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

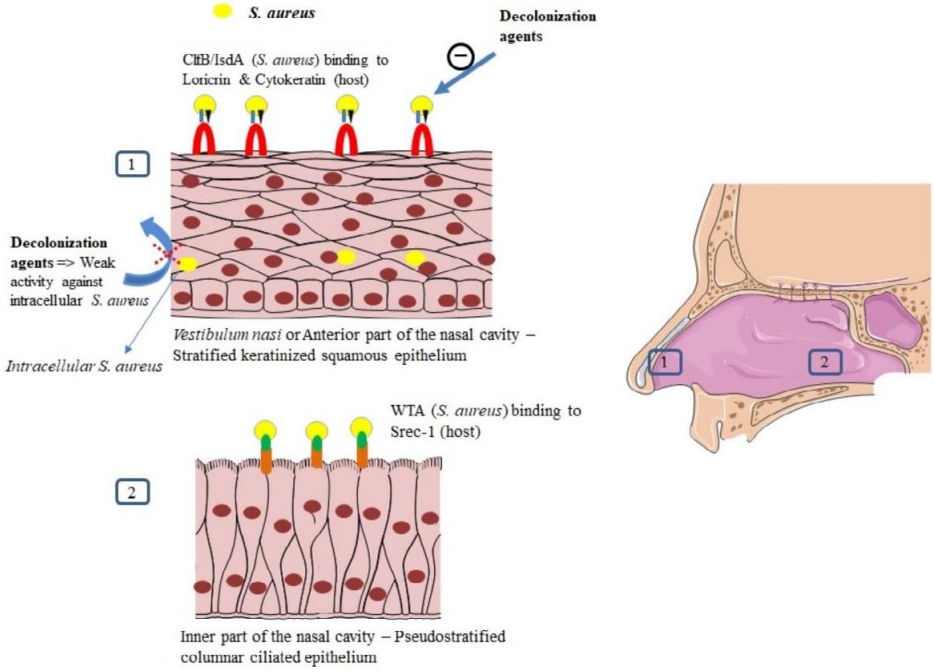
patients suffering from rheumatoid arthritis and granulomatosis with polyangiitis (5). Until now, there is no clear explanation why these populations are at risk for carriage. As far as we know, genetic factors do not play a role in whether or not humans become *S. aureus* carriers (15, 16).

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).

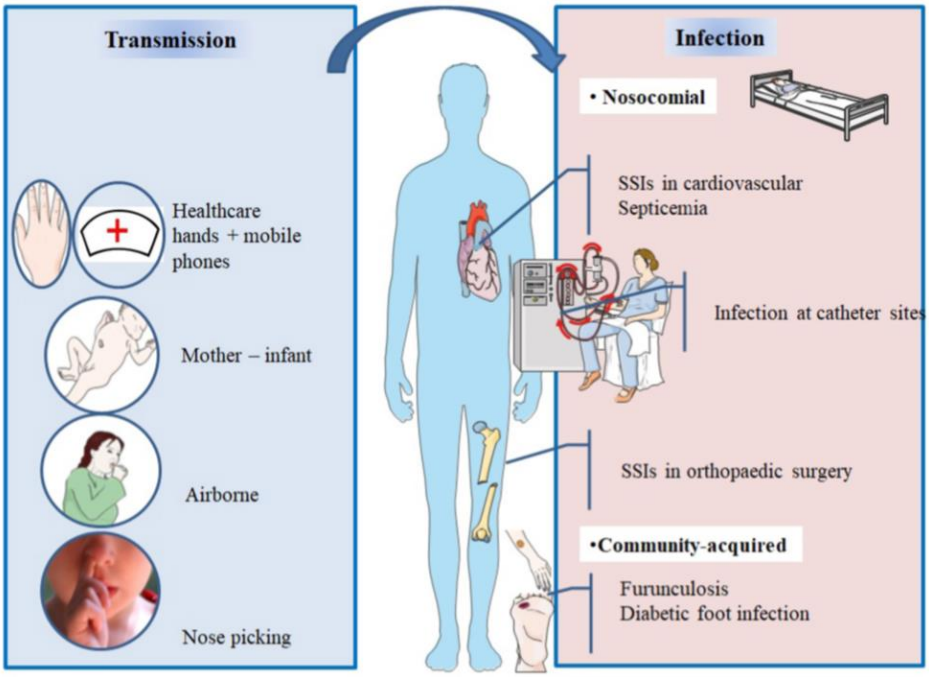
In the Netherlands, the prevalence of MRSA at hospital admission is very low, namely 0.13% between 2010-2017 (54). Due to a Search and Destroy policy (S&D), we are able to keep these numbers low. In Europe, including the Netherlands, for years there has been an increased incidence of carriage of a livestock-associated (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.

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