagttcgatttaaattagaaaatttatataaaatatacaataaagatacggtaacatacaaaaaggatgcattttcttttcattctcataatcatcaagatcaatcattttattatgatttatatataacataaaggttcagtaggagcagagttttttcaattttatagtgataatagaacagttagctcatctaattatcatatcgatgtatttttatataaagattaa

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
Enterotoxin	seo	staphylococcal enterotoxin O	95	494	atgcatatgcaaactgatcataagattctggctaaaatagagagtttgtgtaagaagtcaagtgtagaccctattgctttacataatattaatgatgattatataaataatcgatttacgacagtaaaatcaattgtatcaactacagaaaaattcttagacttcgatttattatttaaaagtattaattggttagatggaatatctgctgaatttaaagatttaaaagtggaatttagctcatcagcgatttctaaagaatttctaggaaagactgttgatatttatggtgtttactataaagcacattgtcatggtgagcatcaagtggatactgcctgtacatatggtggggtaacacctcatgaaaataataaattaagcgagcctaaaaatatagggagtagctgtgtataaggataatgtaaatgttaatacatttatcgttactacagataaaaagaaagttactgcacaagaacttgatattaaagtaagaacaaaattaaataatgcatataaattgtatgacagaatgactagtgatgtacaaaaaggttatattaaatttcattctcattcggagcataaagaatcattttattatgatttatttttaa
Enterotoxin	sep	staphylococcal enterotoxin P	90	362	atgaatggccagtgtaacactaaatacatatatggtggagtcacattggcgaatcaaaatttagataaatctagaaaatatacctataaatatatggatcaatggaaatcacaaaactatttctaccaataaagtttcgacaaacaaaaaattagttaccgctcaagagattgatgtcaaattaagaaagtaccttcaagaagaatacaacatttatggacataacggcactaaaaaaggagaagaatatggtcataaatcaaaatttttattctggatttaatattggtaaagtaacgttccatttaaataatgaaccttcttttacatatgatttattctacacaggaactggtcaagcagagagttttttaaaaatttacgaagacaataaaactgtagagtctgagaaattccatttggatgtcgatatttcttataaagaaacgatataa
Enterotoxin	seq	staphylococcal enterotoxin Q	95	616	atgcctatatggcgttgtaatataaaaaaaggagccatcaaaatgaataaaatatttagaatactcactgttagcttgtttttcttcacatttttaataaaaaacaatctagcatatgctgatgtagggggtaatcaaccttagaaacttttatgctaattatgaacctgaaaagcttcaaggagttagttctggaaatttttctacttctcatcaattagagtatattgatggaaaatacactttatattcacagtttcataatgaatatgaagcgaagagattaaaagatcataaagtagatatctttggaataagttactcaggtctttgtaatacaaaatatatgtatggtggaattacgttggcgaatcaaaatttagataaacctagaaatatacctattaatctctgggtcaatggtaagcaaaatactatatctacagacaaagtttctactcaaaaaaaagaggtaactgctcaagagattgatattaagttacgaaaatacctacaaaacgaatacaatatatatggttttaataaaacaaaaaaaggtcaggaatatggatatcagtcaaaatttaattctgggtttaacaaaggaaaaattactttccatttaaataatgaaccttctttttacatacgatttgttttacaccggaactggtcaagcagagagttttttaaaaatttacaatgatataataaactatagatgcagagaattttcatttggatgtagagatttcatatgagaaaactgaataa
Enterotoxin	ser	staphylococcal enterotoxin R	95	624	atgttaaataaaatcttacttttattgttctctgttacttttatgttgttatttttctctttacattcagtaagtgctaaaccagatccaaggcctggagaattgaatagagttagtgattataaaaaaaacaaaggtactatggggaatgttgaatccttatataaggataaagcggtaatagcagaaaatgttaaaaatactagacaattcttaggacatgatttgatttttcctattccttattcagaatacaaagaagttaaaagcgaatttataaataaaaaaacagctgataaatttaaagacaaacggttagatgtgtttggaatacccttatttttatacatgtttagtacctaagaatgaatcaagagaagaatttatttttgatggagtttgcatatacgggggcgttacaatgcactccacagctgatagtatatccaaaaatataattgttccagttactgtagataataaacaacagttctctttttacaattagtacaaataaaaaaacggttacggtacaagagctagattataaagtaaggaattggttgactacaatataaaaattatatgaatttgatgggtcggcatatgagacgggttatatcaaatttattgaacaaaataaagatagttttggtacgatttgtttcctaaaaaggatttggttcccttttataccttataaatttgtaaatatttatggagataataaaactatagatgcttcgagtgttaagatagaagttcacctgactacaatgtga
Enterotoxin	ses	staphylococcal enterotoxin S	95	618	attgggaatataaataaacgtctatgtgtaaatttgaagagtttatagttttattatcattgtagaatttttaagaaatctacactatatttccctttaaaattaaataaatcataataaaactggatgaattagagtggaattccatatatccttttttgtatgtttcctgtctctgagttatatatttttatattgattttgaataacttttcttgttttaaggtctaactcttgaattgtaggttcctttttgtcagtagtaatcataaaaccttttgttgagagccgtctttgaaaacattcacacctattgaattctttggtctatttttttcttcttcatgaagtgttattccgccatatatacaaccggttctttttagatacttctccaatgcaatttggccccatagtatattccgaatatatctcacctttttttcctttgaaatgattggctatttctgaactattaaactcagttttaggctgtttttattccattctttaagtgtaatatcatcaaatattaaatcgtttgatagatagttgtgtggagtttgttttattttaatatctaaaataatattgtcctgcgaatatgaagttttaatattatatagtgcggttgaatctaattcagatttttttctttaggctttctattttaggtctagattcatctgcttgaacaaagcggttgaaggaaaattcaaatgagaagagtagtacaaagtaatataataattagagttactgttatttttgagtaattcat
Enterotoxin	set	staphylococcal enterotoxin T	95	521	atgaaaaaatgtattttatttatatttttctataatttttattagtttcaatttctactttatttatacccaaatgctaagtctgattctcgtgaaggtttaaaagactttttattcaaaaaaaatagatgtatacacaaataaaaaaataaatgaaaaagataaatatagcgttgatattgaagttgacaattatgtttatcgaatcaatacattagacgataaaatattaaaccaatttaaggttggtgattatgtagatgcttggggacatataataaataataaacccattggcaaagtaattaaatttatgatggagatattagtaaacatagccctttagacaagcctacaaatatatcttatcgaattaatttatttaaagaacaaaagcaaacagaagtaacacccgaaaaagagttagaaataggaaatagatatttaactatgaaacagattgattatcgaattaaaagttatttagtaaaaaaacaacagttatatactgaattctacaatggaaaaattgaaataagcatgttagatggaaaaaacattttattgatttgagtacatactattatgatcctagcaacttttacacttgattacacaaaaatgaagtcattttgatatatatatggaaaaatag
Enterotoxin	seu	staphylococcal enterotoxin U	92	629	atgaagttatttgcttttatcttcatatgtgttaagtcttgcagcttactatttatgttaaatggcaatcctagaccagaacaattgaataaagcgagtgaattccactggtctaatggataatatgaggtatttgtatgacgataaacacgtatcagaaacaaacattaaagcccaagagaagtttttacaacatgatttattatttaaaataaatggctctaaaattgatggttctaaaatttaaaaacagaatttaatataaaagcctttcggataaatacaaaaataaaaacgtagatttgtttgggacaaactattataatcaatgctatttttcagcggataatatggaattaaatgatggtagactaattgaaaaaacgtgtatgtatggcggtgtgaccgagcatgatggaaatcaaatagatataaaaatatctaactgataactctcataatatcttaattaaagtatacgaaaacgaaagaaacacattatcttttgatatatctaccaatagaaaaacataacagcacaagaaatagattataaagttagaaactatttacttaagcataaaaatttatatgaatttaatagttcgccttatgagactggctatataaagtttatcgaaggaaatagtcattcattttggtatgatatgatgcctgaatctggtgaaaaattttatccgactaaatatttactaatttataatgataataagacagttgagagtaaatctattaatgtagaagttcatttaaccaaaaaataa

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
Enterotoxin	sey	staphylococcal enterotoxin Y	95	533	atgaaagcgaaactatggtttttattgacaactttggcatttttgattgcagtaacggaatctattggaatagcagaagtaaaagcaaaaacaactggattgattacagaaaatagtaatgacagtttaaaagagcattatgcacaaaaatttgaagtttatacgaataaagaagtaacaggagttggggagaattatatagacacgaaagttgacacctacaatgtacggacagtgctctacaacactgatatatttgaaacagtttaaaaatcaagacaaagttaatatatggggaacattatatgaaaaccaacaatctaaagtatatcgtggcacagtagttaagtatgatcctatatcaaaagtaactaacttatctatatagaatgaacttgtttgttaacggtcatcaaactaaagtgaatccagacagtttattagaagttaagaataaacagatttctctaaaagaaacagatttttagaattagaaaatatttattagaaaaagaacacctatatagtaattacaattcaggggaactaattatagaaatgaaaaatggagcaagacatataaatagatttaggcgacatattgagtgactcacaagaaaaaacgtttgattttgataatattagtcatatagatatttatatgaaatag
Exoenzyme	aur	aureolysin	95	1224	gtgaggaaattttcaagatatgcatttacaagtatggcagcagtaacattgttgagcactttatcaccagcagcattagcgattgattcaaaaaataaaccagctaattctgatattaaatttgaggtgactcaaaagagtgatgcggtcaaagcattaaaagaattgcctaaatccgaaaatgtaaaaaatatttatcaagattacgctgttactgatgtaaaaactgataaaaaaggatttacgcattatacattgcaaccgagttgttgatggtgttcatgcacctgacaaagaagtgaaagtacacgcagacaaatcaggaaaagtcgttttaatcaatggggatactgatgcgaagaaagtaaagccaacgaataaagtgacattaagtaaagatgacgcagccgacaaagcatttaaagcagttaagattgataagaataaagcgaaaaatcttaaagataaagtcattaaagaaaacaaagttgaaatcgatggtgacagtaataaatacgtttatatgttgagttaattacagtgacaccagaaatttcacattggaaagttaaaattgatgctcaaactggcgaaattttagaaaaaatgaacttagttaaagaagctgcagaaactggtaaaggaaaaggtgtacttggcgatacaaaagatatcaatatcaatagtattgacggtggatttagcctagaagatttaacgcatcaaggtaaattatcagcatttagctttaatgatcaaacaggtcaagcaacattgattactaatgaagatgaaaacttcgtaaaagatgagcaacgtgctggcgtagatgcaaattattacgctaaacaaacatatgattattacaaagacacatttggtcgtgaatcatatgacaaccaaggtagtccaattgtttcattaacgcatgttaataactacggtggtcaagataacagaaataatgccgcatggattggtgacaaaatgatctatggtgatggtgatggtcgcacattcacaagtttatcgggtgcaaatgacgtagtagcacacgaattaacacacggtgtgacacaagagactgcaaacttagaatataaggaccagtcaggcgctctaaatgaaagcttttcagatgttttggatactttgtagatgacgaggatttcttaatgggtgaagatgtctacacacctggaaaagagggagacgctttacgcagcatgtcaaacccagaacaatttggtcaaccagctcatatgaaagactatgtattcactgaaaaagataatggtggcgtacatacgaattctggaattccaaataaagcagcttataacgtgattcaagcaatagggaaatctaaatcagaacaaatttactaccgagcattaacggaatacttaacaagtaattcaaacttcaaagattgtaaagatgcattataccaagcggccaaagatttatatgacgagcaaacagctgaacaggtgtatgaagcatggaatgaagtaggcgtggagtaa
Exoenzyme	coa	staphylocoagulase	75	956	atgaaaaagcaaataatttcgctaggcgcattagcagttgcatctagcttatttacatgggataacaaagcagatgcgatagtaacaaaggattatagtgggaaatcacaagttaatgctgggagtaaaaatgggaaacaaattgcagatggatattattggggaataattgaaaatctagaaaaccagttttacaatatttttcatttactggatcagcataaatatgcagaaaaagaatataaagatgcagtagataaattaaaaactagagttttagaggaagaccaatacctgctagaaagaaaaaagaaaaatacgaaatttatataaagaactatataaaaaatacaaaaaagagaatcctaatactcaagttaaaatgaaagcatttgataaatacgatcttggcgatttaactatggaagaatacaatgacttatcaaaattattaacaaaagcattggatgaactttaagttagaagtaaagaaaattgaatcagagaatccagatttaaaaccatattctgaaagcgaagaaagaacagcatatggtaaaatagattccttgttgatcaagcatatagtgtatattttgcctacgttacagatgcacacaatcaaaacagaagcattaaatcttagggcgaaaattgatttgattttaggtgatgaaaaagatccaattagagttacgaatcaacgtactgaaaaagaaatgattaaagatttagaatctattattgatgatttcttcattgaaaccaagttgaatagacctaaacacattactaggtatgatggaactaaacatgattaccatataacataaagatggatttgatgctctagttaaagaaacaagagaagcggttgcaaaggctgacgaatcttggaaaaataaaactgtcaaaaaatacgaggaaactgtaacaaaatctccagttgtaaaagaagagaagaaagttgaagaacctcaatcacctaaatttgataaccaacaagaggttaaaattacagttgataaagctgaagaaacaacacaaccagtggcacagccattagttaaaattccacagggcacaattacaggtgaaattgtaaaaggtccggaatatccaacgatggaaaataaaacgttacaaggtgaaatcgttcaaggtccagatttcccaacaatggaacaaaacagaccatctttaagcgataattatactcaaccgacgacaccgaaccctatttagaaggtcttgaaggtagctcatctaaacttgaaatataaaccacaaggtactgaatcaacgttaaaaggtactcaaggagaatcaagtgatattgaagttaaacctcaagcatctgaaacaacagaagcatcacattatccagcaagacctcaatttaacaaaacacctaaatatgttaaatatagagatgctggtacaggtatccgtgaatacaacgatggaacatttggatatgaagcgagaccaagattcaataagccatcagaaacaaacgcatacaacgtaacgacaaatcaagatggcacagtaacatatggcgctcgcccaacacaaaacaaaccaagcaaaacaaatgcatacaacgtaacaacacatgcaaatggtcaagtatcatatggcgctcgcccgacacaaacaagccaagcaaaacaaatgcatataacgtaacaacacatgcaaatggtcaagtatcatacggagctcgcccgacacaaaacaagccaagcaaaacaaatgcatataacgtaacaacacacgcaaacggtcaagtgtcatacggagctcgcccgacatacaagaagccaagtaaaacaaatgcatacaatgtaacaacacatgcagatggtactgcgacatatgggcctagagtaacaaaataa

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
Exoenzyme	geh	lipase	95	1658	atgttaagaggacaagaagaaagaaagtatagtattagaaagtattcaataggcgtggtgtcagtgttagcggctacaatgtttgttgtgtcatcacatgaagcacaagcctcggaaaaaacaccaac-taatgcagcggtacaaaaagaaacactaaatcaaccgggagaacaagggaatgcgataacgtcacatcaaatgcagtcaggaaagcaattagacgatatgcataaagagaatggtaaaagtg-gaacagtgacagaaggtaaagatacgcttcaattatcgaagtatcaatcaacacaaaatagtaaaacaatcagaacgcaaaaatgataatcaagtaaagcaagattctgaacgacaaggttctaaa-cagtcacaccaaaataatgcgactaataatactgaacgtcaaaatgatcaggttcaaaatacccatcatgctgaacgtaatggatcacaatcgacaacgtcacaatcgaatgatgttgataaatca-caaccatccattccggcacaaaaggttatacccaatcatgataaagcagcaccaacttcaactacaccccgtctaatgataaaactgcacctaaatcaacaaaagcacaagatgcaaccacggga-caaacatccaaatcaacaagatacacatcaacccgcgcatcaaatcatagatgcaaagcaagatgatactgttcgccaaagtgaacagaaaccacaagttggcgatttaagtaaacatatcgatggt-caaaattccccagagaaaccgacagataaaaatactgataataaacaactaatcaaagatgcgcttcaagcgcctaaaacacgttcgactacaaatgcagcagcagatgctaaaaaggttcgac-cacttaaagcgaatcaagtacaaccacttaacaaatatccagttgtttttgtacatggatttttaggattagtaggcgataatgcacctgctttatatccaaattattggggtggaaataaatttaaagt-tatcgaagaattgagaaagcaaggctataatgtacatcaagcaagtgtaagtgcatttggtagtaactatgatcgcgctgtagaacttattattacattaaaggtggtcgcgtagattatggcgcag-cacatgcagctaaatacggacatgagcgctatggtaagacttataaaggaatcatgcctaattgggaacctggtaaaaaggtacatcttgtagggcatagtatgggtggtcaaacaattcgtttaatg-gaagagtttttaagaaatggtaacaaagaagaaattgcctatcataaagcgcatggtggagaaatatcaccattattcactggtggtcataacaatatggttgcatcaatcacaacattagcaacac-cacataatggttcacaagcagctgataagtttggaaatacagaagctgttagaaaaatcatgttcgctttaaatcgatttatgggtaacaagtattcgaatatcgatttaggattaacgcaatggggctttа-aacaattaccaaatgagagttacattgactatatataaaacgcgttagtaaaagcaaaatttggacatcagacgacaatgctgccatgatttaacgttagatggctctgcaaaattgaacaacatgacaag-tatgaatcctaatattacgtatacgacttatacaggtgtatcatctcatactggtccattaggttatgaaaatcctgatttaggtacatttttcttaatggatacaacgagtagaattattggtcatgatg-caagagaagaatggcgtaaaaatgatggtgtcgtaccagtgatttcgtcattacatccgtccaatcaaccatttgttaatgttacgaatgatgaacctgccacacgcagaggtatctggcaagttaaac-caatcatacaaggatgggatcatgtcgattttatcggtgtggacttcctggatttcaaacgtaaaggtgcagaacttgccaacttctatacaggtattataaatgacttgttgcgtgttgaagcgactgaaag-taaaggaacacaattgaaagcaagttaa
Exoenzyme	hysA	hyaluronate lyase	90	1958	atgacatatagaataaagaaatggcaaaaattatccaccattacgttattaatggctggtgtgattactttgaatggtggtgaattcagaagtattgataaatatcaaatcgctgtggctgatac-gaatgttcaaacgccagattatgaaaagttgaggaacacatggctggacgttaactatggttatgataagtatgatgagaagaatgacgcaatgaagaagaagtttgaggctacggagaatgag-gcaaagaaattacttagtgagatgaaaactgaaagtgataggaaatacttgtgggaaaactcaaaagatttagatacgaagtctgcggatatgactcgtacctatcgtaatattgagaaaatcg-cagaagcgatgaagcataaagatactaagttaaaaatagatgaaaacaagaagaaagtgaaagatgcccttgagtggctgcataaaaatgcatatggaaaagaaccagataaaaaagttgct-gatttgacctcaaactttaaaaataaaacttctagaaatacccaatttaaattggtgggattatgaaattggaacacctagagcattaacaaatacccttatattgctgaatgatcaatttttcaaatgac-gaaaagaaaaaatacactgcccctatcaaaactttcgcccagaaagtgacaaaatattatcatcagtaggacaacctgaacaagctaaaggggaaatttagtagacattgctaaagtaaaacttt-ttagaaagtattatcgaagaagataaagatatаactaaaaattcaatagatgcatttaataaagtcttcacttacgttcaaagtaatgcaactggtaaagaacgtaatggattctatаaagacggctct-tatattgatcatcaagacgtcccatacactggtgcttatggcgttgtactcttagaggggatttctcaaatgatgccgatgataaaagaaacacctttaatgataaaacccaaaatgatacaacctta-aagtcatggattgacgacggatttatgccactcatttataaaggtgaaatgatggatttatcaagaggtagagctatcagtcgtgaaaatgaaacgagtcatcagcgtcagcgactgtaatgaaatcatt-gttgagattgagtgatgccatggatgattcaacaaaagctaagtataagcaaatcgttaaaacttctgttaaatctgattcaagttacggccaaaatgatacattaagctcttattcggatataagcaaaat-gaagtctttaatggaagacagcactatttctactaacggtttaacacaacaacttaaaatatatatgacatggatcgtgtcacctatcacaataaagacttagactttgcatttggtttaagtatgacgtc-gaaaaacgtcgcacgttacgaaagtatcaacggagagaacttaaaaggttggcacactggtgctggaatgtcttatttatacaatagcgatgtgaaacactaccgtgataacttctgggcgacagct-gatatgaaacgtttagcaggtacaacaacttagaaaatgaagaacctaaaggtacggatgttaaaaagtcgagtaaaactttgtaggcggaacaaaattcgatgaccaacatgctagtatcg-gaatggattttgaaaatcaggacaaaactttaactgccaaaaatcatatttcatattaacgataaaattgtcttcttaggaactggcattaaaagtactgattcatcaagaatccagttacaacgatt-gaaaatcgcaaagcgaatgggtatacgttatatacagacgataaacaaacaaccgcttccgataatcagggaaccaattccgtctttttagagtcaaccaataaacctaaaaacaatatagggctat-cattttttaaacgaatcgaaaataactgtaaaaaaagaaagtcatactggtaagtggagtgatataaataaaagtcaaaagcaagacagtaaaactaatcaatattatgaagtaactcaaaagcattc-taatacagatagtaaatatgcatatgtgttgtatccaggcttatctaaagatgactttaatacaaaaaaagataaagtaactgtcgttaaacaagatgatgacttccacgttgtgaaagataatgaatcg-gtttgggctggtgttaattacagcgatagcactcaaacttttatcattaacaacactaaagtcgaagttaaagcaaaaggtatgtttatacttaaaaagaaagatgataaaacttatgaatgtagcttc-tataatcccgaatctacaaatacccgcttcagatattgaatctaaaatttcaatgactggatactctattacaaacaaaaatacgtcgacttctaatgaatccggcgtgcgctttgaattacaacaaacat-taaataaagatgataattaa

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
Exoenzyme	sak	staphylokinase	95	392	atgctcaaaagaagtttattatttttaactgttttattgttattattctcattttcttcaattactaatgaggtaagtgcatcaagttcattcgacaaaggaaaatataaaaaaggcgatgacgcgagttattttgaaccaacaggcccgtatttgatggtaaatgtgactggagttgatggtaaaggaaatgaattgctatcccctcattatgtcgagtttcctattaaacctgggactacacttacaaaagaaaaaattgaatactatgtcgaatgggcattagatgcgacagcatataaagagtttagagtagttgaattagatccaagcgcaaagatcgaagtcacttattatgataagaataagaaaaaagaagaaacgaagtctttccctataacagaaaaaggttttgttgttcccagatttatcagagcatattaaaccctggattcaacttaattacaaaggttgttatagaaaagaaataagatga
Exoenzyme	sspA	staphopain A / V8 protease / serine protease	90	787	atgaaaggtaaatttttaaaagttagttctttattcgttgcaactttgacaacagcgacacttgtgagttctccagcagcaaacgcgttatcttcaaaggctatggacaatcatccgcaacaatcacagtcaagcaaacagcaaacacctaagattcaaaaaggcggtaaccttaaaccattagaacaacgtgaacacgcaaatgttatattaccaaataacgatcgtcaccaaatcacagatacaacgaatggtcatatgcacccgtaacttatattcaagttgaagcacctactggtacatttattgcttccggtgtagttgtaggtaaagatactcttttaacaaataaacacgtcgtagatgctacgcacggtgatcctcatgcttaaaagcattcccttctgcaattaaccaagacaattatccaaatggtggtttcactgctgaacaaatcactaaatattcaggcgaaggtgatttagcaatagttaaattctcccctaatgagcaaaacaacatattggtgaagtagttaaaccagcaacaatgagtaataatgctgaaacacaagttaaccaaaatattactgtaacaggatatcctggtgataaacctgtagcaacaatgtgggaaagtaaaggaaaaatcacttacctcaaaggcgaagctatgcaatatgatttaagtacaactggtggtaactcaggttcacctgtatttaatgaaaaaaatgaagtgatcggaattcattggggcggtgtaccaaatgaatttaatggtgcggtatttattaatgaaaatgtacgcaacttcttaaaacaaaaattgaagatatccatttgccaacgatgaccaacctaataacccagataatcctgataaccctaacaatcctgataaccctaacaaccctaacaaccctgataatccagacaatggcgataacaataatttcagacaaccctgacgctgcataa
Exoenzyme	sspB	staphopain B / cysteine protease	95	946	atgaatagttcatataaatctagagtattcaatattataagcatcataatggtttcaatgcttattttatcactaggcgcatttgctaataacaataaagccaaagccgattcacactctaaacagctagaaattaatgttaagagtgacaaagtacctcaaaaagtaaaagatctagcacaacaacaatttgctggttatgctaaagcattagataaacaaagtaatgcaaaaactggtaaatatgaacttggcgaagctttaaaatttataaatttaatggtgaagaagataatagttactattatccagttatataaagacggtaaaattgtttatactttaacacttagcccttaaaaataaagatgatttaaacaaatcaaaagaagacatgaattacagtgttaaaatttcaaacttcatcgctaaagatttagaccaaattaaagataaaaattcaaatatcactgttcttactgatgaaaaaggattttattttgaagaagatggcaaagttagattagtaaaagctacgccactacctggtaatgtaaaagaaaaagaagtgctaaaacagtttcatcaaaattgaaacaagagttaaaaaatacagtaacacctactaaagttgaagaaaacgaagcgatacaagaagatcaagttcaatatgaaaatacattaaaaaacttcaaaattagagaacaacaattcgataactcatggtgtgcaggatttagtatggcagcattattaaatgcaactaaaaatacagacacttataatgcacatgatattatgcgtacattatacccctgaagtaagtgagcaagaccttcctaattgctcaacattcccttaatcaaatgattgaatacggtaaatcacaaggcagagatattcattatcaagaaggcgtaccatcatatgaacaagttgatcaacttacaaaagataatgtaggaattatgattcttgcacaaagtgtatctcaaaaccctaatgatccacatttaggacatgccctagcagttgttggtaatgctaaaattaatgaccaagaaaaacttatttactggaatccttgggatacagaattatcaatccaagatgcagattcaagcctattacatttatcattcaatcgtgattataactggtatggttcaatgataggttactaa
Exoenzyme	sspC	staphopain C / cysteine protease	95	264	atgtatcaactacaatttataaatttagtttacgacacaaccaaactcacacatctagaacaaaccaatatcaatttattcattggtaattggagtaatcatcaattacaaaaatcaatttgtatacgtcatggcgatgatacaagtcacaatcaatatcatattctttttatagatacggcacatcaacgcattaaattttcatctattgataatgaagaaatcatttatattcttgattatgatgatacacagcatatcctcatgcaaacgtcatccaaacaaggtattggcacttcgcgcccaatcgtttatgagcgcttagtataa
Exoenzyme	vWbp	von Willebrand factor-binding protein	90	787	ttgaaaaataaattgctagttttatcattgggagcattatgtgtatcacaaatttgggaaagtaatcgtgcgagtgcagtggtttctggggagaagaatccatatgtatctgagtcgttgaaactgactaataataaaaataaatctagaacagtagaagagtataagaaaagcttggatgatttaatatggtccttccaaacttagataatgaaagatttgataatcctgaatataaagaagctatgaaaaaatatcaacagagatttatggctgaagatgaggctttgaagaaatttttagtgaagagaaaaaatataaaaatggaaatactgataatttagattatctaggattatctcatgaaagatatgaaagtgtatttaatactttgaaaaaacaaagtgaggagttcttaaaagaaattgaagatataaaaaagatacccctgaattgaaagactttaatgaagaggagcaattaaagtgcgacttagaattaaacaaattagaaaatcagatattaatgttaggtaaaacattttatcaaaactatagagatgatgttgaaagtttatatagtaagttagatttaattatgggatataaagatgaagaaagagcaaatataaaaagcagttaacaaaaggatgttagaaaataaaaaagaagacttagaaaccataattgatgaatttttagtgatatagataaaacaagacctaataatattcctgttttagaagatgaaaaacaagaagagaaaaatcataaaaatatggctcaattaaaatctgacactgaagcagcaaaaagtgatgaatcaaaaagaagcaagagaagtaaaagaagtttaaatactcaaaatcacaaacctgcatctcaagaagtttctgaacaacaaaaagctgaatatgataaaagagcagaagaaagaaagcgagatttttggataatcaaaaaattaagaaaacacctgtagtgtcattagaatatgattttgagcataaacaacgtattgacaacgaaacgacaagaaacttgtggtttctgcaccaacaaagaaaccaacatcaccgactacatatactgaaacaacgacacaggtaccaatgcctacagttgagcgtcaaactcagcaacaaattatttataatgcaccaaaacaattggctggattaaatggtgaaagtcatgatttcacaacaacgcatcaatcaccaacaacttcaaatcacacgcataataatgttgttgaatttgaagaaacgtctgctttacctggtagaaaatcaggatcactggttggtataagtcaaattgattcttctcatctaactgaacgtgagaagcgtgtaattaagcgtgaacacgttagagaagctcaaaagttagttgataattataaagatacacatagttataaagaccgaataaatgcacaacaaaaagtaaatactttaagtgaaggtcatcaaaaacgttttaataaacaaatcaataaagtatataatggcaaataa

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
Immune evasion	adsA	adenosine synthase A	95	1865	atgaaagctttattacttaaaacaagtgtatggctcgttttgctttttagtgtgatgggattatggcaagtctcgaacgcggctgagcagcatacaccaatgaaagcacatgcagtaacaacgatagacaaagcaacaacagataggcaacaagtaccgccaacaaaggaagcggctcatcattatggtgaagaagcggcaaccaacgtatcagcatcagcgcagggaacagctgatgatacaaacaacaaagtaacatccaacgcaccaactaacaaaccaactacagcagtttcaacaacaggaaacaaaagacacgatgtagatgcacaacaagcctcaacacaaaaaccaactcacacagcaacattcaaattatcaaatgctaaaacagcatcactttcaccacgaatgtttgctgctaatgtaccacaaacaacacacatcaaaatattacatacaaatgatatccatggccgactagccgaagaaaaagggcgtgtcatcggtatggctaaattaaaaacagtaaagagaacaagaaaagcctgatttaatgttagacgcaggagacgccttccaaggtttaccactttcaaatcaatctaaaggtgaagaaatggctaaagcaatgaatgcagtaggttatgatgctatggcagtcggtaaccatgaatttgactttggatacgatcagttgaaaaagttagagggtatgttagacttcccgatgctaagtactaacgtttataaagatggaaaacgcgcgtttaagccttcaacgattgtaacgaaaaatggtattcgttatggaattattggcgtaacgacaccagaaacaaagacgaaaacaagacctgaaggcattaaaggtgttgaatttagagatccattacaaagtgtgacagcggaaatgatgcgtatttataaagatgtagatacatttgttgttatatctcatttaggtattgatccttcaacacaagaaacatggcgtggcgattacttagtgaaacaattaagtcaaaatccacaattgaagaaacgtattacagtgattgacggtcattcacataccgtacttcaaaatggtcaaatttatatacaatgatgcattagtacaaacaggtacagcacttgcgaatatcggtaagattacatttaattatcgcaatggagaagtatcgaatattaagccgtcattgattaatgttaaagacgttgaaaatgtaacaccgaacaaagcattagctgaacaaattaatcaagctgatcaaacatttagagcacaaacagcagaggttattattccaaataatacgattgatttcaaaggagaaagagatgacgttagaacgcgtgaaacaaatttaggaaacgcgattgcagatgctatggaagcatatggcgttaagaatttctctaaaaagactgactttgccgtgacaaatggtggaggtattcgtgcctctatcgcaaaaggtaaggtgacacgctatgatttaatctcagtattaccatttggaaatacgattgcgcaaattgatgtaaaggttcagacgtctggacggctttcgaacatagtttaggtgcaccaacaacacaaaaagacggtaagacagtgttaacagcgaatggcggttttattacatatctctgattcaattcgtgtttactatgatatgaataaaccgtctggcaaacgaattaatgctattcaaatttaaataaagagacaggtaagtttgaaagtattgatttaaaacgtgtatatcatgtaacgatgaatgacttcacagcatcaggtggagacggatatagtatgttcggtggccctagagaagaaggtatttcattagatcaagtactagcaagttatttaaaaacagctaacttagctaagtatgatatgacagaaccacaacgtatgttattaggtaaaccagtagcaagtgaacaaccagctaaaggacaacaaggtagcaaaggtagtgagtctggtaaagatacacaaccaattggtaaagagaaagtgatgaatccagcgaaacaaccagcgacagggtaaagttgtgttgttaccagcgcatagaggaactgttagtagcggtacagaaggttctgatcgcgcattagaaggaactgctgtatcaagtaagagtgggaacaattggctaacatgtcagcgcctaaaggtagcgcacatgagaaacagttaccaaaaactggaactgatcaaagttcaagcccagcagcgatgtttgtattagtaacaggtatagggtttaatcgcgactgtacgacgtagaaaagctagctaa
Immune evasion	chp	chemotaxis inhibitory protein	95	200	atgtgtataagagacagagcaacaacagttttagcattaagtttttttaacggcaggaatcagtacacaccatcattcagcgaaagctttttacttttgaaccgtttcctacaaatgaagaaatagaatcaaataagaaaatgttagagaaagaaaaagcttatataagaatcatttaaaaatagtggtcttcctacaacgctaggaaaattagatgaacgtttgagaaattatttaaagaaaggcacaaaaaaattctcgctcaatttgaaaaaatggttattttaactgaaaataaaggttactatacagtatatctgaatacaccacttgctgaagatagaaaaaatgttgagttactaggtaaaatgtataaaacatacttctttaaaaaaggagagtctaaatcatcttatgtaattaatggtcctggaaaaactaatgaatatgcatactaa
Immune evasion	sbi	staphylococcal binder of immunoglobulin	90	1050	atgaaaaataaatatatctcgaagttgctagttggggcagcaacaattactttagctacaatgatttcaaatggggaagcaaaagcgagtgaaaacacgcaacaaacttcaactaagcaccaaacaactcaaaacaactacgtaacagatcaacaaaaagcttttatcaagtattacatctaaaaggtatcacagaagaacaacgtaaccaatacatcaaaacattacgcgaacacccgaacgtgcacaagaagtattctctgaatcacttaaagacagcaagaacccagaccgacgtgttgcacaacaaaacgcttttacaatgttcttaaaaatgataacttaactgaacaagaaaaaaatattacattgcacaaattaaagaaaaccctgatagaagccaacaagtttgggtagaatcagtacaatcttctaaagctaaagaacgtcaaaatattgaaaatgcggataaagcaattaaagatttccaagataacaaagcaccacacgataaatcagcagcatatgaagctaactcaaaattacctaaagatttacgtgataaaaataaccgctttgtagaaaaagtttcaattgaaaaagcaatcgttcgtcatgatgagcgtgtgaaatcagcaaatgatgcaatctcaaaattaaatgtaaagattcaattgaaaacagacgtttagcacaacgtgaagttaacaaagcacctatggatgtaaaagagctatttacagaaacaattagacgcattagtagctcaaaagatgctgaaaagaaagtggcgccaaaagttgaggctcctcaaattcaatcaccacaaattgaaaaacctaaagcagaatcaccaaaagttgaagtccctcaaatccaatcaccaaaagttgaggttcctcaatctaaattattaggttactaccaatcattaaaagattcatttaactatggttacaagtatttaacagatacttataaaagctataaagaaaaatatgatacagcaaagtactactataatacgtactatataatacaaaggtgcgattgacaaagctgtattaacttttacttggcgatggttctaaatcttatatccaaccattgaaagttgatgataaaaatggctatttagctaaatcatatgcacaagtaagaaactatgtaactgagtcaatcaatactggtaaagtattatatactttctaccaaaacccaacattagtaaaaacagctattaaagctcaagaaactgcatcatcaatcaaaaatacaataactggattatttaactcattctggaaataa
Immune evasion	scn	staphylococcal complement inhibitor	95	276	atgacaacacaaatgaaaatcaaaacatatttagttgctggtattaaagcggcgctccttgatacgactggtattaaattagcaagcaaatctgaaactacatcacatacgtatcaacatcaagcgcttgtagatcaattatatgaattaatagcaaacactgacttaaataaattatcgtacctaaatttagatgcgtttcaaaaacgcgatattttagctgcgcactatattgcaaaatcagctatacgcactaaaaatttggatcaaatgactaaagcgaaacatagattagaaagtatttacgattcaatttctaacccttgcattcacaaaaaaattaa

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
MSCRAMM	clfA*	clumping factor A	85	1368	atgaatatgaagaaacaagaaaaacacgcaattcgtaaaaaatcgattggcgtggcttcagtgcttgtaggtacgttaatcggttttggactactcagcagtaaagaagcagatgcaagtgaaaatagtatgacacaaacggaaaatacgagtaatgagagcaaaagtaatgatccaagtagcgttaatgctgcacctaaaacagacaacacaaacgtgagtgattctaatacaacgacaaacactaatagtgacgaaacgaatgtagcgcaaaatccagcacaacaggaaacgacacaatcagcatcaacaaatgcaactacagaagaaacaccggtaactggtgaagttactactacggcaacgaatcaagctaatacaccggcaacaactcaatcaagcaatacaaatgcggaagaatcagtgaatcaaacaagtaatgaaacgacttctaatgatactaatacagtatcatctgtaaattcacctcaaaattctacaaatgcggaaaatgtttcaacaacgcaagacatttcaactgaagcaacaccttcaaacaatgaatcagctccacagagtacagatgcaagtaataaagatgtagttaatcaagcggttaatacaagtgcgcctagaatgagagcatttagtttagcggctgtagctgcagatgcaccggctgctggcaaagatattacgaatcagttgacgaatgtgacagttggtattgactctggagatacagtttatccgcaccaagcaggctatgtcaaactgaattatgggttctcagtaccaaatgaggctgttcaaggtgacacattcaaaataactgtgcccaaagaattaaacttaaatggtgtaacttcaactgctaaagtgccaccaattatggccggagatcaagtattggcaaatggtgtaatcgatagtgatggtaatgttatttatacatttacagactatgtaaatactaaagatgatgttaaagcaactttgaccatgcccgcttatattgaccctgaaaatgttacaaagacaggtaatgtgacattggctactggcataggtagtacaacagcaaacaaaacagtattagtagattatgaaaaatatggtaagttttataacttatctattaaaggtacaattgaccaaatcgataaaacaaataatacgtatcgtcagacaatttatgtcaatccaagtggagataacgttattgcgccggttttaacaggtaatttaaaaccaaatacggatagtaatgcattaatagatgcacaaaatactagtattaaagtatataaagttgataatgcatcagacttgtctgaaagttattatgtgaatccagataactttgaagatgtcactgatagtgtgaatattacattcccaaatccaaatcaatataaagtagagttcaatacgcctgatgatcaaataacaacaccatatattgtagttgttaatgggcatattgatcctaatagtaaaggtgatttagctttacgttcaactttatatggatataattcgaatataatttggcgatcaatgtcatgggataatgaagtagcatttaataacggatcaggttctggtgacggtatcgataaacctgttgttcctgaacaacctgatgagccgggtgaaattgaaccaattccagag
MSCRAMM	clfB*	clumping factor B	90	1418	ttgaaaaaaagaattgattatttgtcgaataagcagaataagtattcgattagacgttttacagtaggtaccacatcagtaatagtaggggcaactatactatttgggataggcaatcatcaagcacaagcttcagaacaatcgaacgatacaacgcaatcttcgaaaaatagtcaagtgcagattccgaaaaaaacaatatgatagaaacacctcaattaaatacaacggctaatgatacatctgatattagtgcaaacacaaacagtgcgaatgtagatagcacaacaaaaccaatgtctacacaaacgagcaatacctactacaacagagccagcttcaacaaatgaaacacctcaaccgacggcaattaaaaatcaagcaactgctgcaaaaatgcaagatcaaactgttcctcaagaaggaaattctcaagtagataataaaacaacgaatgatgctaatagcatagcaacaaacagtgagcttaaaaattctcaaacattagatttaccacaatcatcaccacaaacgatttccaatgcgcaaggaactagtaaaccaagtgttagaacgagagctgtacgtagtttagctgttgctgaaccggtagtaaatgctgctgatgctaaaggtacaaatgtaaatgataaagttacggcaagtaatttcaagttagaaaagactacatttgaccctaatcaaagtggtaacacatttatggcggcaaattttacagtgacagataaagtgaaatcaggggattattttacagcgaagttaccagatagtttaactggtaatggagacgtggattattctaattcaaataatacgatgccaattgcagacattaaaagtacgaatggcgatgttgtagctaaagcaacatatgatatcttgactaagacgtatacatttgtctttacagattatgtaaataataaagaaaatattaacggacaattttcattacctttatttacagaccgagcaaaggcacctaaatcaggaacatatgatgcgaatattaatattgcggatgaaatgtttaataataaaattacttataactatagttcgccaattgcaggaattgataaaccaaatggcgcgaacatttcttctcaaattattggtgtagatacagcttcaggtcaaaacacatacaagcaaacagtatttgttaaccctaagcaacgagttttaggtaatacgtgggtgtatattaaaggctaccaagataaaatcgaagaaagtagcggtaaagtaagtgctacagataacaaactgagaatttttgaagtgaatgatacatctaaattatcagatagctactatgcagatccaaatgactctaaccttaaagaagtaacagaccaatttaaaaatagaatctattatgagcatccaaatgtagctagtattaaatttggtgatattactaaaacatatgtagtattagtagaagggcattacgacaatacaggtaagaacttaaaaactcaggttattcaagaaaatgttgatcctgtaacaaatagagactacagtattttcggttggaataatgagaatgttgtacgttatggtggtggaagtgctgatggtgattcagcagtaaatccgaaagacccaactccagggccgccggttgacccagaaccaagtccagacccagaaccagaaccaacgccagatccagaaccaagtccagacccagaaccggaaccaagcccagaccggatccg

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
MSCRAMM	cna*	collagen binding protein	95	1280	atgaacaagaacgtgttgaagtttatggtctttataatgttattaaatatcatcacgcctttatttaataaaaatgaagcattggcagcacgagatatttcatcaacgaatgttacagatttaactgtat-caccgactaagatagaagatggtggtaaaacgacagtaaaaatgacattcgacgataaaagtgtaaaaatacaaaatggtgacacgattaaagtggcatggccgacaagcggtacagtaaa-gatagagggttatagtaaaacagtaccattaactgttaaaggtgaacaggtgggtcaagcagttattacaccagacggagcaacaattacattcaatgataaagtagaaaaatttaagtgatgtttc-gggatttgcagaatttgaagtacaaggaagaaatttaacgcaaacaaatacttcagatgacaaagtagctacgataacatctgggaataaatcaacgaatgttacggttcataaaagtgaagc-gggaacaagtagtgttttctattataaaacgggagatatgctgccagaagatacgacacatgtacgatggttttaaatattaataatgaaaaaagatatgtatcgaaagatattactataaaggatca-gattcaaggtggacagcagttagatttaagcacattaaacattaatgtgacaggtacacatagcaattattatagtggatcaaatgcaattactgattttgaaaaagcttttccaggttctaagataactgtt-gataatacgaagaacacaattgatgtaacaattccacaaggctatgggtcatataatagtttttcaattaactacaaaaccaaaattacgaatgaacagcaaaaagagtttgttaataattcacaagcttg-gtatcaagagcatggtaaggaagaagtgaacgggaaatcatttaatcatactgtgcacaatattaatgctaatgccggtattgaaggtactgtaaaaggtgaattaaaagttttaaaacaggataaa-gataccaaggctcctatagctaatgtaaaatttaaactttctaaaaaagatggatcagttgtaaaggacaatcaaaaagaaattgagattataacagatgcaaacggtattgctaatattaaagcgtt-gcctagtggagactatattttaaaagaaatagaggcgccagcaccgtatacatttgataaggataaagaatatccgtttactatgaaagatacagataatcagggatattttacgactattgaaaatg-caaaagagatagaaaaaacaaaagatgtatctgctcaaaaggtttgggaaggcactcaaaaagtgaaaccaacgatttatttcaagttgtacaaacaagatgacaatcaaaacacaacaccagtagaga-caaagcagagattaaaaaattagaagatggaacgacaaaagtgacatggtctaatcttccagaaaatgacaaaaatggcaagactattaaatatttagttaaagaagtaaatgctcaaggtgaagaca-caacaccagaaggatatactaaaaaagaaaatggtttagtggttactaatactgaaaaaccaatcgaaa
MSCRAMM	eap-map	extracellular adherence protein / MHC analogous protein	80	1412	atgaaatttaagtcattgattacacaacttttagcattaggtgttttagcatcaacaggtgcaaacttaataataatgaagcgtctgccgcagctaagccattagataaatcatcaagttcgttacaccatg-gatattctaaagtccatgttccatatgcaatcactgtgaacggtacaagccaaaatatttttatcaagcttaacatttaataagaatcaaaatattagttataaagatttagaggatagagttaaatcagttt-taaaatcagacagaggtattagtgatatagatttaagactatcgaagcaagcgaaatatactgtttacttttaaaaatggaacaaagaaagttatcgatttgaaagtaggtatttacacagctgatttaatta-atacaagtgaaattaaagctattaatattaacgtagatactaaaaagcaagttgaagataaaaagaaagataaagcaaattaccaagttccatacacaatcactgtgaacggtacaagccaaaatatttt-tatcaaacttaacatttaataagaatcaaaatattagttacaaagatttagaggataaagttaaatcagttttagaatcaaatagaggtattaccgatgttgatttaagattatcgaagcaagc-gaaatatacagttaattttaaaaatggaacgaagaaagttatcgatttgaaatcaggtatttacacagcgaatttaataaattcaagtgatattaaaagtatcaatattaacgtagatacaaaaaaa-catatcgaaaataaagctaaaagaaactatcaagttccatattccaattaatttaaatggtacatctacaaacattttatcgaatctttcattttcaaataaaccttggacaaattacaaaaatttaactagt-caaataaaatcagtactgaagcatgatagaggtattagtgaacaagaatttaaaatatgctaagaaagcttattatactgtttattttaaaaatggtggtaaaagaatcttacagttgaattcaaaaaatta-cacagcaaacttagttcatgcgaaagatgttaagagaattgaaattactgttaaaacaggaactaaagcgaaagcagacagatatgtaccatacacaattgcagtaaatggcacatcaacaccaattt-tatcagatttaaaatttacaggtgacccacgtgtaggctacaaagatatctctaaaaaagttaaatcagtactgaagcatgatagaggtatcggggaacgtgaattaaaatatgcaaaaaaagctact-tacgcagtacattttaaaaatggaacgaaaaaagtgattaacataaatttcaaatattagccaactgaatctgctttatgttcaagatattaaaaagatagatattgatgttaaaacaggaactaaagc-gaaagcggatagctatgtaccatatacaattgcagtaaatggcacatcaacaccaatttttatcaaaacttaaaatttcgaataaacaattaattagttacaaatatttaaacgacaaagtgaaatctgtat-taaaaagtgaaagaggcatcagtgatcttgacttaaaatttgcgaaacaagcaaaatatacagtatatttcagaaatggaaagaaacaagtagtgaatttaaaatcagacatctttacacctaatttatt-tagtgccaaagatattaaaagattggtattgatgtaaaacaatacactaaatcaaaaaaaaataaataa
MSCRAMM	ebp	elastin binding protein	95	1200	atgtctaataattttaaagatgactttgaaaaaaatcgtcaatcgatagacacaaattcacatcaagaccatacggaagatgttgaaaaagaccaatcagaattagaacatcaggatacaatagaga-atacggagcaacagtttccgccaagaaatgcccaaagaagaaaaagacgccgtgatttagcaacgaatcataataaacaagttcacaatgaatcacaaacatctgaagacaatgttcaaaatgag-gctggcacaatagatgatcgtcaagtcgaatcatcacacagtactgaaagtcaagaacctagccatcaagacagtacacctcaacatgaagaggaatattatataagaatgcttttgcaatggata-aatcacatccagaaccaatcgaagacaatgataaacacgagactattaaagaagcagaaaataacacctgagcattcaacagtttctgataagagtgaagctgaacaatctcagcaacctaaac-catattttgcaacaggtgctaaccaagcaaatacatcaaaagataaacatgatgatgttaactgttaagcaagacaaagatgaatctaaagatcatcatagtggtaaaaaaggcgcagcaattggtgctg-gaacagcgggtgttgcaggtgcagctggtgcaatgggtgtttctaaagctaagaaacattcaaatgacgctcaaaacaaaagtaattctgacaagtcgaataactcgactgaggataaagtgtctcaa-gataagtctaaagatcatcataatggcaaaaaaggtgcagcgatcggtgctggaacagcaggtttggctggaggcgcagcaagtaaaagtgcttctgccgcttcaaaaccacatgcctctaataat-gcaagccaaaaccatgatgaacatgacaatcatgacagagataaagaacgtaaaaaaggtggcatggccaaagtattgttaccattaattgcagctgtactaattatcggtgcattagcgatatttg-gaggcatggcattaaacaatcatataataatggtacaaaagaaaataaaatcgcgaatacaaataaaaaataatgctgatgaaagtaaagacaaagacacatctaaagacgcttctaaagataaat-caaaatctacagacagtgataaatcaaaagaggatcaagacaaagcgactaaagatgaatctgataatgatcaaaacaacgctaatcaagcgaacaatcaagcacaaaatatcaaaatcaacaa-caagctaatcaaaatcaacaacagcaacaacaacgtcaaggtggtggccaaagacatacagtgaatggtcaagaaaacttataccgtatcgcaattcaatactacggttcaggttcaccggaaaatgtt-gaaaaaattagacgtgccaatggtttaagtggtaacaatattagaaacggtcaacaaatcgttattccataa

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
MSCRAMM	fnbpA	fibronectin binding protein A	80	1534	gtgaaaagcaatcttagatacggcataagaaaacacaaattgggagcggcctcagtattcttaggaacaatgatcgttgttggaatgggacaagaaaaagaagctgcagcatcggaacaaaa-caatactacagtagaggaaagtgggagttcagctactgaaagtaaagcaagcgaaacacaaacaactacaaataacgttaatacaatagatgaaacacaatcatacagcgcgacatcaactgag-caaccatcaaaatcaactcaagtaacaacagaagaagcaccaacaactgtgcaagcaccaaaagtagaaaccgaaatgaaatcacaagaagatttaccatcagaaaaagttgctgataaggaaac-tacaggaactcaagttgacatagctcaaccaagtaacgtctcagaaattaaaccaagaatgaaaagatcagctgacgttacagcagtttcagagaaagaagtagcggaagaagctaaagcga-caggtacagatgtaacaaataaagtggaagttactgaaagctctttagaaggacataataaagattcgaatattgttaatccgcataatgctcaaagagtaactttaaaatacaaatggaaatttg-gagaaggaattaaggcaggagattattttgatttcacattaagtgataatgttgaaacacatggtatatcaacactgcgtaaagttccggagataaaaagttcaacagaagataaagttatggcaaat-ggtcaagttataaatgaacgtacaattcgctatacatttactgattatataaatacaaaaaagatttaactgctgaatttaaacttaaacctattcattgacccaacaacagtgacaaagcaagggagt-caaaaagttgaagtaacactaggtcaaaataaagtctcaaaagaatttgatatcaaatatttagacggcgttaaagatagaatgggtgttactgttaatggtcgtattgatactttgaataaagaagaggg-taaatttagccatttttgcatatgtgaagcctaacaaccagtcgttaacttctgtcacagtaactggtcaagtaacatctggatataaacaaagtgctaataatccaacagtcaaagtatataaacacattg-gttcagatgaattagctgaaagtgtttatgcaaagcttgatgatcaccagtaaatttgaagatgtgactgaaaaagtaaatctatcttacacaagtaatggtgggtacacattgaaccttggcgatt-tagataattcgaaagactatgtaattaaatatgaaggtgaatatgatcaaaatgctaaggatctaaatttccgaacacatctttcaggatatcataaatactacccatactatcctattacccgtattatc-cagttcaattaacttggaacaacggtgttgcattttactctaataatgctaaaggcgatggtaaagataaaccaaatgatcctatcattgagaagagtgaaccaattgatttagacattaaatcagagccac-cagtggagaagcatgaattgactggtacaatcgaagaaagtaacgattctaagccaattgattttgaatatcatacagctgttgaaggtgcagaaggtcatgcagaaggtattattgaaactgaagaa-gattctattcatgtggattttgaagaatctacacatgaaaattcaaaacatcacgctgatgttgttgaatatgaagaggatacaaacccaggtggtggccaagtaacaactgagtctaacttagttgaattt-gacgaagagtctacaaaaggtattgtaactggcgcagtgagcgaccatacaacaattgaagatacgaaagaatatacgactgaaagtaatctgattga
MSCRAMM	fnbpB	fibronectin binding protein B	80	1670	gtgaaaagcaatcttagatacggcataagaaaacacaaattgggagcggcctcagtattcttaggaacaatgatcgttgttggaatgggacaagaaaaagaagctgcagcatcggaacaaaa-caatactacagtagaggaaagtgggagttcagctactgaaagtaaagcaagcgaaacacaaacaactacaaataacgttaatacaatagatgaaacacaatcatacagcgcgacatcaactgag-caaccatcacaatcaacacaagtaacaacagaagaagcaccgaaaactgtgcaagcaccaaaagtagaaacttcgcgagttgatttgccatcggaaaaagttgctgataaggaaactacaggaact-caagttgacatagctcaaccaagtaacgtctcagaaattaaaccaagaatgaaaagatcaactgacgttacagcagttgcagagaaagaagtagtggaagaaactaaagcgacaggtacagatgtaa-caaataaagtggaagtagaagaaggtagtgaaattgtaggacataaacaagatacgaatgttgtaaatcctcataacgcagaaagagtaaccttgaaatataaatggaaatttggagaaggaatta-aggcgggagattattttgatttcacattaagcgataatgttgaaactcatggtatctcaacactgcgtaaagttccggagataaaaagtacagatggtcaagttatggcgacaggagaaataattggaga-aagaaaagttagatatacgtttaaagaatatgtacaagaaaagaaagatttaactgctgaattatctttaaatctatttattgatcctacaacagtgacgcaaaaaggtaaccaaaatgttgaagtta-aattgggtgagactacggttagcaaaatatttaatattcaatatttaggtggagttagagataattgggggagtaacagctaatggtcgaattgatactttaaataaagtagatgggaaatttagtcatttt-gcgtacatgaaacctaacaaccagtcgttaagctctgtgacagtaactggtcaagtaactaaaggaaataaaccaggggttaataatccaacagttaaggtatatataaacacattggttcagacgatt-tagctgaaagcgtatatgcaaagcttgatgatgtcagcaaatttgaagatgtgactgataatatgagtttagattttgatactaatggtggttattctttaaactttaataatttagaccaaagtaaaaat-tatgtaataaaatatgaagggtattatgattcaaatgctagcaacttagaatttcaaacacacctttttggatattataactattattatacaagtaatttaacttggaaaaatggcgttgcattttactc-taataacgctcaaggcgacggcaaagataaactaaaggaacctattatagaacatagtactcctatcgaacttgaatttaaatcagagccgccagtggagaagcatgaattgactggtacaatc-gaagaaagtaatgattctaagccaattgattttgaatatcatacagctgttgaaggtgcagaaggtaccattgaaactgaagaagattctattcatgtggattttgaagaatcgaca-catgaaaattcaaaacatcatgctgatgttgttgaatatgaagaagatacaaacccaggtggtggtcaggttactactgagtctaacctagttgaatttgacgaagattctacaaaaggtattgtaactg-gtgctgttagcgatcatacaacaattgaagatacgaaagaatatacgactgaaagtaatctgattgaactagtagatgaactacctgaagaacatggtcaagcgcaaggaccaatcgaggaaattact-gaaaacaatcatcatatttctcattctggtttaggaactgaaaatggtcacggtaattatggcgtgattgaagaaatcgaagaaaatagccacgtggatattaagagtgaattaggttag

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
MSCRAMM	sdrC*	Ser-Asp rich protein C	85	1733	atgaataatgaaaagacagcaacaaatagaaaaggcatgataccaaatcgattaaacaaattttcgataagaaagtattctgtaggtactgcttcaattttagtagggacaacattgatttttgggt-taagtggtcatgaagctaaagcggcagaacatacgaatggagaattaaatcaatcaaaaaatgaaacgacagccccaagtgagaataaaacaactaaaaaagttgatagtcgtcaactaaaaga-caatacgcaaactgcaactgcagatcagcctaaagtgacaatgagtgatagtgcaacagttaaagaaactagtagtaacatgcaatcaccacaaaacgctacagctaatcaatctactacaaaaac-tagcaatgtaacaacaaatgataaatcatcaactacatatagtaatgaaactgataaaagtaatttaacacaagcaaaagatgtttcaactacacctaaaacaacgactattaaaccaagaactt-taaatcgcatggcagtgaatactgttgcagctccacaacaaggaacaaatgttaatgataaagtacatttttcaaatattgacattgcgattgataaaggacatgttaatcagactactggtaaaact-gaattttgggcaacttcaagtgatgttttaaaattaaaagcaaattacacaatcgatgattctgttaaagagggcgatacatttacttttaaatatggtcaatatttccgtccaggatcagtaagat-taccttcacaaactcaaaatttatataatgcccaaggtaatattattgcaaaaggtatttatgatagtacaacaaacacaacaacatatacttttacgaactatgtagatcaatatacaaatgttagag-gtagctttgaacaagttgcatttgcgaaacgtaaaaatgcaacaactgataaaacagcttataaaatggaagtaactttaggtaatgatacatatagcgaagaaatcattgtcgattatggtaata-aaaaagcacaaccgcttatttcaagtacaaactatattaacaatgaagatttatcgcgtaatatgactgcatatgtaaatcaacctaaaaatacatatactaaacaaacgtttgttactaatttaactg-gatataaatttaatccaaatgcaaaaacttcaaaatttacgaagtgacagatcaaaatcaatttgtggatagtttcaccctgatacttcaaaacttaaagatgttactgatcaattcgatgttatttatag-taatgataataaaacagctacagtcgatttaatgaaaggccaaacaagcagcaatacaacatacatcattcaacaagttgcttatccagataatagttcaacagataatggaaaaattgattatactt-tagacactgacaaaactaaatatagttggtcaaatagttattcaaatgtgaatggctcatcaactgctaatggcgaccaaaagaaatatcaatctaggtgactatgtatgggaagatacaaataaagatg-gtaaacaagatgccaatgaaaaagggattaaaggtgtttatgtcattcttaaagatagtaacggtaaagaattagatcgtacgacaacagatgaaaatggtaaatatcagttcactggtttaagcaatg-gaacttatagtgtagagttttcaacaccagccggttatacaccgacaactgcaaatgtaggtacagatgatgctgtagattctgatggactaactacaacaggtgtcattaaagacgctgacaacatga-cattagatagtggattctacaaaacaccaaaatatagtttaggtgattatgtttggtacgacagtaataaagatggtaaacaagattcgactgaaaaaggaattaaaggtgttaaagttacttgcaaaac-gaaaaaggcgaagtaattggtacaactgaaacagatgaaaatggtaaatacccgtttgataatttagatagtggtaaatacaaagttatctttgaaaaacctgctggcttaactcaaacaggtacaaata-caactgaagatgataaagatgccgatggtggcgaagttgatgtaacaattacggatcatgatgatttcacacttgataatggctactacgaagaagaaaca
MSCRAMM	sdrD*	Ser-Asp rich protein D	80	2713	atgctaaacagagaaaatataaacggcaataacaaggaaaggcatggtatcccaatcgattaaataaatttttcgattagaaagtacacagtgggaacagcatcaattttagtaggtacaacat-taatttttggtctggggaaccaagaagcaaaggctgcagaaagtactaataaagaattgaacgaagcgacaacttcagcaagtgataatcaatcgagtgataaagttgatatgcagcaactaaat-caagaagacaatactaaaaatgatatcaaaaagaaatggtatcatctcaaggtaatgaaacgacttcaaatgggaataaattaatagaaaaagaaagtgtacaatctaccactggaaataaagtt-gaagtttcaactgccaaatcagatgagcaagcttcaccaaaatctacgaatgaagatttaaacactaaacaaactatagtaatcaagaagcgttacaacctgatttgcaagagaataaatcagtg-gtaaatgttcaaccaactaatgaggaaaacaaaaaggtagatgccaaaactgaatcaactacattaaatgttaaaagtgatgctatcaagagtaatgatgaaactcttgttgataacaatagta-attcaaataatgaaaataatgcagatatcattttgccaaaaagtacagcacctaaacgtttgaatacaagaatgcgtatagcagcagtacagccatcatcaacagaggctaaaaatgttaatgatttaat-cacatcaaatacaacattaactgtcgttgatgcagataaaacaataaatcgtaccagcccaagatttattcattaaaatcacaaattacagttgatgacaaagttaatcaggtgattatttcacaat-taaatactcagatacagtacaagtatatggattgaatccggaagatattaaaaatattggtgatattaaagatccaaataatggtgaaacaattgcgactgcaaaacatgatactgcaaataatttaat-tacatatacatttacagattatgttgatcgatttaattctgtacaaatgggaattaattattcaatttatatggatgctgatacaattcctgttagtaaaaacgatgttgagtttaatgttacgatagggtaatac-tacaacaaaaacaactgctaacattcaatatccagattatgttgtaaatgagaaaaattcaattggatcagcgttcactgaaacagtttcacatgttggaaataaagaaaatccagggtactataaa-caaacgatttatgtaaatccatcggaaaattctttaacaaatgccaaactaaaagttcaagcttaccactcaagttatcctaataatatcgggcaaataaataaagatgtaacagatataaaaatatat-caagttcctaaaggttatacattaaataaaggatacgatgtgaatactaaagagcttacagatgtaacaaatcaatacttgcagaaaattacatatggcgacaacaatagcgctgttattgattttg-gaaatgcagattctgcttatgttgtaatggttaatacaaaattccaatatacaaatagcgaaagcccaacacttgttcaaatggctactttatcttcaacaggtaataaatccgtttctactggcaatgctt-taggatttactaataaccaaagtggcggagctggtcaagaagtatataaaattggtaactacgtatgggaagatactaataaaaacggtgttcaagaattaggagaaaaaggcgttggcaatgtaact-gtaactgtatttgataatacaaatacaaaagtaggagaagcagttactaaagaagatgggtcatacttgattccaaacttacctaatggagattaccgtgtagaatttcaaacttaccaaaaggt-tatgaagtaacccctcaaaacaaggtaataacgaagaattagattcaaacggcttatcttcagttattacagttaatggcaaagataacttatctgcagacttaggtatttacaaacctaaatacaact-taggtgactatgtctgggaagatacaaataaaaatggtatccaagaccaagatgaaaaaggtatatctggcgtaacggtaacattaaaagatgaaaacggtaacgttgttaaaaacagttacaacagac-gctgatggcaaatataaatttactgatttagataatggtaattataaagttgaatttactacaccagaaggctatacaccgactacagtaacatctggtagcgacattgaaaaagactctaatggtt-taacaacaacaggtgttattaatggtgctgataacatgacattagatagtggattctacaaaacaccaaaatatatttaggtaattatgtatgggaagatacaaataaagatggtaagcaggattcaact-gaaaaaggtatttcaggcgtaacagttacattgaaaaatgaaaacggtgaagttttacaaacaactaaaacagataaagatggtaaatatcaatttactggattagaaaatggaacttataaagtt-gaattcgaaacaccatcaggttacacaccaacacaagtaggttcaggaactgatgaaggtatagattcaaatggtacatcaacaacaggtgtcattaaagataaagatacgatactattgactctg-gtttctacaaaccgacttacaacttaggtgactatgtatgggaagatacaaataaaacggtgttcaagataaagatgaaaagggcatttcaggtgtaacagttacgttaaaagatgaaaacga-caaagttttaaaaacagttacaacagatgaaaatggtaaatatcaattcactgatttaaacaatggaacttataaagttgaattcgagacaccatcaggttatacaccaacttcagtaacttctggaaat-gatactgaaaaagattcttaatggtttaacaacaacaggtgtcattaaagatgcagataacatgacattagacagtggtttctataaaacaccaaaatatagtttaggtgattatgtttggtacgacagta-ataaagacggcaaacaagattcaactgaaaaaggtatcaaagatgttaaagttacttttattaaatgaaaaaggcgaagtaattggaacaactaaaacagatgaaaatggtaaatactgctttgataatt-tagatagcggtaaatacaaagttatttttgaaaagcctgctggcttaacacaaacaggtacaaatacaactgaagatgataaagatgcagatggtggcgaagttgacgtaacaattacggatcatgat-gatttcacacttgataatggctactacgaagaagaaaca

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
MSCRAMM	sdrE*	Ser-Asp rich protein E	95	2281	atgattaacagggataataaaaaggcaataacaaaaaagggtatgatttcaaatcgcttaaacaaatttttcgattagaaagtatactgtaggaactgcatcgattttagtaggtacgacattgatttttg-gtctagggaaccaagaagctaaagctgctgaaaacactagtacagaaaatgcaaaacaagatgatgcaacgactagtgataataaagaagtagtgtcggaaactgaaaataattcgacaa-cagaaaataattcaacaaatccaattaagaaagaaacaaatactgattcacaaccagaagctaaaaaagaatcaacttcatcaagtactcaaaaacagcaaaataacgttacagctacaactgaaac-taagcctcaaaacattgaaaaagaaaatgttaaaccttcaactgataaaactgcgacagaagatacatctgttatttttagaagagaagaaagcaccaaaataatacaaataacgatgtaactacaaaac-catctacaagtgaaccatctacaagtgaaattcaaacaaaaccaactacacctcaagaatctacaaatattgaaaattcacaaccgcaaccaacgccttcaaaagtagacaatcaagttacagatg-caactaatccaaaagaaccagtaaatgtgtcaaaagaagaacttaaaaataatcctgagaaattaaaagaatggttagaaatgatagcaatacagatcattcaactaaaccagttgctacagctccaa-caagtgttgcaccaaaacgtgtaaacgcaaaaatgcgctttgcagttgcacaaccagcagcagttgcttcaaacaatgtaaatgatttaattaaagtgacgaagcaaacaatcaaagttggcgatggta-aagataatgtggcagcagcgcatgacggtaaagatattgaatatgatacagagtttacaattgacaataaagtcaaaaaaggcgatacaatgacgattaattatgataagaatgtaattccttcggatt-taacagataaaaatgatcctatcgatattactgatccatcaggagaggtcattgctaaaggaacatttgataaagcaactaagcaaatcacatatacatttacagactatgtagataaatatgaagatata-aaatcacgcttaactctatattcgtatattgataaaaaaacagttccaaatgagacaagtttgaatttaacatttgctacagcaggtaaagaaacaagccaaaatgtcactgttgattatcaagatccaatg-gtccatggtgattcaaacattcaatctatctttacaaaattagatgaagataagcaaactattgaacaacaaatttattgttaacccattgaaaaaatcagcaaccaacactaaagttgatatagctggtagt-caagtagatgattatggaaatattaaactaggaaatggtagcaccattattgaccaaaatacagaaataaaggtttataaagttaactctgatcaacaattgcctcaaagtaatagaatctatgattttagt-caatacgaagatgtaacaagtcaatttgatataataaaaatcatttagtaataatgtagcaacattggattttggtgatattaattcagcctatattatcaaagttgttagtaaatatacacctacatcagatg-gcgaactagatattgcccaaggtactagtatgagaacaactgataaatatggttattataattatgcaggatattcaaacttcatcgtaacttctaatgacactggcggtggcgacggtactgttaaacct-gaagaaaagttatacaaaattggtgactatgtatgggaagacgttgataaagacggtgttcaaggtacagattcaaaagaaaaccaatggcaaacgttttagttacattaacttacccggacggtac-tacaaaatcagtaagaacagatgctaatggtcattatgaattcggtggtttgaaagacggagaaacttatacagttaaattcgaaacgccaactggatatcttccaacaaaagtaaatggaacaact-gatggtgaaaaagactcaaatggtagttcggttactgttaaaattaatggtaaagatgatatgtctttagatactggttttacaaagaacctaaatacaacttaggtgactatgtatgggaagatacta-ataaagatggtatccaagatgcaaatgagccaggaatcaaagatgttaaggttacattaaaagatagtactggaaaagttattggtacaactactactgatgcctcgggtaaatataaatttacagatt-tagataatggtaactatacagtagaatttgaaacaccagcaggttacacgccaacggttaaaaatactacagctgatgataaagattctaatggtttaacaacaacaggtgtcattaaagatgcagata-atatgacattagacagggggtttctataaaacaccaaaatacagtttaggtgattatgtttggtacgacagtaataaagacggcaaacaagattcaactgaaaaaggtatcaaagatgtgacagttacatt-gcaaaacgaaaaaggcgaagtaattggaacactaaaacagatgaaaatggtaaatatcgtttcgataatttagatagcggtaaatacaaagttatttttgaaaagcctgctggcttaacacaaacagt-tacaaatacaactgaagatgataaagatgcagatggtggcgaagttgacgtaacaattacggatcatgatgatttcacacttgataacggatacttcgaagaagataca
Surface/Adherence	efb	extracellular fibrinogen binding protein	89	400	atgaaaaataaattgatagcaaaatctttattaacaatagcggcaattggtattactacaactacaattgcgtcaacagcagatgcgagcgaaggatacggtccaagagaaaagaaaccagtgagtat-taatcacaatatcgtagagtacaatgatggtactttaaatatcaatctagaccaaaatttaactcaacacctaaatatattaaattcaaacatgactatataatattttagaatttaacgatggtacattc-gaatatggtgcgcgtccacaatttaataaaccagcagcgaaaactgatgcaactattaaaaaagaacaaaaattgattcaagctcaaaatcttgtgagagaatttgaaaaaacacatactgtcagtgca-cacagaaaagcacaaaaggcagtcaacttagtttcgtttgaatacaaagttaagaaaatggtcttacaagagcgaattgataatgtattaaagcaaggattagtaaaataa
Surface/Adherence	icaA	intracellular adhesion protein A	95	991	ttgcaatttttaacttttgctttttatcctgtatttatgtctatttactggattgtcggttcaatttatttctattttaccagagaaattagatattcattgaacaagaagcctgacataaatgtggat-gaattagaaggcattacatttttacttgcctgttataacgaaagtgcacgcgattgaagatacgttgtctaatgttcttgcactcaaatacgagaagaaagaaaattattatcattaatgatggaagttcag-ataatacagcagaactcatctataaaatcaaagaaaataatgactttatttttcgtcgatttacaagaaaacagaggtaaagccaacgcactcaatcaaggcattaaacaggcttcatatgatttatgta-atgtgcttggatgcagatactatcgttgatcaagatgcaccatattatatgattgagaatttcaaacatgatccaaaacttggtgcagttacaggtaatcctagaattcgaaataagagttctatttta-gtaaaattcaaacgatagaatatgcaagtttaattggctgtattaagcgaagtcagacacttgctggcgcagtcaatactatttcgggtgttcttcactcatttaaaaaaagtgcagttgtcgacgttg-gctactgggatactgatatgattaccgaagatattgcagtttcttggaaattgcatttacgtggatatcgtattaagtatgaaccgcttgccatgtgttggatgttggttccagaaacattgggaggtctttg-gaagcaacgcgtgagatgggctcaaggggacacgaagtattactacgagacttttttagcacaatgaaaacgaaaaggtttccttatatatttgatgtttgagcaaatcatctcgatttttatggg-tatatatagtgcttctatatttaggctatttgttcataacagcaaacttcttagactatacatttatgacatatagttttttcaatatttctactatcatcatttactatgactttataaacgttattcaatttacagtc-gcactctttattgatagtcgctacgagaaaaagaatatggctggactcatatttgtaagttggtatccgacagtatactggattattaacgcagcagtagttcttgtcgcatttccaaaagcattaaaacgta-agaaaggtggttacgcaacatggtcaagcccagacagagggaatacccaacgctaa
Surface/Adherence	icaB	intracellular adhesion protein B	95	698	gtgaagtatagaaaatttatatatttagtgttgagtatcttgatcatattgcctgtaagcacactggatggtcatcatattgcaaatgcagatgacgattcacctaaaaaactgaaatataaagaaaatagt-gctctggcattaaattatcaccgtgtaagaaaagcgaattttctgaataatttttatttacttctttttctagtagtaaagaaattaaaaattatagtgttagtcaatcacaatttgaatctcaaataaaatggcta-aaatcacatgatgctaaatttttaaccttgaaagaatttttatattacaagaaaaaaggtaagtttccaaaacgaagtgtatggattaactttgatgatatggatgaaactatttatgaaaatgcttatc-caatcttaaaaaaatataaaatacggcaactgggtttattatcacaggtcatgttggggagaaaactttcacaacctcgatatgattagtaaaaagaactaaaagaaatgtataaaactgggttat-gggaatttgaaacacatacccacgatttgcataacttatctaaaaataataagtcaaaattaatgaaagcttctgaagctacaatcataaaagatttaaacaaaagtgaaaaatatctaactaaaaactt-taaaaagtcgcagaaaactatagcctatccttatggcttgatgaatgacgataaattaccggtaatcaaaaaagctgggttaaaatacggttttcattagaggaaaaagcagtcactccgaactccaat-gattattacatccctagaatattaattagtgatgatgcttttgagcatttaattaagagatgggacggattccatgaaaaagattag

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	gene symbol	descrip-tion	minimum percent-age identity	minimum alignment length	Query sequence
Surface/Ad-herence	icaC	intracel-lular adhesion protein C	95	692	atgaaaaagattagacttgaactcgtatatttacgtgctattatatgtgcaattattattatcacacatttacttacacaaattactttaaaacatgaaaatatggagggtggatccttagtgttacaatttt-tacattcgtaatattgtgatttttggtacaccttgctttattatcttgtcacagttactgacaaccttgaattaccaaaaagtcacctatagatacttaactacacgcgtaaaatatatacttattccttacatat-taatgggattgttttacagttatagtgaatcattattaacagattcaagtttcaataaacaattcattgaaaatgtcctattaggtcaatggtatggctattttatcgttgttatcatgcaattctttatttttgagt-tatatcatttttaaaattaactataacctattcaacagtaaaatattattattgttatctttttattttacagcaatcatttttatattactttacgaacaacacagcgtttcacgatacgctgctacactattatc-cattaagtgaaaatactataatattcggatggatttttttatttcttcttaggtgcatatatgggttataactacgaacgtgtattaaatttcttagaacgttatttagttattatgattgttattagctgtagctact-tattttgtgtttattgcgttagcaaatggagactattggaacgttaccagcttttcatattcattaacaccatataatagtattatgtttattgttatcttgggtatttgcacgcattttaaaacaatgttatttta-atacgattcaaatgattagtgctttctcattctttatttatttattacatc
Surface/Ad-herence	icaD	intracel-lular adhesion protein D	95	244	atggtcaagcccagacagagggaatacccaacgctaaaatcatcgctaaatattgtaagagaaacagcacttatcgctatatcttgtgtcttttggatatattgtttagttgttctactcgtttatattggtac-tatatttgaaattcatgacgaaagtatcaatacaatacgtgttgctttaaacattgaaaatactgaaatttttagatatatttgaaactatgggcattttcgcgattatcatttttgtatttttttacaattagcatatt-gattcaaaaatggcagagaggaagagaatcgtga
Surface/Ad-herence	icaR	intracel-lular adhesion protein R	95	449	ttgaaggataagattattgataacgcaataaccttattttcagagaaggggtatgacggtacaacacttgatgatatagctaaaagtgtaaatataaagaaagcgagtttatattaccattttgactc-gaaaaaaagtatttacgaacaaagtgttaaatgttgttttgattaccttaataatattattatgatgaatcaaaataaatcgaactattcaattgatgctttatatcaattcttatttgagtttatttttcgacatc-gaagaaaggtatattagaatgtacgttcaattatctaatacgcctgaggaattttctggaaatatttacggacaaatacaagatttaaatcaatcattaagtaaagagatagcaaaattctatgatgaat-caaagataaagatgacaaaagaagactttcagaatttaatattgctgtttcttgaaagttggtatttgaaagcatccttttcgcaaaaatttggagcagtggaagaaagtaaaagtcaattcaaagat-gaagtgtattcgctactaaatatatttttgaagaaataa
Surface/Ad-herence	isaA	immu-nodomi-nant staphy-lococcal antigen A	95	561	atgaaaaagacaattatggcatcatcattagcagtggcattaggtgtaacaggttacgcagcaggtacaggacatcaagcacacgctgctgaagtaaacgttgatcaagcacacttagttgact-tagcgcataatcaccaagatcaattaaatgcagctccaatcaaagatggtgcatatgacatccacttttgtaaaagatggtttccaatataacttcacttcaaatggtactacatggtcatggagctat-gaagcagctaatggtcaaactgctggtttctcaaacgttgcaggtgcagactacactacttcatacaaccaaggttcaaatgtacaatcagtaagctacaatgcacaatcaagtaactcaaacgtt-gaagctgtttcagctccaacttaccataactacagcacttcaactacttcaagttcagtgagattaagcaatggtaatactgcaggtgctactggttcatcagcagctcaaatcatggctcaacgtactggt-gtttcagcttctacatgggctgcaatcatcgctcgtgaatcaaatggtcaagtaaatgcttacaacccatcaggtgcttcaggtttattccaaactatgccaggttggggtccaacaaacactgttgaccaa-caaatcaacgcagctgttaaagcatacaaagcacaaggtttaggtgcttggggattctaa
Surface/Ad-herence	isdA	iron-reg-ulated surface deter-minant protein A	90	842	atgacaaaacattatttaaacagtaagtatcaatcagaacaacgttcatcagctataaaaaagattacaatgggtacagcatctatcattttaggttcccttgtatacataggcgcagacagccaacaagt-caatgcggcaacagaagctacgaacgcaactaataatcaaagcacacaagtttctcaagcaacatcacaacccaattaatttccaagtgcaaaaagatggctcttcagagaagtcacacatggatgac-tatatgcaacaccctggtaaagtaattaaacaaaataataaatattatttccaaaccgtgttaaacaatgcatcattctggaaagaatacaaattttacaatgcaaacaatcaagaattagcaacaact-gttgttaacgataataaaaaagcggatactagaacaatcaatgttgcagttgaacctggatataagagcttaactactaaagtacatattgtcgtgccacaaattaattacaatcatagatatactacg-catttggaatttgaaaaagcaattcctacattagctgacgcagcaaaaccaaacaatgttaaaccggttcaaccaaaaccagctcaacctaaaacacctactgagcaaactaaaccagttcaaccta-aagttgaaaaagttaaacctactgtaactacaacaagcaaagttgaagacaatcactctactaaagttgtaagtactgacacaacaaaagatcaaactaaaacacaaactgctcatacagttaaaa-cagcacaaactgctcaagaacaaaataaagttcaaacacctgttaaagatgttgcaacagcgaaatctgaaagcaacaatcaagctgtaagtgataataaatcacaacaaactaacaaagtta-caaaacataacgaaacgcctaaacaagcatctaaagctaaagaattaccaaaaactggttttaacttcagttgataactttattagcacagttgccttcgcaacacttgccctttttaggttcattatctttat-tacttttcaaaagaaaagaatctaaataa

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
Surface/Adherence	isdH	iron-regulated surface determinant protein H	90	2153	atgaacaaacatcacccaaaattaaggtctttctattctattagaaaatcaactctaggcgttgcatcggtcattgtcagtacactatttttaattacttctcaacatcaagcgcaagcagcagaaaatacaaatacttcagataaaatctcggaaaatcaaaataatgcaactacaactcagccaccaaaggatacaaatcaaacacaacctgctacgcaaccagcaaacactgcgaaaaactatcctgcagcggatgaatcacttaaagatgcaattaaagatcctgcattagaaaataaagaacatgatataggtccaagagaacaagtcaatttccaattattagataaaaacaatgaaacgcagtactatcactttttcagcatcaaagatccagcagatgtgtattacactaaaaagaaagcagaagttgaattagacatcaatactgcttcaacatggaaaaagtttgaagtctatgaaaacaatcaaaaattgccagtgagacttgtatcatatagtcctgtaccagaagaccatgcctatattcgattcccagtttcagatggcacacaagaattgaaaattgtttcttcgactcaaattgatgatggagaagaaacaaattattgattatactaaattagtatttgctaaacctatttataacgatccttcacttgtaaaatcagatacaaatgatgcagtagtaacgaatgatcaatcaagttcagtcgcaagtaatcaaacaaacacgaatacatctaatcaaaatacatcaacgatcaacaatgctaataatcaaccgcaggcaacgaccaatatgagtcaacctgcacaaccaaaatcgtcaacgaatgcagatcaagcgtcaagccaaccagctcatgaaacaaattctaatggtaatactaacgataaaacgaatgagtcaagtaatcagtcggatgttaatcaacagtatccaccagcagatgaatcactacaagatgcaattaaaaacccggctatcatcgataaagaacatacagctgataattggcgaccaattgattttcaaatgaaaaatgataaaggtgaaagacagttctatcattatgctagtactgttgaaccagcaactgtcatttttacaaaaacaggaccaataattgaattaggtttaaagacagcttcaacatggaagaaatttgaagtttatgaaggtgacaaaaagttaccagtcgaattagtatcatatgattctgataaagattatcgcctatattcgtttcccagtatctaatggtacgagagaagttaaaattgtgtcatctattgaatatggtgagaacatccatgaagactatgattatacgctaatggtctttgcacagcctattactaataacccagacgactatgtggatgaagaaacatacaatttacaaaaattattagctccgtatcacaaagctaaaacgttagaaagacaagtttatgaattagaaaaattacaagagaaattgccagaaaaatataaggcggaatataaaaagaaattagatcaaactagagtagagttagctgatcaagttaaatcagcagtgacggaatttgaaaatgttacacctacaaatgatcaattaacagatttacaagaagcgcattttgttgttttgaaagtgaagaaaatagtgagtcagttatggacggctttgttgaacatccattctatacagcaactttaaatggtcaaaaatatgtagtgatgaaaacaaaggatgacagttactggaaagatttaattgtagaaggtaaacgtgtcactactgtttctaaagatcctaaaaataattctagaacgctgattttcccatatatacctgacaaagcagtttacaatgcgattgttaaagtcgttgtggcaaacattggttatgaaggtcaatatcatgtcagaattataaatcaggatatcaatacaaaagatgatgatacatcacaaaataacacgagtgaaccgctaaatgtacaaacaggacaagaagataaggttgctgatacagatgtagctgaaaatagcagcactgcaacaaatcctaaagatgcgtctgataaagcagatgtgatagaaccagagtctgacatggttaaagatactgatagtagtgttgacaaagatgcgcatctaaatattgatcattcatcaacaaatatgatgaataacactcacttcgataagtatgatcttaaagaaatggatactcaaattgctaaagacactgatagaaatgtggataaagatgccgataatagcgttggtatgtcatctaatgtcgatactgataaagactctaataaaaataaagacaagtcatacagctgaatcatattgccgataaaaatatcatactggaaaagcagcaaagcttgacgtagtgaaacaaaattatataatacagacaaagttactgacaaaaaaacaactgaacatctgccgagtgatattcataaaactgtagataaaacagtgaaaacaaaagaaaagcggcacaccatcgaaagaaaacaaactagtcaatctaaaatgctaccaaaaactggagaaacaacttcaagccaatcatggtggggcttatatgcgttattaggtatgttagctttattcattcctaaattcagaaaagaatctaaataa
Surface/Adherence	sasG*	staphylococcus aureus surface protein G	95	1040	atgagagataagaaggaccggtaaataaaagagtagattttctatcaaataaattgaataaatattcaataagaaaatttacagttggaacagcatctattttaattggctcactaatgtatttgggaactcaacaagaggcagaagcagctgaaaacaatattgagaatccaactactaattaaaagataatgtccaatcaaaagaagtgaagattgaagaagtaacaaacaaagacactgcaccacagggtgtagaagctaaatctgaagtaacttcaaacaaagacacaatcgaacatgaaccatcagtaaaagctgaagatatatcaaaaaaggaggatacaccaaaagaagtagctgatgttgctgaagttcagccgaaatcgtcagtcactcataacgcagagacacctaaggttagaaaagctcgttctgttgatgaaggctcttttgatattacaagagattctaaaaatgtagttgaatctaccccaattacaattcaaggtaaagaacatttgaaggttacggaagtgttgatatacaaaaaaaaccaacagatttaggggtatcagaggtaaccaggtttaatgttggtaatgaaagtaatggtttgataggagctttacaattaaaaaataaaatagattttagtaaggatttcaattttaaagttagagtggcaaataaccatcaatcaaataccacaggtgctgatggttgggggttcttatttagtaaaggaaatgcagaagaatatttaactaatggtggaatccttggggataaaggtctggtaaattcaggcggatttaaaattgatactggatacatttatacaagttccatggacaaaactgaaaagcaagctggacaaggttatagaggatacggagcttttgtgaaaaatgacagttctggtaattcacaaatggttggagaaaatattgataaatcaaaaactaatttttaaactatgcggacaattcaactaatacatcagatggaaagtttcatgggcaacgtttaaatgatgtcatcttaacttatgttgcttcaactggtaaaatgagagcagaatatgctggtaaaacttgggagacttcaataacagatttaggtttatctaaaaatcaggcatataatttcttaattacatctagtcaaagatggggccttaatcaagggataaatgcaaatggctggatgagaactgacttgaaaggttcagagtttacttttacaccagaagcgccaaaaacaataacagaattagaaaaaaaagttgaagagattc
Toxin	edn	epidermal cell differentiation inhibitor protein	90	605	atgaatttaaatatgaaagatacaattgtaaaatttctatcagcattccttgtgatttcaagtattagtttgatagatacatcttttagctctaaatataataaaatctcaatagctgccgagactaaaaatttttacagacttagttgaagctactaaatggggaaactcattaataaagtcagccaagtattcttctaaagataagatggctatttataattatacaaaaaatagttcacccataaatactcctctaagatcagcaaatggtgacgtgaacaaattatccgaaaacattcaagagcaggttagacaattagactcaacgatatctaaatctgtaacaccagattcagtctatgtatatagattattaaatttagactacttatcaagtataactggctttacgcgagaagatttacatatgctacaacaaactaacaatggtcaatataatgaagcgcttgtgtcaaaactaaataatcttatgaatagtagaatttacagagaaaatggctactctagtacacaactagttagtggtgcagcactagcaggtaggccaattgaattaaaattagaattacctaaaggtactaaagcagcatatattgattctaaagagttaacagcatacccaggtcaacaagaagttcttttgcctagagggacagaatatgctgtaggcagtgttaaacttttctgataacaaaagaaagataattataactgctgtagttttaaaaaataa

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
Toxin	etA	exfoliative toxin A	95	800	atgaataatagtaaaattatttctaaagttttattgtctttatctctatttactgtaggagctagtgcatttgttattcaagacgaactgatgcaaaaaaaccatgcaaaagcagaagtttcagcagaagaaatataaaaaaacatgaagagaaatggaataagtactatggtgtcaatgcatttaatttaccaaaagagctttttagtaaagttgatgaaaaagatagacaaaagtatccatataatactatagggtaatgtttttgtaaaaggacaaacaagtgcaactggtgtgttaattggaaaaaatacagttctaacaaatagacatatcgctaaatttgctaatggagatccatctaaagtatctttagaccttctataaatacagatgataacggtaatactgaaaccaccatatggagagtatgaagtcaaagaaatattacaagaaccatttggtgcaggtgttgatttagcattaatcagattaaaaccagatcaaaacggtgtttcattaggcgataaaatatcgccagcaaaaatagggacatctaatgatttaaaagatggagacaaactcgaattaatggcatatccattcgatcataaagttaaccaaatgcacagaagtgaaattgagttaacaacttttatcaagaggattaagatactatggatttacagttccgggaaattctggatcaggtatatttaattcaaatggagaattagttggtatacattctagcaaagtgtctcatcttgatagagagcatcaaataaattatggtgttggtattgggaattatgtcaagcgcattataaacgagaaaaatgagtaa
Toxin	etB	exfoliative toxin B	95	800	atggataaaaatatgtttaaaaaaattattttagcagcgtcaatttttactatttccttacctgtgattccttttgaaagtacattacaagcaaaagaatacagcgcagaagaaatcagaaaattaaaacaaaaatttgaggttccacctacagataaagagctttatacacacattacggatatgcaagaagtccttataattctgttggtacagtgtttgtcaaaggtagtacattagctaccggagttttaattggtaaaatacaattgttactaattaccacgttgcaagagaagcagccaaaaacccatcgaatattatttttacacccgctcaaaatagagatgcagaaaaaaatgaattccctactccgtatggaaaatttgaagctgaagaaattaaagaatctccgtatggacaaggactcgatttagctataataaaattaaaaccaaacgaaaaaggggaatcagcgggagatttaattcaaccagctaatatacctgatcatattgatatacaaaaggagacaaatattctttattaggatatcctatataattcattcagcttactctttatatcaaagtcagattgaaatgttcaatgattctcaatatttttggatatactgaggtaggaaactctggatcaggtatatttaatttaaaaggagaattaataggtattcacagtggtaaaggcggacaacatatcttccaataggagtgtttttcaatagaaagataagttcactctattcggttgataatactttggagacactttggggaacgatttgaaaaagagagcaaaattagatataaataa
Toxin	etD	exfoliative toxin D	95	678	atgactaaaaatatattaaaaaagttatttataacattatctcttgcaatatttctctcccgttgattagtcatgcagaaaataaattattcgcaaatacatatgaagaatctgaaatttaaaaaagagagaaaaagtacaatgcagccccttccacattaagtgaggaagttttttcaaaagtttcaaatactatgaaaagcccctataattcagttggaacagttttttattaaggggagactatagcttctggtgtattaattggcaaaaatacaattataacaaactatcatgtatcaaggatggctaaaaaagatccgactaaagttatattcacacctggttcgactaaaacagaagatggagtatataaaactccatatggacaatttgtagcagaagaaattaatgaacacccatatggacaaggaactgatttgtctataattaaactaaaacccaataaagacggaaagtctgcaggtgatttaattccccctgcaaagattgcagattctatagatttgcaacaaggtgacaaaataagcttgctcggataccctataactttctactaattctttatatagaagtgaaattgaaatctttaatttaaattctggtcaatatttgggtatactgagtcgggaaattctggatccgggttgtttaatttaaaaggagagttagttggtattcatgttggaaaaggaggaaaatataatttaccgataggtaaatttttttaatactgaaattggctcattgtattcagtagataattcattaagcactttaggtagtgatttaaagaaaagagctgaattacagtctcattag
Toxin	hla	alpha hemolysin	90	772	atgaaaacacgtatagtcagctcagtaacaacaacactattgctaggttccatattaatgaatcctgtcgctaatgccgcagattctgatattaatattaaaaccggtactacagatattggaagcaatactacagtaaaaacaggtgatttagtcacttatgataaagaaaatggcatgcacaaaaaagtattttatagttttatcgatgataaaaatcacaataaaaaactgctagttattagaacgaaaggtaccattgctggtcaatatagagtttatagcgaagaaggtgctaacaaaagtggtttagcctggccttcagccttaaggtacagttgcaactacctgataatgaagtagctcaaatatctgattactatccaagaaattcgattgatacaaaagagtatatgagtactttaacttatggattcaacggtaatgttactggtgatgatacaggaaaaattggcggccttattggtgcaaatgtttcgattggtcatacactgaaatatgttcaacctgatttcaaaacaattttagagagcccaactgataaaaaagtaggctggaaagtgatatttaacaatatggtgaatcaaaattggggaccatatgatagagattcttggaacccggtatatggcaatcaacttttcatgaaaactagaaatggttctatgaaagcagcagagaacttccttgatcctaacaaagcaagttctctattatcttcagggttttcaccagacttcgctacagttattactatggatagaaaagcatccaaacaacaaacaaatatagatgtaatatacgaacgagttcgtgatgactaccaattgcattggacttcaacaaattggaaaggtaccaatactaaagataaatggacagatcgttcttcagaaagatataaaatcgattgggaaaaagaagaaatgacaaattaa
Toxin	hlb	beta hemolysin	90	816	atgatggtgaaaaaaacaaaatccaattcactaaaaaaagttgcaacacttgcattagcaaatttattattagttggtgcacttactgacaatagtgccaaagccgaatctaagaaagatgatactgatttgaagttagttagtcataacgtttatatgttatcgaccgttttgtatccaaactgggggcaatataaacgcgctgatttaatcggacaatcttcttatattaaaaatatgatgtcgtaatattcaatgaagcatttgataatggtgcatcagacaaattattaagtaatgtgaaaaaagaatatccttatcaaacacctgtactcggccgttctcaatcagggtgggacaaaactgaaggtagctactcatcaactgttgcagaagatggtggcgtagcgattgtaagtaaatatcctattaaagaaaaatccagcatgttttcaaaagcggttgtggattcgataatgatagcaacaaaggctttgtttatacaaaaatagagaaaaatggtaagaacgttcacgttatcggtacacatacacaatctgaagattcacgttgtggtgctggacatgatcgaaaaattagagctgaacaaatgaaagaaatcagtgactttgttaaaagaaaaatatcccttaaagatgaaacggtatatataggtggcgacctttaatgtcaataaaggcactccagagttcaaagatatgcttaaaaacttgaatgtaaatgatgttctatatgcaggtcatatagcacatgggaccctcaatcaaattcaattgcgaaatataattaccctaatggtaaaccagaacatttagactatatatttacagataaagatcataaacaaccaaaacaaattagtcaatgaagttgtgactgaaaaacctaagccatgggatgtatatgcgttcccatattactacgtttacaatgatttttcagatcattaccccaatcaaagcctatagtaaatag
Toxin	hld	delta hemolysin	95	109	atgagttgtttaattttaagaatttttatcttaattaaggaaggagtgatttcaatggcacaagatatcatttcaacaatcagtgacttagtaaaatggattatcgacacagtgaacaaattcactaaaaaataa

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
Toxin	hlgA	gamma hemolysin A	90	749	atgattaaaaataaaatattaacagcaactttagcagttggtttaatcgccccttagccaatccatttatagaaatttctaaagcagaaaataagatagaagatatcggccaaggtgcagaaatcatcaaaagaacacaagacattactagcaaacgattagctataactcaaaacattcaatttgattttgtaaaagataaaaaatataacaaagatgccctagttgtttaagatgcaaggcttcatcagctctagaacaacatattcagacttaaaaaaatatccatatattaaaagaatgatatggccatttcaatataatatcagtttgaaaacgaaagactctaatgttgatttaatcaattatcttcctaaaaataaaattgattcagcagatgttagtcagaaattaggctataatatcggcggaaacttccaatcagcgccatcaatcggaggcagtggctcattcaactactctaaaacaattagttataatcaaaaaaactatgttactgaagtagaaagtcagaactctaaaggtgttaaatggggagtgaaagcaaattcattcgttacaccgaatggtcaagtatctgcatatgatcaatacttatttgcacaagacccaactggtccagcagcaagagactatttcgtcccagataatcaactacctcctttaattcaaagtggctttaatccatcatttattacaacattgtcacacgaaaaaggtaaaggtgataaaagcgagtttgaaatcacttacggcagaaacatggatgctacatatgcatacgtgacaagacatcgtttagccgttgatagaaaacatgatgcttttaaaaaccgaaacgttacagttaaatatgaagtgaactggaaaacacatgaagtaaaattaaaagcatcacacctaagtaaa
Toxin	hlgB	gamma hemolysin B	90	788	atgaaaatgaataaattagtcaaatcatccgttgctacatctatggcattattattactttctggtactgctaatgctgaaggtaaaataacaccagtcagcgtaaaaaaagtcgatgacaaagttacttatatacaaaacaacagccacagcagattctgataaatttaaaatttcacagattttaacatttaatttcatcaaagataaaagttatgataaagatactttagtacttaaagctactgggaatattaactcaggctttgtgaaacctaatcctaatgactatgacttttcaaaattatattggggagctaaatacaatgtatctataagctcacaatctaatgattcagtaaacgtcgttgattatgcaccaaaaaatcaaaatgaagagtttcaagttcaaaatactttaggctatacatttggtggtgacattagtatctctaatggtttatctggtggacttaatggaaatacagctttttctgaaacaattaattataaacaagaaagttacagaacaacattaagtcgcaacacaaattataaaaatgttggctggggagttgaagcacataaaattatgaataatggttggggaccttatggacgagatagcttccacccaacatatggtaatgaactcttcttagctggcagacaaagcagtgcatacgctggccaaaacttcatagcgcaacaccaaatgccattattatctagaagtaacttcaatccagaatttttaagcgtactatcacacagacaagatggcgctaaaaaatctaaaattacagtaacttatcaacgtgaaatggatttatacccaaattcgttggaatggcttctactgggcaggcgcaaattataaaaactttaaaactagaacatttaaatcaacatatgaaattgattgggaaaatcacaaagtgaaattgttagatacaaaagaaactgaaaacaataaatag
Toxin	hlgC	gamma hemolysin C	90	764	atgcttaaaaataaaatattagccacaactttatctgtgagcttacttgcccctcttgccaatccgttattagaaaatgctaaagctgccaacgatactgaagacatcggtaaaggaagcgatatagaaattatcaaaaggacagaagataaaacaagtaataaatggggcgtgactcaaaatattcaatttgattttgtgaaggataaaaaatataacaaagatgctttgatattaaagatgcaaggattcattagctctagaacaacatattacaactataaaaaaactaatcatgttaaagctatgcgatggccattccaatataatattggtttaaaaacaaatgataaatatgtttcttttaattaattatttacctaaaaataaaattgaatctacaaacgtgagtcagacattaggatacaatatcggtggtaatttccaatcagccccatcactcggtggtaatggatcatttaactattctaaatcgattagctatacacaacaaaattatgtaagtgaagtagaacaacaaaactcaaaagtgttttatggggcgtcaaagcgaattcattcgccactgaatcaggtcaaaaatcagcatttgatagcgatttatttgtaggctacaaacctcatagtaaagatcctagagattatttcgttccagacagtgagttaccacctcttgtacaaagtggatttaaccccttcatttatcgccacagtatctcatgaaaaaggttcaagcgatacaagcgaatttgaaattacttacggaagaaacatggatgtcactcatgccattaaaagatcaacgcattatggcaacagtttatttagacggacatagagtccataatgcatttgtaaatagaaactatactgtgaaatacgaggtcaattggaagactcatgaaatcaaggtgaaaggacagaattga
Toxin	lukD	leukocidin D	90	791	atgaaaattgaaaaattaggcaaatcatcagttgcttcatcaattgcactgcttttgctatcgaatacagttgatgcagctcaaaatatcacacctaaaagagagaaaaaagtagatgacaaaatcacttatatacaaaacaacagcaacatctgataatgataaattgaatatttttcaaatttaacgtttaatttcattaaggataaaagttatgacaaagatacgttagtacttaaggcagccggaaacattaattcaggttataaaaattctaatccaaaagattacaattactcacagttttattggggcggtaagtataatgtttcggttagttcagaatcaaatgatgctgtaaatgttgttgactatgcacctaaaaatcaaaatgaagaattccaagttcaacaaacattaggttattcttatggcggagatattaatatatctaatggcttatcaggtggattaaatggatcaaaatcattttcagaaacgataaattataaacaagaaagttacagaactacgattgatagaaaaacaaatcataaatcaattggctggggtgttgaggcccacaaaattatgaataatggttggggaccatatggtagagatagttatgacccaacatatggtaatgaactgtttttaggcggtgacaaaagtagttcaaatgctggtcaaaatttcttgccaacacatcaatcccttattggcgcgtggtaactttaacccagaatttataagcgtactttctcataaacttttttgatacaaaaaaatctaaaatcaaagtaacttaccaaagagagaaatggatagatatactaatcaatggaatcgatcacactgggttggtaataactacaaaaatcaaaatacagtaacgtttacatctacttatgaagttgactggcaaaacatactgttaaaattaatcggtacggattctaaagaaactaatcctggagtataa

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
Toxin	lukE	leukocidin E	90	757	atgtttaagaaaaaaatgttagctgcaagtttgtcagtaggactgattgcaccctttagcatctccgattcaagaatctagagcaaatactaatattgaaaatattggtgatggtgctgaagtaatcaaacgtacggaggatgtaagtagtaagaaatggggcgttactcaaaatgtccaattcgactttgtaaaagataaaaaatataacaaagacgctttaattgttaaaatgcaaggttttattaattccagaacttcattttcggatgtgaagggtagaggatatgaattaactaaacgtctgatttggccattccaatataatatatggactgacgactaaagatccaaatgttagcttaatcaattcaattacctctacctaaaaccaaaatagaaactactgatgttggtcaaacattaggatataacattggaggtaatttccagtcagcaccatctataggtggcaatggctcatttaattattctaaaacaattagttatacccaaaagagttatgtcagtgaagtagacaagcaaaactcaaaatctgttaaatggggtgttaaagcaaacaaatttgttacccctgatggaaaaaaatttgcgcatgatagatatttattcgtacaaagtccaaatggtccaacaggttcagcaagagaatattttgctcctgataatcaattgccaccttagttcaaagtggctttaatccatcgtttatcactacactatcacatgaaaaggttcaaagttgatacgagtgaatttgaaatttttcatatggtagaaacttagatattacatatgcgacttttattccctagaactggtatttacgcagaaagaaagcataatgcatttgtaaatagaaacttttgtagttagatataaagttaattggaaaacacacgaaattaaagtgaaaggacataattaa
Toxin	lukF	leukocidin F	90	790	atgaaaaaaatagtcaaatcatcagttgttacatcaattgcattgcttttgctatccaatacagttgatgcagctcaacatatcacacctgtaagtgagaaaaaggttgatgataaaattactttgtacaaaacaactgcaacatcagattccgataagttaaaaatttctcagattttaacttttaatttattaaagataaaagttatgataaagatacattaatactcaaagctgctggaaacatttattctggctatacaaagccaaatccaaaagacactattagttctcaattttattgggttctaagtacaacatttcaattaattcagattctaatgactcagtaaacgttgtagattatgcacctaaaaatcaaaatgaagaatttcaagtacaacaaacggtaggttattcttatggtggagatattaatatctctaacggcttatcaggtggaggtaatggttcaaaatctttttcagagacaattaactataaacaagaaagctatagaactagcttagataaaagaactaatttcaaaaaaattggttgggatgttgaagcacataaaattatgaataatggttggggaccatatggcagagatagttatcattcaacttatggtaatgaaatgtttttaggctcaagacaaagcaacttaaatgctggacaaaacttcttggaatatcacaaaatgccagtgttatccagaggtaacttcaatccagaatttattggtgtcctatctcgaaaacaaaacgctgcaaaaaaatcgaaaattactgttacttatcaaagagaaatggatagatatacaaacttttggaatcaacttcactggataggtaataattataaagatgaaaatagagcaactcatacatcaatttatgaagttgattgggaaaatcatacagttaaattaatagatactcaatctaaggaaaaaaatcctatgagctaa
Toxin	lukM	leukocidin M	90	754	atgtttaagagaaaattattagttacaactttgtcgctaggtctaattgtccctatagctacaccatttcaaggctctaaggctactactaatgcagaagatattggcgacgatgcagaagtgattaaacgtacggaagatgtaagtagtaggaaatggggtgtaacacaaaatgtccaatttgatttcgtaaaagataaaaaatatatacaaagacgcattaattattaagatgcaaggttttatcaattctaggacaactttcaatgatgttaaacaaaatagagcaaatataaagaatggtttggccatttcaatataatatcggtcttacatcaaaagaccaaaatacgagcttaatcaattatcttcctaaaaataaaatagaaacagttgatgttggtcaaactttaggatataacattggaggtaaattccagtcagtaccatctataggcggaaatggatcatttaattattctaagagtattaaatattcccaaaagagttatgtcagcgaagttgaacaacaagctcaaaaactattaagtgggggttaaagcaaattcttttgttatagcagggcatcgatggtctgcttacgatgaattattgtttataagaaatacgacaagaggaccatatgctagagactattttgtagacgataatgaattgcccccttaataacaagtggatttaatccgtcttttatcgcgacagtatctcacgaaaaagattcaggcgatacgagcgaatttgaaattacttacggtagaaatatggatgttacctatgcaacctaccttcctaaacttggtctttatccagaaagaaacataatgaatttgtaaacagaaactttgtggtcaaatatgaagtgaattggaaaacgtacgaaattaaagtaaaggggcacaactaa
Toxin	lukS	leukocidin S	90	770	atgatttttatggtcaaaaaaagactattagctgcaacattgtcgttaggaataatcactcctattgctacttcgtttcatgaatctaaagctgataacaatattgagaatattggtgatggcgctgaggtagtcaaaagaacagaagatacaagtagcgataagtgggggtcacacaaaatattcaggttgattttgttaaagataaaaagtataacaaagacgctttgattttaaaaatgcaaggttttatcaattcaaagactacttattacaattacaaaaacacagatcatataaaagcaatgaggtggcctttccaatacaatattggtctcaaaacaaatgaccccaatgtagatttaataaattatctacctaaaaataaaatagattcagtaaatgttagtcaaacattaggttatacatcaggtggtaatttttaatagtggtccatcaacaggaggtaatggttcatttaattattcaaaaacaattagttataatcaacaaaactatatcagtgaagtagaacgtcaaaattcaaaaagtgttcaatggggaataaaagctaattcatttatcacatcattaggtaaaatgtctggacatgatccaaatttattttgttggatataaaccatatagtcaaaatccgagagactattttgttccagacaatgaattacccccattagtacacagtggtttcaatccttcatttattgcaactgtttctcatgaaaaaggctcaggagatacaagtgaatttgaaataacgtatggcagaaatatggatgttactcatgctactagaagaacaacacactatggcaatagttatttagaaggatctagaatacacaacgcatttgtaaacagaaattacacagttaaatatgaagtgaactggaaaactcatgaaattaaagtgaaaggacataattga

Table S2. *S. aureus* virulence genes (*continued*)

			BLAST search parameters		
Category	**gene symbol**	**description**	**minimum percentage identity**	**minimum alignment length**	**Query sequence**
Toxin	PVL	Panton-Valentin leukocidin	90	1555	atgatttttatggtcaaaaaaagactattagctgcaacattgtcgttaggaataatcactcctattgctacttcgtttcatgaatctaaagctgataacaatattgagaatattggtgatggcgctgaggtagtcaaaagaacagaagatacaagtagcgataagtgggggtcacacaaaatattcaggttgattttgttaaagataaaaagtataacaaagacgctttgattttaaaaatgcaaggttttatcaattcaaagactacttattacaattacaaaaacacagatcatataaaagcaatgaggtggcctttccaatacaatattggtctcaaaacaaatgaccccaatgtagatttaataaattatctacctaaaataaaatagattcagtaaatgttagtcaaacattaggttataacataggtggtaattttaatagtggtccatcaacaggaggtaatggttcatttaattattcaaaaacaattagttataatcaacaaaactatatcagtgaagtagaacgtcaaaattcaaaaagtgttcaatggggaataaaagctaattcatttatcacatcattaggtaaaatgtctggacatgatccaaatttatttgttggatataaaccatatagtcaaaatccgagagactattttgttccagacaatgaattacccccattagtacacagtggtttcaatccttcatttattgcaactgtttctcatgaaaaaggctcaggagatacaagtgaatttgaaataacgtatggcagaaatatggatgttactcatgctactagaagaacaacacactatggcaatagttatttagaaggatctagaatacacaacgcatttgtaaacagaaattacacagttaaatatgaagtgaactggaaaactcatgaaattaaagtgaaaggacataattgatatgaaaaaaatagtcaaatcatcagttgttacatcaattgcattgcttttgctatccaatacagttgatgcagctcaacatatcacacctgtaagtgagaaaaaggttgatgataaaattactttgtacaaaacaactgcaacatcagattccgataagttaaaaatttctcagattttaacttttaatttttattaaagataaaagttatgataaagatacattaatactcaaagctgctggaaacatttattctggctatacaaagccaaatccaaaagacactattagttctcaatttttattggggttctaagtacaacatttcaattaattcagattctaatgactcagtaaacgttgtagattatgcacctaaaaatcaaaatgaagaatttcaagtacaacaaacggtaggttattcttatggtggagatattaatatctctaacggcttatcaggtggaggtaatggttcaaaatctttttcagagacaattaactataaacaagaaagctatagaactagcttagataaaagaactaatttcaaaaaaattggttgggatgttgaagcacataaaattatgaataatggttggggaccatatggcagagatagttatcattcaacttatggtaatgaaatgtttttaggctcaagacaaagcaacttaaatgctggacaaaacttcttggaatatcacaaaatgccagtgttatccagaggtaacttcaatccagaatttattggtgtcctatctcgaaaacaaaacgctgcaaaaaaatcgaaaattactgttacttatcaaagagaaatggatagatatacaaacttttggaatcaacttcactggatagggtaataattataaagatgaaaatagagcaactcatacatcaatttatgaagttgattgggaaaatcatacagttaaattaatagatactcaatctaaggaaaaaaatcctatgagctaa
Toxin	tsst-1	toxic shock syndrome toxin-1	90	566	atgaatnaaaaattactaatgaaatttttcatcgtaagccctttgttgcttgcgacaatcgctacaggttttaccccctgttcccttatcatctaatcaaataatcaaaactgcaaaagcatctacaaacgatatataaaggatttgctagactggtatagtagtgggtctgacgctttacaaanagtgaagttttagataattccttaggatctatgcgtataaaaaacacagatggcagcatcagccttataatttttccgagtccttattatagccctactttttcaaaaggggaaaaagttgacttaaacacaaaaaggactaaaaaaagccaacatactagcgaaggaacttggattcatttccaaataagtggtgttacaaatactgaaaaattacctactccaatagaactacctttaaaagttaaggttcatggtaaagatagtccattaaagtattggccaaagttcgataaaaaacaattagctatatcaacttagactttgaaattcgtcatcagctaactcaaatacatggattatatcgttcaagcgataaaacgggtggttattggaaaatacaatgaatgacggatccacatatcaaagtgatttatctaaaaagtttgaatacaatactgaaaaacacacctataaatattgatgaaataaaaactatagaagcagaaattaattaa

Sequences marked with an asterisc contain repetitive elements. Only the largest non-repetitive part was used for querying.

4

Summarizing discussion

SUMMARIZING DISCUSSION

In this thesis, colonization, carriage and transmission of *S. aureus* were studied and the most important findings and conclusions are explicated in this chapter. In spite of all the research that has been performed on nasal *S. aureus* (de)colonization in animals, probably the best way to study this is by using the *S. aureus* human inoculation model (1-4). Unfortunately, we encounter ethical problems regarding the use of this human model, and a proper human-like animal model is still missing. Furthermore, in order to prevent carriage of and infection with *S. aureus*, especially in risk groups, understanding carriage and detecting reservoirs in hospitals is essential.

The first aim of this thesis was to gain more insights into the colonizing capacity of *S. aureus*. Therefore, a novel experimental decolonization and carriage model in rhesus macaques (*Macaca mulatta*) was set up, and a study with the human inoculation model was performed. A secondary aim was to investigate, using whole-genome sequencing (WGS), whether nosocomial acquisition of *S. aureus* via healthcare workers (HCWs) occurred in neonates admitted to a neonatal intensive care unit (NICU). We also studied whether transmission and the presence of specific virulence genes support the occurrence of neonatal bloodstream infections.

MAJOR FINDINGS AND CONCLUSIONS

In **Chapter 2** *S. aureus* colonization and carriage were investigated to enhance existing knowledge about the colonizing capacity of emerging, livestock-associated *S. aureus* strains in humans. In addition, the ethical restrictions of the human inoculation model challenged us to an experiment, to develop a new animal model in rhesus macaques. In **Chapter 2.1** we developed an experimental decolonization and inoculation procedure in rhesus macaques. Rhesus macaques are natural hosts of *S. aureus* (5). They were therefore considered to be an excellent model, compared to other animal models where sometimes the importance of the animal being a natural host seems to be ignored. Twenty rhesus macaques, all nasal *S. aureus* carriers, were split up into two groups for a decolonizing treatment. To check for nasal *S. aureus* carriage, animals were swabbed 4–5 times with one-week intervals. We defined carriage as animals in which at least 80% of the nasal cultures were *S. aureus* positive. For decolonization, the nose was approached with either a topical treatment with mupirocin nasal ointment only (treatment A), or a combination treatment of mupirocin nasal ointment with systemic trimethoprim/sulfadiazine (treatment B). **In this novel rhesus macaque model, we showed that we could decolonize the nose of *S. aureus* carriers, using standardized protocols, for a period of 10 weeks or more. There was no significant difference between the two treatments, as all animals became negative for *S. aureus* in the nose**

between days 7-21. After 63 days, 60% of all animals in both treatment groups persistently cultured negative in the nose. Furthermore, with respect to the time of first recurrence of *S. aureus*, there was no significant difference between the two treatment groups when only the nose was analyzed. However, analysis of recurrence of *S. aureus* at any site (throat or rectum) showed a trend, suggesting that treatment B was more effective than treatment A. Following decolonization, the anterior nares were inoculated with a human *S. aureus* strain 8325–4, to investigate whether carriage could be induced. **We were able to inoculate the noses of the rhesus macaques with human *S. aureus* strain 8325–4, but we did not observe stable, long-term colonization with this strain.** Contrary to this observation, in previous studies, *S. aureus* 8325–4 was shown to persist for at least four weeks in the human nose, despite the presence of a defect in the sigma factor (SigB) locus (3, 4, 6). By choosing strain 8325-4, we had the benefit of being able to use our previous experience in human inoculation experiments in which this strain was also used. In addition, this strain is suitable for genetic modification and can be applied for both knockout (3, 4) and knock-in studies.

We conclude that we developed in rhesus macaques, a natural host for *S. aureus*, an experimental decolonization and inoculation procedure, which can be used for *S. aureus* decolonization and inoculation studies in a properly controlled fashion.

In contrast to other *S. aureus* animal models, the major future benefit of this model is that rhesus macaques allow long-term follow-up when using them for (de)colonization studies, as these animals do not need to be sacrificed. Also, the pathogen-host interplay could very well be studied in this animal because of its natural *S. aureus* carrier state and its genetically close relatedness to humans.

In **Chapter 2.2** the intrinsic capacity of a non-human *S. aureus* strain (ST398) to colonize the human nose was studied. This strain emerges in livestock and people in close contact with livestock are presumed to be at risk for carriage, but data on its colonizing capacity in humans is lacking. An artificial human nasal inoculation experiment was performed with a mixed inoculum (10*7 bacteria per strain per nostril) of a bovine methicillin-susceptible *S. aureus* (MSSA) ST398 (CC398) strain and a human MSSA ST931 (CC8) strain. Subsequently, their ability to survive in the anterior nares was determined. The driving force to perform this study was, as earlier mentioned, that methicillin-resistant *S. aureus* (MRSA) strains of lineage ST398 are described in literature as predominantly occurring in livestock, while they have the ability to also colonize humans. Furthermore, an increase of livestock-associated (LA)-MRSA ST398 infections was documented in hospitalized patients without a link to livestock. **We demonstrate in our human inoculation model, that *S. aureus* ST398 of bovine origin is capable of surviving in the nose of 10 healthy volunteers for at least 21 days, when inoculated 7 weeks after an eradication treatment with mupirocin and chlorhexidine-containing soap.** In the first days after inoculation, we observed a rapid decrease in bacterial load of both strains in the nares of all volunteers, resulting in the elimination of both strains within 21 days in 4 volunteers. Interestingly, in the remaining 10 volunteers, ST398 could

still be detected after 21 days. In half of this latter group we observed, after a decrease in bacterial loads of both strains, that the loads stabilized after 21 days. In the remaining five individuals, cell counts for strain ST398 increased at the end of the follow-up period, where in most of these cases the human strain was already eliminated. Our data clearly indicate that in 28.6% of the volunteers *S. aureus* is rapidly eradicated, even when exposed to significant numbers of bacteria. Yet, 71.4% of the volunteers were unable to eradicate any of the inoculated *S. aureus* strains. **Based on microarray analysis, no evidence was found that survival of ST398 in the human host was due to the acquisition of mobile genetic elements (MGEs).** This suggests that animal ST398 is able to survive for several weeks in the human nares without gaining or losing MGEs.

In conclusion, MSSA strain 5062 of bovine origin (ST398, CC398) is capable of surviving in the human nose for at least 21 days and can successfully outcompete a human strain 1036 (ST931, CC8).

It is a matter of debate whether we should fear exposure to *S. aureus* ST398, in relation to the risk of becoming a *S. aureus* carrier. As we studied the colonizing capacity of this strain in a controlled fashion, exposing the volunteers to a significant amount of bacteria, we need to be cautious about drawing conclusions. We think that people in contact with livestock may be exposed to lower numbers of bacteria than in our experimental setting. This may explain the fact that people exposed to LA-MRSA in the community, may lose the strain within 24 hours (7), in contrast to what we have observed. Van Cleef et al. discuss that it could very well be that people who are temporarily in contact with livestock, are just being contaminated instead of being colonized with LA-MRSA (7). Among infection prevention control professionals, it is well-known that the success of a transmission of MRSA should be checked only about eight hours after exposure. The reason for this approach is that there is a high chance of culturing positive for *S. aureus* immediately after exposure. When culturing the day after, the positive results spontaneously turn negative, for instance because often, contamination does not lead to successful colonization. Further research is needed to investigate if colonization with *S. aureus*, whether or not due to inoculation in experimental settings, is dependent on bacterial loads in the way we assume. As we pretreated the volunteers with mupirocin nasal ointment before inoculation, we could only speculate about the possible positive effect of this treatment on the development of *S. aureus* nasal colonization. The treatment could have eradicated more bacteria from the nasal cavity than only *S. aureus*, which may be beneficial for *S. aureus* regrowth and recolonization after a period of time. For example, we know that *Staphylococcus epidermidis* (*S. epidermidis*) has a negative influence on *S. aureus* nasal colonization (8, 9), and by eradicating this bacterium, the chances of *S. aureus* may improve.

In **Chapter 3,** nosocomial transmission of *S. aureus* was studied in the highest-risk population for the development of hospital-acquired infections (HAIs): neonates on a NICU. Neonates are regularly confronted with invasive *S. aureus* infections, in particular bacteremia,

which makes this bacterial species one of the most important pathogens in this high-risk group (10-12). All-cause mortality among neonates suffering from *S. aureus* bacteremia varies between 10 and 20% (13, 14). So how, and from whom or where, do these neonates become contaminated, leading to colonization and infections with this bacterium? It is relatively unknown what the contribution of the parents is in the transmission of *S. aureus* to their newborns. Meanwhile, literature describes a variety of *S. aureus* sources in the hospital that might influence the neonatal *S. aureus* carriage state, from non-carriage to carriage. NICU outbreaks are frequently described with respect to *S. aureus* (15-22), and because of the currently available advanced typing techniques, transmission via HCWs has already been shown (21, 22). By demonstrating possible sources of *S. aureus* and transmission routes, awareness will rise, allowing us to avoid, or at least minimalize, the transmission of *S. aureus* to neonates to prevent HAIs. In **Chapter 3.1,** we performed a retrospective analysis in which *S. aureus* isolates of neonates and HCWs were analyzed by WGS to detect relationships. The isolates of neonates were derived from different patient materials, including blood cultures, but we also included screening cultures. The main goal in this study was to explore whether HCWs could possibly be involved as a source in *S. aureus* transmission to neonates, by showing indistinguishable genomes in both groups in the same time period. **By using WGS, we showed that HCWs might serve as a reservoir for the transmission of methicillin-susceptible *S. aureus* (MSSA) to neonates on a level IV NICU. In 4 WGS clusters, a total number of 4 HCWs and 35 neonates were involved, of which 10 neonates suffered from neonatal bacteremia.** Indistinguishable MSSA isolates, causing 10 of the total number of 20 episodes of neonatal MSSA bacteremia, were found in both neonates and HCWs, and were already present in HCWs or neonates admitted earlier. Data concerning MSSA transmission from HCW to neonate are scarce since, normally, no contact investigation is performed when a neonate is positive for MSSA, in contrast with MRSA findings. In three studies, a MSSA transmission from HCW to patient was described. Gomez-Gonzalez et al. showed, -albeit by use of Pulse Field Gel Electrophoresis, a technique with less discriminatory power -, that HCWs acted as a reservoir for neonates who later on developed a MSSA sepsis (16). In another study, using Raman spectroscopy typing, a HCW proved to be the source of an outbreak in neonates with MSSA bullous impetigo (18). In addition, Price et al., applying WGS, showed indications for the transmission from patients, the environment and HCWs, to other patients on an adult intensive care unit, leading to colonization and infections with genetically identical *S. aureus* isolates (23).

With our data, we can confirm that HCWs might be an important reservoir and link in the transmission of MSSA to neonates.

These data show that more insight is needed into the reservoirs, driving forces and transmission routes of *S. aureus* in the NICU. As long as the reservoirs and routes are unclear, possible intervention measures might focus on the use of personal protective equipment,

the improvement of compliance with hand hygiene and extra (daily) cleaning or disinfection, to prevent transmission from HCWs to patients.

In **Chapter 3.2**, all neonatal *S. aureus* bloodstream isolates, collected over a 7-year period, were analyzed by WGS to detect whether the transmission and genetic makeup of the isolates support the occurrence of neonatal *S. aureus* bacteremia. **By using WGS, among the 104 *S. aureus* isolates studied, 12 different core genome multilocus sequence-typing (cgMLST) clusters of MSSA isolates were found. Seven of these twelve cgMLST clusters included at least two MSSA isolates, cultured from blood of neonates within one month, indicative of transmission.** Transmission should therefore be considered a contributing factor to the frequent occurrence of neonatal *S. aureus* bacteremia, as was recently described by Rouard et al. (24). Although it seems reasonable to assume that transmission, irrespective of the source, can only occur through the hands of HCWs, we cannot prove this, as no culture data of the environment, the HCWs or the parents are present. Besides transmission, it was determined whether the presence of certain virulence factors is associated with neonatal *S. aureus* bacteremia. Since it was difficult to define a suitable control population of neonates, we chose to compare neonatal *S. aureus* bacteremia isolates to all available *S. aureus* genomes from the Refseq Genome Database (N = 10.288 at the time of analysis). **The genes staphylococcal enterotoxin A (*sea*) and toxic shock syndrome toxin 1 (*tsst-1*) were found a factor 2.6 and 3.2 times more often in the MSSA bloodstream isolates, compared to the reference genomes in the Refseq Genome Database.** The overrepresentation of *tsst-1* could not be explained by the frequent presence of MLST ST5 and ST45 in our isolates collection, since *tsst-1* was not associated with these sequence types. On the other hand, 11 of the 25 isolates carrying *sea* were found in ST5 isolates. Still, this cannot be the full explanation for finding an association between *sea* and neonatal MSSA bacteremia. Many studies have been executed on *S. aureus* toxins and their pathogenic roles, particularly on *sea* and *tsst-1*. Previously, it was described that antibody responses to these two specific toxins were higher in patients with *S. aureus* bacteremia, compared to control patients (25). In addition, in a recent publication about a MSSA outbreak on a NICU, *tsst-1* and especially *sea* were found in bloodstream isolates, compared to colonization isolates (24).

The main conclusion from this study is that the occurrence of *S. aureus* bacteremia in neonates could partly be explained by the transmission of MSSA.

The toxins *sea* and *tsst-1* might play a role in neonatal bacteremia. This finding warrants further investigation.

FUTURE PERSPECTIVES

The studies described in this thesis have all contributed to the existing knowledge about *S. aureus* colonization, carriage and transmission in a high-risk population. In the future, the

newly developed animal model might act as model to study the colonizing capacity of different (emerging) *S. aureus* strains such as ST398, and to test the strains' characteristics, for instance. Also, new decolonization strategies can be tested using this model. As mupirocin resistance is increasing, in particular in MRSA strains (26), there is an urgent need to find another effective and easy-to-apply, topical antibacterial drug to eradicate *S. aureus* from the nasal cavity.

From the transmission studies, we have learned that improvements can be made in the field of infection prevention. In future studies in neonatal wards, it would be interesting to prospectively follow up different sources of MSSA in HCWs, the environment and maybe also parents, to detect carriage and to monitor routes of possible transmission, with the main goal to prevent future transmission and infections in neonates. Furthermore, in the future, strains originating from neonates who are colonized by or suffer from *S. aureus* infections, could be compared by using WGS, to determine the number of virulence factors present and to determine whether there are differences between colonization and infection strains. Ideally, it would be interesting to be able to predict whether a strain has more potential to become invasive based on the genetic package, including the toxin genes. The toxins *sea* and *tsst-1* that were found to be more present in neonatal bacteremia isolates, are interesting targets to begin with and figure out what their role is in the pathogenesis of *S. aureus*.

REFERENCES

1. Nouwen J, Boelens H, van Belkum A, Verbrugh H. Human factor in Staphylococcus aureus nasal carriage. Infect Immun. 2004;72(11):6685-8.
2. 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(12):1820-6.
3. Wertheim HF, Walsh E, Choudhurry R, Melles DC, Boelens HA, Miajlovic H, et al. Key role for clumping factor B in Staphylococcus aureus nasal colonization of humans. PLoS Med. 2008;5(1):e17.
4. Verkaik NJ, Benard M, Boelens HA, de Vogel CP, Nouwen JL, Verbrugh HA, et al. Immune evasion cluster-positive bacteriophages are highly prevalent among human Staphylococcus aureus strains, but they are not essential in the first stages of nasal colonization. Clin Microbiol Infect. 2011;17(3):343-8.
5. van den Berg S, van Wamel WJ, Snijders SV, Ouwerling B, de Vogel CP, Boelens HA, et al. Rhesus macaques (Macaca mulatta) are natural hosts of specific Staphylococcus aureus lineages. PLoS One. 2011;6(10):e26170.
6. Giachino P, Engelmann S, Bischoff M. Sigma(B) activity depends on RsbU in Staphylococcus aureus. J Bacteriol. 2001;183(6):1843-52.
7. van Cleef BA, Graveland H, Haenen AP, van de Giessen AW, Heederik D, Wagenaar JA, et al. Persistence of livestock-associated methicillin-resistant Staphylococcus aureus in field workers after short-term occupational exposure to pigs and veal calves. J Clin Microbiol. 2011;49(3):1030-3.
8. Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, Pace NR. The human nasal microbiota and Staphylococcus aureus carriage. PLoS One. 2010;5(5):e10598.
9. Sakr A, Bregeon F, Mege JL, Rolain JM, Blin O. Staphylococcus aureus Nasal Colonization: An Update on Mechanisms, Epidemiology, Risk Factors, and Subsequent Infections. Front Microbiol. 2018;9:2419.
10. Carey AJ, Duchon J, Della-Latta P, Saiman L. The epidemiology of methicillin-susceptible and methicillin-resistant Staphylococcus aureus in a neonatal intensive care unit, 2000-2007. J Perinatol. 2010;30(2):135-9.
11. Dolapo O, Dhanireddy R, Talati AJ. Trends of Staphylococcus aureus bloodstream infections in a neonatal intensive care unit from 2000-2009. BMC Pediatr. 2014;14:121.
12. Ericson JE, Popoola VO, Smith PB, Benjamin DK, Fowler VG, Benjamin DK, Jr., et al. Burden of Invasive Staphylococcus aureus Infections in Hospitalized Infants. JAMA Pediatr. 2015;169(12):1105-11.
13. Hakim H, Mylotte JM, Faden H. Morbidity and mortality of Staphylococcal bacteremia in children. Am J Infect Control. 2007;35(2):102-5.
14. Kaufman D, Fairchild KD. Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. Clin Microbiol Rev. 2004;17(3):638-80, table of contents.
15. Achermann Y, Seidl K, Kuster SP, Leimer N, Durisch N, Ajdler-Schaffler E, et al. Epidemiology of Methicillin-Susceptible Staphylococcus aureus in a Neonatology Ward. Infect Control Hosp Epidemiol. 2015;36(11):1305-12.
16. Gomez-Gonzalez C, Alba C, Otero JR, Sanz F, Chaves F. Long persistence of methicillin-susceptible strains of Staphylococcus aureus causing sepsis in a neonatal intensive care unit. J Clin Microbiol. 2007;45(7):2301-4.
17. Huang YC, Lien RI, Su LH, Chou YH, Lin TY. Successful control of methicillin-resistant Staphylococcus aureus in endemic neonatal intensive care units--a 7-year campaign. PLoS One. 2011;6(8):e23001.
18. Koningstein M, Groen L, Geraats-Peters K, Lutgens S, Rietveld A, Jira P, et al. The use of typing methods and infection prevention measures to control a bullous impetigo outbreak on a neonatal ward. Antimicrob Resist Infect Control. 2012;1(1):37.

19. Lin MF, Huang ML, Lai SH. Investigation of a pyoderma outbreak caused by methicillin-susceptible Staphylococcus aureus in a nursery for newborns. J Hosp Infect. 2004;57(1):38-43.
20. Ramsing BG, Arpi M, Andersen EA, Knabe N, Mogensen D, Buhl D, et al. First outbreak with MRSA in a Danish neonatal intensive care unit: risk factors and control procedures. PLoS One. 2013;8(6):e66904.
21. Regev-Yochay G, Rubinstein E, Barzilai A, Carmeli Y, Kuint J, Etienne J, et al. Methicillin-resistant Staphylococcus aureus in neonatal intensive care unit. Emerg Infect Dis. 2005;11(3):453-6.
22. Reich PJ, Boyle MG, Hogan PG, Johnson AJ, Wallace MA, Elward AM, et al. Emergence of community-associated methicillin-resistant Staphylococcus aureus strains in the neonatal intensive care unit: an infection prevention and patient safety challenge. Clin Microbiol Infect. 2016;22(7):645 e1-8.
23. Price JR, Cole K, Bexley A, Kostiou V, Eyre DW, Golubchik T, et al. Transmission of Staphylococcus aureus between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. Lancet Infect Dis. 2017;17(2):207-14.
24. Rouard C, Bourgeois-Nicolaos N, Rahajamanana L, Romain O, Pouga L, Derouin V, et al. Evaluation of an 'all-in-one' seven-day whole-genome sequencing solution in the investigation of a Staphylococcus aureus outbreak in a neonatal intensive care unit. J Hosp Infect. 2019;102(3):297-303.
25. Verkaik NJ, Dauwalder O, Antri K, Boubekri I, de Vogel CP, Badiou C, et al. Immunogenicity of toxins during Staphylococcus aureus infection. Clin Infect Dis. 2010;50(1):61-8.
26. Poovelikunnel T, Gethin G, Humphreys H. Mupirocin resistance: clinical implications and potential alternatives for the eradication of MRSA. J Antimicrob Chemother. 2015;70(10):2681-92.

5

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Staphylococcus aureus (*S. aureus*) is een bacterie die met name de neus van ongeveer 30% van de gezonde bevolking koloniseert. In deze 30% is de aanwezigheid van de bacterie hardnekkig, dat wil zeggen, over zeer lange tijd. Dit zogeheten *S. aureus* dragerschap is als permanent te beschouwen. De overige 70% van de bevolking draagt af en toe, of zelden tot nooit, deze bacterie bij zich. Naast de neus kunnen ook de keel, oksels, liezen en vagina gekoloniseerd zijn met *S. aureus*. Of iemand deze bacterie bij zich draagt heeft niets te maken met hygiëne. Bij iedereen zijn talloze bacteriën aanwezig op de huid en slijmvliezen. Het permanente dragerschap met *S. aureus* levert doorgaans geen problemen op, vooral niet in mensen met een goede gezondheid. We weten echter wel dat permanente *S. aureus* dragers een hoger risico lopen op het ontwikkelen van (ernstige) infecties met dezelfde *S. aureus* die zij ongemerkt in hun neus bij zich dragen. Voorbeelden van veel voorkomende *S. aureus* infecties zijn: een huidinfectie of een wondontsteking nadat een persoon een operatie heeft ondergaan, een hartklepinfectie bij mensen met hartklepafwijkingen, een bloedbaaninfectie nadat eerst de huid met *S. aureus* geïnfecteerd is geraakt of een infectie (korte of soms langere tijd) na de plaatsing van een kunstheup of -knie.

Onder de microscoop ziet *S. aureus* eruit als een druiventros en op een voedingsbodem in een petrischaal heeft *S. aureus* een gouden kleur. Om deze reden werd de bacterie vroeger 'golden staph' genoemd. Ondanks het feit dat we *S. aureus* al bijna 150 jaar kennen, zijn er ten aanzien van zijn gedrag nog steeds heel veel dingen onbekend. Zo snappen we nog niet goed waarom sommige mensen wél gekoloniseerd raken en sommigen juist niet. Sinds jaren worden meerdere aspecten bestudeerd die van invloed zouden kunnen zijn op het gedrag van *S. aureus*. In het reeds uitgebreid bekende genenpakket van *S. aureus* wordt bijvoorbeeld geprobeerd genen aan te wijzen die ervoor zorgen dat iemand gekoloniseerd raakt. Dit soort experimenten, inoculatiestudies genoemd, worden bijna altijd uitgevoerd in diermodellen (gebruikmakend van bijvoorbeeld muizen of katoenratten). In dit type studie worden levende bacteriën, doorgaans in de neus, aangebracht om overleving en interactie tussen dier en bacterie te testen. Ook kunnen experimenten op de mens worden uitgevoerd. Het Erasmus MC te Rotterdam heeft het enige bestaande, menselijke *S. aureus* inoculatiemodel opgezet én in gebruik, maar door ethische beperkingen wordt het steeds lastiger om dit model toe te passen. Een andere mogelijkheid om meer te leren over het gedrag van *S. aureus*, is het bestuderen van de afweerreactie gericht tegen *S. aureus* in het bloed van personen die permanent drager of juist geen drager zijn. Verder kan er ook gekeken worden naar overdracht (transmissie) van *S. aureus* in het ziekenhuis, met name op afdelingen met kwetsbare groepen patiënten, bijvoorbeeld mensen die een hartoperatie hebben ondergaan of pasgeboren baby's. Met transmissie wordt het ongemerkt verplaatsen van *S. aureus* bedoeld, bijvoorbeeld via de handen van een medewerker in de gezondheidszorg. De met *S. aureus* besmette handen raken vervolgens de omgeving of anderen patiënten aan. Het

grootste risico van deze transmissie is dat een 'ontvangende' patiënt een infectie kan krijgen met de overgedragen *S. aureus*. Deze transmissie moet te allen tijde worden voorkomen, maar uit de praktijk blijkt dat dit niet altijd even goed lukt, omdat we soms niet goed in kaart kunnen brengen wie of wat de bron van *S. aureus* is en hoe deze transmissieroute(s) verloopt/verlopen.

In dit proefschrift hebben we meerdere studies verricht die bijdragen aan de kennis over kolonisatie, dragerschap en transmissie van *S. aureus*. In het eerste deel is gekeken of een nieuw (niet-invasief) diermodel in resusapen opgezet kon worden om kolonisatie en eradicatie (verwijdering) van *S. aureus* te bestuderen (**Hoofdstuk 2.1**). De keuze voor een diermodel met resusapen is gemaakt vanwege de vele genetische overeenkomsten met de mens en het feit dat ze natuurlijke dragers zijn van *S. aureus*. Als eerste werden de resusapen getest op aanwezigheid van *S. aureus* en permanente dragers werden over twee groepen verdeeld. Daarna zijn twee (voor mensen gebruikelijke) behandelingen aan de resusapen gegeven om de bacterie tijdelijk uit de neus te eradiceren: een lokale behandeling met alleen een antibacteriële neuszalf (mupirocine) of een behandeling met mupirocine in combinatie met een systemische behandeling (een behandeling om het hele lichaam aan te pakken) door middel van antibiotica-injecties. Het doel van de eradicatie was om de neus van de resusaap extra aantrekkelijk ('leeg') te maken voor de inoculatiebehandeling die een aantal weken later volgde. Tijdens de inoculatiebehandeling werd *S. aureus* stam 8325-4 in de neus van de resusaap aangebracht. We hebben daarna de overleving van *S. aureus* 8325-4 getest, door middel van de afname van neuskweken met een wattenstok, in een periode van een aantal weken na de inoculatie. Uit deze experimenten kwam naar voren dat het mogelijk is om de oorspronkelijke *S. aureus* stammen uit de neuzen van resusapen te eradiceren met de twee behandelingen, en er was geen verschil in uitkomst tussen de behandelingen wanneer alleen gekeken werd naar de neus. De keel en het rectum zijn ook onderzocht op aanwezigheid van *S. aureus* gedurende de studie waarbij de behandeling met de antibiotica injecties iets effectiever leek in het eradiceren van *S. aureus* uit de keel en het rectum. Tevens is het gelukt om de inoculatiebehandeling uit te voeren, maar de variant 8325-4 bleek niet lang te overleven in de neus van de resusaap. We weten nu dat we het resusapen model kunnen gebruiken voor inoculatiestudies en dat inoculatiebehandeling mogelijk zal slagen als er andere varianten van *S. aureus* stammen gebruikt worden. De resusapen zijn medisch goed gecontroleerd en niet ziek geworden gedurende de studieperiode. In **Hoofdstuk 2.2** beschrijven we hoe we een menselijk inoculatie-experiment hebben uitgevoerd met twee varianten van *S. aureus*. De afgelopen jaren hebben ziekenhuizen steeds meer te maken met een, voor veel antibiotica, ongevoelige variant van *S. aureus*, namelijk methicilline-resistente *S. aureus* (MRSA). Het blijkt om een specifiek type MRSA te gaan, MRSA ST398, dat vooral voorkomt onder mensen die in direct contact staan met vee, met name de varkenshouderij. Echter, steeds vaker wordt gerapporteerd dat ook mensen zonder contacten met de varkenshouderijen, MRSA ST398 bij zich kunnen dragen. Het is onduidelijk hoe deze mensen drager

zijn geworden van MRSA ST398. In de literatuur wordt beschreven dat de neus van mensen gemakkelijk 'gecontamineerd' kan raken met MRSA ST398, maar dit kan zelden permanent neusdragerschap worden genoemd. De motivatie om dit menselijke inoculatie-experiment uit te voeren was om te kijken of het toch mogelijk was om de neus van mensen langdurig met *S. aureus* ST398 te koloniseren. Er werd een (klein volume) vloeibare mix van de twee varianten van *S. aureus* aangebracht in de neus van gezonde vrijwilligers, nadat zeven weken daarvoor een eradicatiebehandeling met mupirocine in combinatie met een ontsmettende huidbehandeling (chloorhexidine zeep) was uitgevoerd. De inoculatiemix bevatte een, voor antibiotica gevoelige, uit een kalf afkomstige, methicilline-gevoelige *S. aureus* (MSSA) ST398 variant en een humane *S. aureus* (ST931) variant. De overleving van beide bacteriën werd bestudeerd in een periode van drie weken na de inoculatie. We hebben gevonden dat MSSA ST398 in staat is om te overleven in de neus van gezonde vrijwilligers voor tenminste drie weken. We kunnen stellen dat deze variant tot meer in staat is dan tot dusver werd aangenomen. Daarbij dient te worden opgemerkt dat in de gebruikte studieopzet heel erg over de details werd nagedacht en de vraag is of de situatie vergelijkbaar is met de praktijkomstandigheden waarin mensen ongemerkt in aanraking komen met bacteriën uit de omgeving of via de handen van andere mensen. Voorafgaand aan de inoculatie zijn de neuzen met mupirocine behandeld, wat ook invloed kan hebben gehad op andere bacteriën uit de neus waardoor de mix extra goed heeft kunnen hechten. Een andere reden voor de succesvolle inoculatie kan zijn geweest, dat een grote hoeveelheid bacteriën (1x10log7) in één keer is aangebracht in de neus, terwijl in de omgeving normaliter niet zoveel bacteriën circuleren op een kleine oppervlakte.

In **Hoofdstuk 3.1** en **3.2** is *S. aureus* transmissie onderzocht op de intensive care voor pasgeboren baby's (neonatale intensive care unit (NICU)). Pasgeboren baby's (neonaten), in het bijzonder te vroeg geborenen, hebben door hun lage geboortegewicht, vaak overige problematiek en een nog niet volledig ontwikkelde afweer, een hoog risico op het ontwikkelen van ziekenhuisinfecties. Neonatale sterfte door bloedbaaninfecties met *S. aureus* komt frequent voor. Er zijn op de NICU uitbraken beschreven met *S. aureus*, van zowel MRSA's als MSSA's, wat suggereert dat de bacterie onbedoeld wordt overgedragen binnen de NICU. Sommige van deze studies tonen aan dat zorgmedewerkers de bron blijken te zijn voor de transmissie. Door middel van een genetische analyse ('whole-genome sequencing') hebben we aangetoond dat de *S. aureus* gekweekt uit 35 neonaten en 4 zorgmedewerkers, in 4 verschillende groepen (clusters) in te delen waren, waarbij in elke groep de *S. aureus* genetisch identiek was. De kweken van al deze personen werden afgenomen in een periode van ruim een jaar. Tien neonaten hebben zelfs een bloedbaaninfectie ontwikkeld met *S. aureus* afkomstig uit een van de vier clusters (**Hoofdstuk 3.1**). We kunnen concluderen uit deze studie dat de zorgmedewerker aantoonbaar een belangrijke rol lijkt te spelen in de transmissie van *S. aureus* op de NICU. De neonaten komen daar immers direct na geboorte terecht, terwijl de zorgmedewerkers al langer op de NICU rondlopen.

In **Hoofdstuk 3.2** zijn in een periode van zeven jaar *S. aureus* stammen, afkomstig van neonaten op de NICU met bloedbaaninfecties, met dezelfde genetische analyse methode bekeken. Er is gekeken of deze stammen aan elkaar verwant waren, wat wederom zou kunnen wijzen op transmissie. Daarnaast is ook gekeken of deze stammen virulenter (schadelijker) waren dan andere stammen uit een controlegroep; een grote referentiedatabase is gebruikt waarin vele *S. aureus* zijn gerapporteerd en opgeslagen. Er is specifiek naar genen gekeken waarvan we weten dat deze extra schade kunnen veroorzaken gedurende een infectie met *S. aureus*. We toonden aan dat er over de jaren meerdere clusters met identieke *S. aureus* circuleerden, wat de suggestie wekt dat er een reservoir of bron binnen de NICU is. Het lijkt dus aannemelijk dat *S. aureus* transmissie, tijdens en door de zorg, bijdraagt aan neonatale bloedbaaninfecties. De route en de bron van transmissie zijn in deze studie niet onderzocht. Daarnaast zijn er twee specifieke *S. aureus* genen gevonden die meer leken voor te komen in de *S. aureus* afkomstig uit het bloed van neonaten, in vergelijking tot de controle groep. Deze twee genen heten 'sea' (staphylococcal enterotoxin A) en 'tsst-1' (toxic shock syndrome toxin-1), en verder onderzoek naar de rol van deze genen is aanbevolen. Interessant is om te vermelden dat van deze genen al is beschreven dat ze vaker voorkomen in *S. aureus* stammen die bloedbaaninfecties veroorzaken.

In toekomstig onderzoek zou het nieuwe resusapen-model gebruikt kunnen worden voor inoculatie- en eradicatiestudies waarbij bijvoorbeeld MRSA ST398 onderzocht kan worden. Daarnaast zou onderzoek van *S. aureus* bronnen op de NICU, en daarmee samenhangend, het voorkomen van neonatale infecties aandacht moeten verkrijgen. Belangrijke toxines van *S. aureus* zijn 'sea' en 'tsst-1', maar hun rol in neonatale bacteriemie is nog onduidelijk. Nader onderzoek naar de rol van deze toxinen en mogelijke andere toxinen is gewenst om zo, hopelijk, beter zicht te krijgen op waarom sommige neonaten, of misschien in het algemeen sommige mensen, sneller een *S. aureus* (bloedbaan)infectie krijgen dan anderen. Hierbij is het uiteraard de vraag wat de bepalende factor is in de *S. aureus* pathogenese (het ontstaan, ontwikkelen en verloop van een infectie): de bacterie, de mens of juist beide.

6

Appendices

Dankwoord
Curriculum Vitae
List of publications
Portfolio

DANKWOORD

Waar een wil is, is een weg, maar zonder de hulp van anderen bewandelt niemand het promotiepad.

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Lieve Andrew, jij was de allerbeste motivatie om dit boekje sneller dan snel af te ronden. Ik heb heel veel zin in onze toekomst!

CURRICULUM VITAE

Bibi Clara Gertruida Céline Slingerland werd geboren op 3 maart 1985 te Breda. In 2004 behaalde zij haar VWO diploma aan het Mencia de Mendoza Lyceum te Breda. Zij werd hetzelfde jaar ingeloot voor de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. Haar afstudeeronderzoek met als onderwerp, de verandering van het intestinale microbioom en het effect van prebiotica op colitis in HLA-B27 transgene ratten onder supervisie van prof. dr. L.A. Dieleman en prof. dr. R. Benner, verrichte zij op de afdeling Maag-, Darm- en Leverziekten van The University of Alberta, Edmonton, Canada in 2008. In 2010 behaalde zij het artsexamen en begin 2011 startte zij met het beschreven promotieonderzoek onder leiding van copromotor dr. W.J.B. van Wamel en em. prof. dr. H.A. Verbrugh, later prof. dr. M.C. Vos, op de afdeling Medische Microbiologie en Infectieziekten van het Erasmus MC. Daarnaast startte zij eind 2011 ook met de opleiding tot arts-microbioloog onder supervisie van em. prof. dr. H.A. Verbrugh, mw. dr. A.G. Vonk en dhr. dr. J.M. Ossewaarde. In september 2016 rondde zij de opleiding met 3 maanden korting af. Haar carrière als arts-microbioloog begon in oktober 2016 bij het Regionaal Laboratorium Microbiologie, een laboratorium verbonden aan het Albert Schweitzer Ziekenhuis te Dordrecht en het Beatrix Ziekenhuis te Gorinchem. Na 3,5 jaar perifeer te hebben gewerkt, is zij in maart 2020 teruggekeerd naar het Erasmus MC, waar zij tot op heden werkzaam is op de unit Klinische Virologie van de afdeling Viroscience.

LIST OF PUBLICATIONS

Slingerland, B.C.G.C, Vos, M.C, Bras, W, Kornelisse, R.F, De Coninck, D, van Belkum, A, Reiss, I.K.M, Goessens, W.H.F, Klaassen, C.H.W, & Verkaik, N.J. (2020). *Whole-genome sequencing to explore nosocomial transmission and virulence in neonatal methicillin-susceptible Staphylococcus aureus bacteremia.* Antimicrobial Resistance and Infection Control*,* 9*(1). doi:10.1186/s13756-020-0699-8*

Slingerland, B.C.G.C, Verkaik, N.J, Klaassen, C.H.W, Zandijk, W.H.A, Reiss, I.K.M, & Vos, M.C. (2019). *Neonatal Staphylococcus aureus acquisition at a tertiary intensive care unit.* American Journal of Infection Control*. doi:10.1016/j.ajic.2019.11.011*

Sarink, M.J, Koelewijn, R, **Slingerland, B.C.G.C**, Tielens, A.G.M, van Genderen, P.J.J, & van Hellemond, J.J. (2018). *Performance of the commercially available SERION ELISA classic Echinococcus IgG test for the detection of cystic echinococcosis in clinical practice.* Journal of Helminthology*, 1–4. doi:10.1017/S0022149X18000536*

Thai, K.T.D, Götz, H.M, **Slingerland, B.C.G.C**, Klaasse, J, Schutten, M, & Geurts van Kessel, C.H. (2018). *An analysis of the predictive value of the HIV Ag/Ab screening assay within the performance characteristics of the DiaSorin LIAISON XL for the detection of blood-borne viruses*. Journal of Clinical Virology*,* 102*, 95–100. doi:10.1016/j.jcv.2018.02.018*

Slingerland, B.C.G.C, Keehnen, M, Ouwerling, B, Tavakol, M, Snijders, S.V, Verbrugh, H.A, Vos, M.C, Remarque, E.J, Langermans, J.A.M, & van Wamel, W.J.B. (2018). *An experimental Staphylococcus aureus carriage and decolonization model in rhesus macaques (Macaca mulatta).* PLoS ONE*,* 13*(4). doi:10.1371/journal.pone.0194718*

K. van Dijk, M.B.B. McCall, **B.C.G.C. Slingerland,** O. Pontesilli, M.J. Schijffelen, J. Jansen, B. Vlaminckx, J.E.M. de Steenwinkel. *Brucella – een vaak onverwachte verwekker van persisterende koorts.* NTMM, 22e jaargang, maart 2014.

Slingerland, B.C.G.C, Tavakol, M, McCarthy, A.J, Lindsay, J.A, Snijders, S.V, Wagenaar, J.A, van Belkum, A, Vos, M.C, Verbrugh, H.A, & van Wamel, W.J.B. (2012). *Survival of Staphylococcus aureus ST398 in the Human Nose after Artificial Inoculation.* PLoS ONE*,* 7*(11). doi:10.1371/journal.pone.0048896*

PHD PORTFOLIO

PhD student: Bibi Clara Gertruida Céline Slingerland
Erasmus MC Department: Medical Microbiology and Infectious Diseases
PhD period: 2011 – 2020
Promotor: Prof. Dr. M.C. Vos
Co-promotor: Dr. W.J.B. van Wamel

	Year
Courses	
- Biostatistics & Survival Analysis, Molmed, Erasmus MC, Rotterdam	2011, 2012
- BROK (Basiscursus Regelgeving Klinisch Onderzoek), Erasmus MC, Rotterdam	2012
- Phenotypic interpretation of the antimicrobial susceptibility determination, Molmed, Erasmus MC, Rotterdam	2013
- Openbare Gezondheidszorg, RIVM, Bilthoven	
- Gezondheidsrecht, Erasmus MC Desiderius School, Rotterdam	
- Communicatie, Erasmus MC Desiderius School, Rotterdam	
- Klinische mycologie, Radboud Universitair Medisch Centrum, Nijmegen	2014
- Laboratorium diagnostiek van humane parasitaire infecties, Leids Universitair Medisch Centrum, Leiden	
- Samenwerking, Erasmus MC Desiderius School, Rotterdam	
- Ziekenhuismanagement, Erasmus MC Desiderius School, Rotterdam	2015
- Medische Ethiek, Erasmus MC Desiderius School, Rotterdam	2016
- Infectiepreventie, Amphia Academy Infectious Disease Foundation, Utrecht	
- Basiscursus Ziekenhuismanagement voor AIOS (NVMM), Epe	
- Serologische Diagnostiek van Infectieziekten, VU Medisch Centrum, Amsterdam	2019
(Inter)national conferences/presentations	
- Pilgrim Concord Joint Symposium, Brussels, Belgium (*oral presentation*)	2011
- NVAMM symposia, Amsterdam and Utrecht, The Netherlands	2012 - 2016
- Scientific Spring Meeting NVMM, Papendal, The Netherlands (*poster presentation*)	2012
- International Symposium on Staphylococci and Staphylococcal Infections (ISSSI) Lyon, France (*poster presentation*)	
- Scientific Spring Meeting NVMM, Papendal, The Netherlands (*poster presentation*)	2013
- Scientific Spring Meeting NVMM, Papendal, The Netherlands	2014
- Scientific Spring Meeting NVMM, Papendal, The Netherlands	2015
- Scientific Spring Meeting NVMM, Papendal, The Netherlands (*poster presentation*)	2016
- European Congress on Clinical Microbiology and Infectious Diseases (ECCMID), Amsterdam, The Netherlands	2017
- NVAMM symposium, Rotterdam, The Netherlands	
- Teach-the-teacher III, Erasmus MC, Rotterdam, The Netherlands	2018
- Scientific Spring Meeting NVMM, Papendal, The Netherlands	
- RODIN bijeenkomst: thema Influenza, Rotterdam, The Netherlands	
- SWAB meeting: Invasieve Mycosen, Utrecht, The Netherlands	
- Netwerkdag ABR Zorgnetwerk Zuidwest Nederland, Dordrecht, The Netherlands	
- Symposium 'The clinical utility of bacteriophages; theory, science and practice', Universitair Medisch Centrum Utrecht, The Netherlands	
- Scientific Spring Meeting NVMM, Papendal, The Netherlands	2019
- Prosthetic Joint Infections (PJI) Workshop, Berlin, Germany	
- International Symposium on Staphylococci and Staphylococcal Infections (ISSSI), Kopenhagen, Denmark (*poster presentation*)	
- NVAMM symposium, Den Bosch, The Netherlands	
- OOR symposium: Docentprofessionalisering, Het Congresbureau Erasmus MC	2020
- NVMM wetenschappelijke najaarsvergadering, Utrecht, The Netherlands	
- NVAMM symposium, Utrecht, The Netherlands	

Teaching activities	
- Supervision of 2nd year medical students: vaardigheidsonderwijs Thema 'Infectieziekten'	2012 – 2016
- Education/supervision of residents in Medical Microbiology/Infectious Diseases	2016 – present
Other activities	
- Member of the NVAMM Opleidingscommissie	2014 – 2016
- Member of the NVMM Commissie Nascholing	2017 – present
Grant	
- Erasmus MC Travel Grant, Trustfonds	2012