Molecular Epidemiology of *Staphylococcus aureus* Nasal Carriage and Wound Colonization in a Burn Centre

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Molecular Epidemiology of *Staphylococcus aureus* Nasal Carriage and Wound Colonization in a Burn Centre

Moleculaire epidemiologie van *Staphylococcus aureus* neusdragerschap en wondkolonisatie op een brandwondencentrum

Proefschrift

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Aan mijn ouders

Voor André en mijn lieve kids

CONTENTS

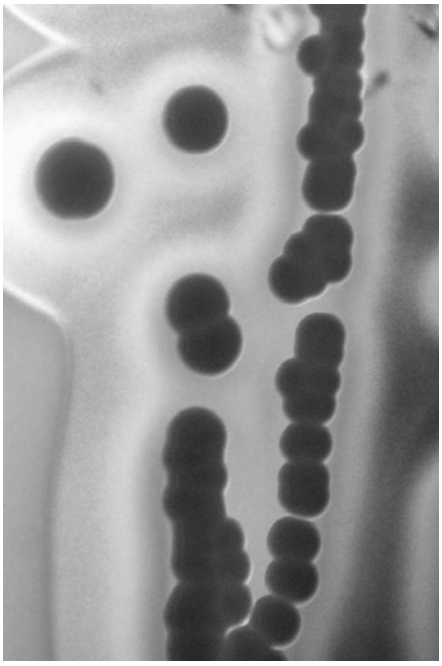
Part III Discussion

PART I

INTRODUCTION

CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS



INTRODUCTION

Human skin is vital to the preservation of body fluid homeostasis, thermoregulation, protection against the harmful effects of UV-irradiation, and the prevention of tissue invasion by micro-organisms. Organisms of diverse species naturally colonise different layers of the skin. They interact via specific ligands with receptors in host tissues (1). Thermal injury creates a breach in the surface of the skin and alters the variety and exposition of specific host' receptors (2, 3). The wound resulting from such injury is a protein-rich environment consisting of avascular necrotic tissue that provides an even more favourable niche for microbial colonization and proliferation than healthy skin does (4-6). Although immediately following thermal injury, burn wounds are sterile, these wounds will soon become colonized with micro-organisms (4, 7). Within hours or days, Gram-positive bacteria, including *Staphylococcus aureus* (*S. aureus*) will colonize the burn wound; Gram-negative species are isolated from wounds at a later stage post thermal injury (4, 8).

The major human pathogen *S. aureus* asymptomatically colonizes the skin and mucosal membranes of approximately 30% of the healthy human population (9). Although recently debated (van Belkum *et al*., submitted), three carriage patterns can be distinguished when cohorts of individuals are sampled repeatedly: persistent, intermittent and non-carriage (10). The prime ecological niche of *S. aureus* in humans is the anterior nares. However, from this niche *S. aureus* may spread to various other parts of the body, especially to the throat and gastrointestinal tract and to the skin of the hands, arms and other sites. Secondary seeding of these body sites is especially noted in individuals persistently colonized with *S. aureus* (Figure 1).

S. aureus cells also contaminate the inanimate environment when shed from the body of persistent carriers. Squames of skin slough off constantly to settle as dust and micro droplets. Direct contact between contaminated hands and surfaces in the environment provide other means for *S. aureus* ' dissemination.

Nasal carriage of *S. aureus* appears to play a key role in the epidemiology and pathogenesis of staphylococcal infection in that carriers have a 3-6 fold increased risk of developing *S. aureus* infection compared to individuals who do not carry *S. aureus* or do so only intermittently (9, 11, 12). *S. aureus* may cause a wide spectrum of diseases ranging from simple skin infections and food poisoning, to major life-threatening severe infections such as pneumonia, sepsis and endocarditis (13). *S. aureus* is one of the most common causes of wound infection. Hundreds of *S. aureus* virulence factors and their coding genes have been described. Many of these genes are variably present among *S. aureus* strains and define a strains' individual invasive capacities (14, 15).

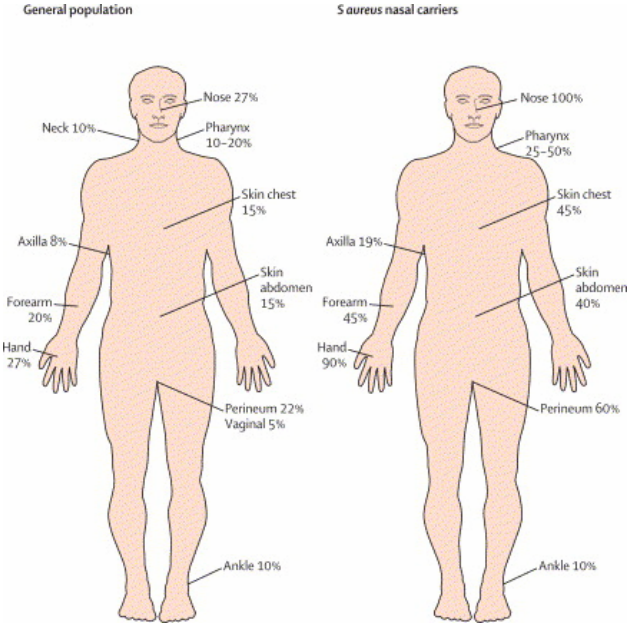


Figure 1: S. aureus carriage rates per body site in adults. (Adapted from Wertheim et al. (10)).

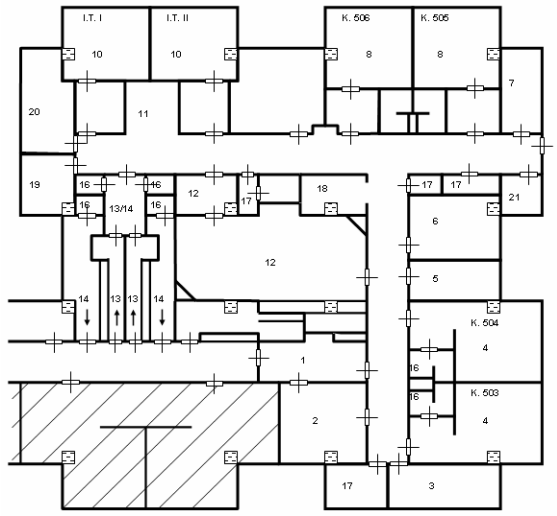
Burn wound colonization by *S. aureus* can be derived from the host when the particular strain was already present in the upper respiratory and/or gastrointestinal flora prior to the injury. In these cases, colonization can be classified as *endogenous*. The other sources of colonization of burn wounds may be *S. aureus* carriers in the direct vicinity of the patient, including the health care workers (HCWs) and other patients, the environment (air and surfaces), and animals. This type of colonization can be classified as *exogenous*. Thus, in a hospital setting essentially, five clinical reservoirs of *S. aureus* may exist (simultaneously or separately): the patient him/herself, the health care workers, other patients, contaminated air, and surfaces in the inanimate environment. Colonization of the nose, throat, skin or wound in a given individual can give rise to additional contamination of individuals and the environment. This may lead to transmission and to new cases of colonized wounds.

Patients with burns are known to be at a high risk of contracting nosocomial pathogens and, hence, infection. Several studies have shown that the rate of burn wound colonization with *S. aureus* varies considerably (18-19). Colonization of burn wounds with *S. aureus* has been associated with delayed wound healing and prolonged length of patient' stay at the burn centre (6, 17).

To reduce the risk of *S. aureus* colonization, a variety of preventive measures have been implemented. At the Burn Centre in Groningen, located within the Martini Hospital, these consisted of the following. First, this Burn Centre, at the time of the study, was a closed centre including an operating theatre. Entry into the Burn Centre was limited via an anteroom, and involved the use of specialized clothing. The centre provided four rooms with two beds each and two one-bed Intensive Treatment (IT) rooms, accommodating a total of 10 patients (Figure 2). A dedicated team of HCWs, who have no healthcare duties outside the Burn Centre, took care of the patients. In addition to this team, other HCWs (e.g. psychologists and dieticians) visited the center daily to tend to patients. Burn patients were cohorted, and strict contact precautions were implemented. Patients remained at their room during the entire hospital stay, except for bathing and for surgical interventions. Secondly, burn wounds were routinely covered with ointment containing silver sulphadiazine in combination with cerium nitrate (Flammacerium®). Finally, adults with more than 20% TBSA and children aged 16 years or younger with more than 15% TBSA received an antibiotic regimen for selective decontamination of the digestive tract (SDD). SDD was given from admission until wounds were healed spontaneously or 5 days after the last surgical intervention. Hand washing procedures, barrier isolation, waste management, equipment and environmental cleansing and disinfection were daily routines. During wound dressing changes, precautions were brought to an even higher level. Additional measures involved the use of gowns, surgical masks and gloves by all HCWs in contact with the patients or the patients' environment. However, despite all these preventive measures *S. aureus* burn wound colonization still occurred frequently at this and most other centres.

To eliminate nasal *S. aureus* topical mupirocin has been widely used, particularly during outbreaks caused by methicillin resistant strains (MRSA) (21, 22). Mupirocin prophylaxis has been shown to reduce the nasal carriage rate, and hence, clinical infection in surgical, dialysis and in AIDS patients (25-26). The efficacy of nasal mupirocin on *S. aureus* burn wound colonization has been evaluated only in one historically controlled trial; Mackie *et al.* (27) found a significant reduction in *S. aureus* burn wound colonization when using nasal mupirocin and selective decontamination of the digestive tract in patients with more than 30% TBSA. It is important to elucidate all reservoirs and transmission routes of *S. aureus* at a Burn Centre in order to prevent colonization and infection with this species. When transmission routes between reservoirs are carefully mapped, effective clinical interventions can be designed. For example, for vancomycin resistant enterococci (VRE) careful mapping led to mathematical models explaining infection dynamics in intensive care settings (28, 29).

The Groningen Burn Centre is a semi-closed setting with a dedicated team taking care of patients with burns only, making it an ideal setting for studying the *S. aureus* population



1 Entrance and exit for visitors
2 Admission room
3 Office nurse manager
4 2-beds patients' room, no sluice
5 Secretariat
6 Dayroom
7 Kitchen
8 2-beds patients' room, sluice
9 Meeting room
10 Intensive Treatment rooms
11 Reception
12 Operating theatre
13 Entrance health care workers
14 Exit health care workers
15 Exit for waste materials
16 Toilets and showers
17 Supply closet
18 Room for rinsing materials
19 Room for medicaments
20 Room for physicians
21 Ergotherapy

Figure 2. Floor plan of the Groningen Burn Centre during the studyperiod (20).

dynamics and kinetics in colonization and infection.

This thesis focuses on *S. aureus* burn wound colonization in relation to *S. aureus* nasal carriage in patients and HCWs of a Burn Centre.

The main aim of the research described in this thesis was to more precisely delineate the reservoirs and transmission routes of *S. aureus* in this dedicated Burn Centre, and to estimate the effect of targeted interventions on the rate of burn wound colonization and infection by *S. aureus*. In **Chapter 2** a review is presented of the literature regarding the determinants of burn wound colonization of *S. aureus* nasal carriage and the effect of several prophylactic measures on *S. aureus* burn wound colonization. In **Chapter 3** we describe the first extensive bacterial genotyping study carried out at the Groningen Burn Centre. This study helped phrase 3 new research questions:

1. What would be the effect of a short course of intranasal mupirocin treatment at admission for all patients on *S. aureus* burn wound colonization?
2. What would be the effect of a short course of intranasal mupirocin treatment for HCWs on *S. aureus* burn wound colonization?
3. What is the genetic population structure of *S. aureus* isolates prospectively obtained from patients and HCWs?

For research question 1 and 2 intervention studies were performed at the Groningen Burn Centre. After the first intervention, i.e. intranasal application of mupirocin in all patients upon admission, a washout period of 6 months was introduced in order to dilute

the effect of this intervention, and assess whether a steady state situation was again reached.

The first two research questions are addressed in **Chapter 4** and **Chapter 5,** respectively. In **Chapter 6** genotyping data of all serial *S. aureus* strains that were isolated over a period of 5 years from burn wounds of patients during their stay at the Groningen Burn Centre, are compared with the sets of isolates obtained from patients at admission and from HCWs. Diversity at the gene level was assessed by DNA array technology for endemic versus incidental *S. aureus* isolates.
For designing prophylactic strategies it is most helpful to design and test a mathematical model of the epidemiology of *S. aureus* colonization in a dedicated Burn Centre. **Chapter 7** describes a study in which the microbiological and clinical data gathered during the preceding intervention studies was used to construct such a mathematical model.

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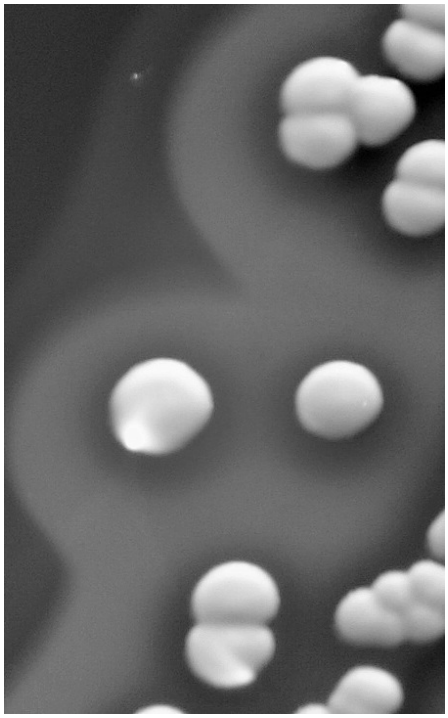
CHAPTER 2

THE ROLE OF NASAL CARRIAGE IN *STAPHYLOCOCCUS AUREUS* BURN WOUND COLONIZATION

A.M.D. Kooistra-Smid, M.K. Nieuwenhuis, A. van Belkum, H.A. Verbrugh



INTRODUCTION

The skin or integument covers the entire external surface of the human body and is the principle site of interaction with the surrounding world. It serves as a protective barrier preventing internal tissues from exposure to trauma, ultraviolet radiation, temperature extremes, hazardous chemicals, toxins, and, not in the least, micro-organisms. Other important functions include sensory perception, immunologic surveillance, thermoregulation, and control of insensible fluid loss (1). Thermal injuries of the skin and concomitant depression of local and systemic host cellular and humoral immune responses are important factors which contribute to colonization and infection of the burn wound (2, 3). Micro-organisms colonizing the burn wounds may originate from the patient's endogenous respiratory and gastrointestinal flora but may also be transferred to a patient's skin surface via contact with contaminated external environmental surfaces, the hands of health care workers and the air (4, 5, 6). One of the most common burn wound pathogens is *Staphylococcus aureus* (*S. aureus*). *S. aureus* is both a human commensal and a frequent cause of infections ranging from mild to life-treatening diseases. Colonization with *S. aureus* has been associated with delayed wound healing, increased need for surgical interventions and prolonged length of stay at burn centres (7,8).
Nasal carriage of *S. aureus* plays a key role in the development of *S. aureus* infection. In several recent reviews the mechanisms, risks and treatment of *S. aureus* nasal carriage and infection have been described (9-12). In this chapter we focus on *S. aureus* nasal carriage in relation to *S. aureus* burn wound colonization and subsequent infection, and its impact on strategies for infection control.

GENERAL

The skin

Skin, the largest organ of the body, weighing about one sixth of the total bodyweight, is supplied with one third of the circulating blood volume. Human skin is the interface between the external environment and the internal environment composed of organs, connective tissue, and bones. Skin is a multifunctional organ that provides protection, sensation, thermoregulation, biochemical, metabolic, and immune functions. Human skin is colonized with micro-organisms. Many of these organisms actually provide resistance to pathogenic microorganisms through bacterial interference (13).
The normal flora of the skin can be thought of as either resident or transient. Resident bacteria are those that normally persistently inhabit an individual's skin. Grice *et al*. (14)

recently reported a 16s RNA gene survey of the resident skin microbiota of the inner elbow regions of healthy humans. They used three sampling methods (swab, scrape and punch biopsy) to survey the microbiota at different penetration levels of the skin. They reported the same dominant phylotypes of macrobiota at all depths of sampling. *Proteobacteria* dominated all skin microbiota followed by *Actiniobacteria* and *Firmicutes*. Apparently, in the division *Proteobacteria* the genera *Pseudomonas* and *Janthinobacterium* were predominant.

Moist areas and covered skin surfaces such as the axilla, perineum, and toe webs are especially attractive to bacteria. The hair follicles, nail beds, and sweat glands are other areas were bacteria like to live. Microcolonies of bacteria can also be found at the edges of the squames as halos in the upper loose epidermal surface layers of the skin or stratum corneum. Several species of bacteria are found by culture in human skin including *Staphylococcus* spp., *Micrococcus* spp., *Peptococcus* spp., *Corynebacterium* spp., *Brevibacterium* spp., *Propionibacterium* spp., *Streptococcus* spp., *Neisseria* spp., and *Acinetobacter* spp. (5). Not all of these are found on any one individual, but most humans carry at least five of these genera.

Transient bacteria are those species not normally found on a particular person's skin, but which are lost through daily hygienic measures such as hand washing and bathing. Transient bacteria are acquired through contact with other individuals or exposure to bacteria-laden surfaces.

Data on normal viral flora is scarce, although viruses have been detected in damaged skin or in immunocompromised individuals (15, 16).

Thermal injury of the skin

Several important physiological functions of the skin are severely compromised by thermal injury. Disruption of the skin can result in infection, fluid loss, hypothermia, scarring, and compromised immunity (5).

Pathogenesis and etiology of burns

Breaches in the skin barrier are the main hallmark of thermal injury. The body tries to maintain homeostasis by initiating a process of contraction, retraction, and coagulation of blood vessels immediately after a burn injury. Three distinct zones of a burn wound have been described by Jackson in 1953 (17). The zone of coagulation comprises the dead tissue that forms the burn eschar that is located at the centre of the wound nearest to the heat source. The zone of stasis comprises tissues adjacent to the area of burn necrosis that are still viable but remain at risk for ongoing ischemic damage due to decreased perfusion. Finally, the zone of hyperemia comprises normal skin with minimal cellular

injury that predominantly has vasodilatation and increased blood flow as a response to injury (18-19) (Figure 1).

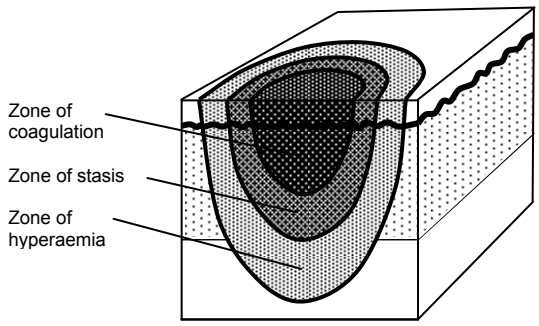


Figure 1. Jackson's burns zones

A superficial (first degree) burn, the least serious type, is one in which the epidermis of the skin has been burned slightly. These burns produce pain, redness, and swelling of the skin. In partial thickness (second degree) burns the epidermis and the dermis are destroyed, causing pain, redness, swelling, and blistering. These wounds can become infected more easily than superficial burn wounds. Also, if the partial thickness burn affects more than 10% of the skin surface, a patient may go into shock because large quantities of fluid are lost via the burned area. Damage from full thickness (third degree) burns extends into the hypodermis, causing destruction of the full thickness of skin with its nerve supply; the skin becomes whitened, blackened or even charred. Full thickness burns leave scars and may cause persisting loss of function and/or sensation (21).

Because of the importance of the skin as a barrier to host' microbial invasion, it is not surprising that the risk of subsequent burn wound colonization and infection, and subsequent systemic infection correlates with the size of burn injury (22, 23).

The extent of a burn wound, expressed as the percentage of the total body surface area (TBSA) that is burned, and the depth of the burn wound are the most important predictors of clinical outcome. The percentage of TBSA affected is used to calculate the patient's fluid and nutritional needs, which can be enormous for those with severe burns. Burn depth, on the other hand, dictates subsequent local and surgical treatment of burn wounds (21). An estimate of the percentage TBSA can be made by a method in which the patient's own hand is used as a complementary, readily available template (25, 26). The entire palmar surface area of the hand (including the fingers) is approximately 1% of the total body surface area. Another method to estimate the percentage of TBSA is by

using e.g. a Lund-Browder chart (27). The Lund-Browder system uses fixed percentages for the feet, arms, torso, neck, and genitals, but the values assigned to the legs and head vary with a persons' age.

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Patient' demographics

Very young children and the elderly have an increased risk of being burned and they have worse clinical outcomes compared to patients in other age groups (28-34). In the United States, approximately two-thirds of children who required emergency care for burn related injuries sustained thermal injuries, while children <4 years are particularly prone to scald injury (33). Obese adults, AIDS patients and those who have an underlying medical condition such as diabetes have also been shown to suffer higher morbidity and mortality (35-41).

Individuals with deliberate self-inflicted burn injuries and the disabled have been shown to have more severe injuries and, on average, longer hospital stays than those with accidental injuries (42-44).

Microbial colonization of the burn wound

The burn wound surface is a protein-rich environment consisting of avascular necrotic tissue that provides a favorable niche for microbial colonization and proliferation (4, 45-48). The avascularity of the eschar results in impaired migration of host immune cells and restricts delivery of systemically administered antimicrobial agents to the area. Therefore topical burn treatment is essential; the gold standard in topical burn treatment is silver-sulfadiazine (Ag-SD), a useful broad spectrum antibacterial agent.

Although burn wound surfaces are sterile immediately following thermal injury, these wounds will soon be colonized with a variety of micro-organisms (4, 5). Micro-organisms colonizing the burn wounds originate from the patient's endogenous skin, respiratory and

gastro-intestinal flora, but the bacteria may also be transferred to a patient's skin surface via contact with contaminated external environmental surfaces, the hands of health care workers and even air (4, 5, 6).
Immediately following injury, predominant Gram-positive bacteria (e.g. *S. aureus*, coagulase-negative staphylococci, *Enterococcus* spp.) from the patient's endogenous flora or the external environment start to colonize the burn wound (5, 45, 49). Endogenous Gram-negative bacteria from the gastro-intestinal flora (e.g. *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter* ssp.) also rapidly colonize the burn wound surface in the first few days after injury (46, 50-52). Wound colonization by yeasts and fungi usually occurs later due to the clinically imposed selective powers of broad-spectrum antibiotic therapy (e.g. *Candida* spp., and *Aspergillus* spp.) (53-55). The most common burn wound pathogens are *S. aureus* and *P. aeruginosa*. This review focuses on *S. aureus.*

STAPHYLOCOCCUS AUREUS

S. aureus is a common bacterium that often resides quite harmlessly on the skin or in the nose of a healthy person. However, a breach in the skin or a weakened immune system can trigger *S. aureus* to cause minor skin infections or sometimes even life-threatening diseases. *S. aureus* cells also contaminate the inanimate environment when excreted from the body of (persistent) carriers. Staphylococci are known to survive on inanimate surfaces (e.g. surfaces and medical equipment) for weeks or even months (56-58).

***S. aureus* carriage**

S. aureus colonizes the skin and mucosal surfaces of humans and also of several animal species. The anterior nares of the nose are the most frequent carriage site for *S. aureus* (10, 59). Other sites which can harbor this organism are the skin in general, perineum, the gastro-intestinal tract and the pharynx (59-62). Less frequently, *S. aureus* can be cultured from vagina (63) and axilla (59, 64).
Several longitudinal studies reported that *S. aureus* nasal carriage patterns differ between individuals; 10-35% of individuals carry *S. aureus* persistently, 20-75% carry intermittently, and 5-50% never carry *S. aureus* in the nose (60, 65-70). Furthermore, the number of *S. aureus* cells in the nose is significantly higher in persistent carriers than in intermittent carriers (71, 72) resulting in an increased risk of *S. aureus* infections in the first category of individuals (73-75). Persistent carriers are often colonized by only one single strain over extended periods, up to 10 years, while intermittent carriers carry many different strains over time (65, 66, 70, 76, 77).

Determinants of *S. aureus* nasal carriage

For determining *S. aureus* nasal carriage both bacterial and host factors play a role. Hydrophobic interactions and surface charge provide forces that are probably involved in mediating staphylococcal binding to epithelial cells (78-80). Specific non proteinaceous staphylococcal cell wall components (78, 81), surface proteins (76), microbial surface components recognizing adhesive matrix molecules (MSCRAMMS) (82-84), and staphylococcal factors facilitating interactions with mucus components (85, 86) are important in colonization efficacy. Hundreds of *S. aureus* virulence factors and putative virulence genes have been described, including those involved in adherence to human tissue, evasion of the immune response, and regulation of virulence gene expression (87).

The fact that different nasal *S. aureus* colonization patterns can be discerned, suggests a clear host influence. A study in which volunteers (noncarriers and persistent carriers) were artificially inoculated with a mixture of *S. aureus* strains showed that noncarriers quickly eliminated the inoculated *S. aureus* strains, whereas persistent carriers primarily reselected their original resident strain from the inoculation mixture (88). The authors concluded that host factors substantially co-determine the *S. aureus* carriage state in an individual. This conclusion is supported by other studies which showed that *S. aureus* carriage rates vary between different ethnic groups, with higher rates in white people, in men and lower rates in elderly people (59, 60, 70, 89-92). Increased carriage rates are found in hospitalized patients (10, 11, 67, 93-96). Another recently discovered nasal determinant is smoking status: current smoking was negatively associated with nasal *S. aureus* carriage (97). Emonts *et al*. showed that persistent carriage of *S. aureus* is influenced by genetic variation in host inflammatory response genes; Interleukin 4 -524 C/C host genotype was associated with an increased risk of persistent *S. aureus* carriage, whereas C-Reactive Protein (CRP) haplotype 1184C; 2042C; 2911C was overrepresented in individuals who were not colonized (98). Van den Akker *et al.* reported that genotype-dependent variation in the sensitivity to glucocorticoids is associated with tolerance toward staphylococcal nasal colonization (99).

The noncarrier state may, in part, be explained by the phenomenon of bacterial interference; when an ecological niche is already occupied with other bacteria, such as coagulase-negative staphylococci, newly arriving wild type *S. aureus* can not replace the resident bacterial population (66, 100-102). Bacterial interference between *S. aureus* and *Streptococcus pneumoniae* in the nasopharynx of children was recently documented (103). Uehara *et al.* artificially implanted a strain of *Corynebacterium* spp. into the nares of 17 *S. aureus* carriers. After 15 inoculations in 71% of the carriers *S. aureus* was completed eradicated. Thus, *Corynebacterium* spp. interfered with *S. aureus* (104).

Finally, underlying diseases have been associated with a higher *S. aureus* nasal carriage rate and infection rate as reviewed by Kluytmans *et al*. (10) and Nouwen *et al*. (11).

What are the risks of *S. aureus* nasal carriage for patients with burn wounds?

The nose is regarded as the ecological niche from where *S. aureus* can spread to other parts of the body. In 1959 the first reports were published that investigated the relation between nasal carriage and the development of surgical wound infection. Furthermore, phage typing determined that a clonal relation was often found between nasal strains and infectious strains (105, 106). Further studies showed a significantly risk for development of a autologous wound infections by nasal carriers (10, 75). Hence, *S. aureus* carriage has been identified as a risk factor for the development of infections in various settings.

The burn wound itself and the accompanying immunosuppression are two major factors that predispose burn patients to colonization and infection. Several studies have shown that the rate of burn wound colonization with *S. aureus* varies considerably. The risk of colonization seems to be determined, at least in part, by the TBSA, the age of the patient, and nasal and pharyngeal *S. aureus* carriage of patients as well as of their health care workers (7, 107-109). Colonization of burn wounds with *S. aureus* has been associated with delayed wound healing and prolonged length of stay at the burn centre (7, 8).

Clinical effect of burn wound colonization

Burn wound colonization describes the presence of micro-organisms in a wound that appears clinically uninfected. Following colonization, the organisms on the surface start to penetrate the burn eschar to a variable extent, depending on their invasive capacity, local wound factors, and the degree of patient's immunodepression (110). Non-invasive burn wound infection involves microbial growth in the wound or eschar with purulent drainage and diffusion of microbial products into the surrounding viable tissue. This can cause a systemic response in the patient. Invasive burn infection describes microbial growth in the wound or eschar with invasion into and necrosis of the surrounding and potentially viable tissue (111).

Infection of burn wounds with *S. aureus* can lead to a hypertrophic scar. A hypertrophic scar is "a widespread red, raised, sometimes itchy scar that remains within the borders of the injury" (112). These scars can be disfiguring and even painful. If such scars are sited across joints, the almost inevitable contractures can impair function and results in painful fissures. Burn scars may have a dramatic influence on a patient's quality of life. They have been associated with anxiety, social avoidance, depression, a disruption in

normal daily activities, the onset of sleep disturbances, and all of the consequent difficulties in returning to normal life after physical rehabilitation. The factors involved in pathological scar formation are still under debate, and their complexity, especially with burn scars, is well known. Some studies suggest that genetics play an important role in the pathogenesis of scarring (113-118). It seems that local factors as burn depth, the presence of infection in the wound bed, and healing delay are of relevance as well (113). Concerning the causes of postburn pathologic scarring, it has been hypothesized that hypertrophy is a systemic inflammatory disease of central origin, regulated by local influence factors (118). Some studies have pointed out the key role played by lymphocytes and the skins' immune system in general in the maintenance of a continuous activated inflammatory state of hypertrophic tissue (119-121). A delay in re-epithelialization increases the risk of wound infection and prolongs the inflammatory phase, consequently leading to eschar abnormalities (122).

Hypertrophic scars are particularly common following burns; however, the prevalence of hypertrophic scars is unknown. A limited number of groups studied the prevalence of hypertropic scars and found pravalences ranging from 32 to 67% (Table 1). Infection increases the likelihood of hypertrophic scarring. Baker *et al*. showed that there is a significant association between bacterial colonization of burn wounds and hypertrophic scarring (123). The association between colonization and hypertrophic scars was significant for several genera, namely *Staphylococcus* spp., fecal *Streptococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Enterococcus* spp. and *Escherichia coli.* Colonization may elicit a subclinical inflammatory response, which may increase the stimulus to the formation of hypertrophic scar tissue (123).

***S. aureus* reservoirs and transmission routes**

Important sources of *S. aureus* in burn centres are colonized burn wounds and carriage sites of patients and personnel. These are the places were the micro-organisms multiply, and from these they are transmitted to other patients, health care workers, and the air and surfaces in the inanimate environment. The principal *S. aureus* transmission route is most likely from patient to patient via transiently contaminated hands of the HCWs who have acquired the micro-organism by direct patient contact or by handling contaminated materials (128, 129). Compliance rates of HCWs in hand hygiene are known to be around 50%, which essentially is too low to block transmission in full (130).

Table 1. Overview of studies that illustrate the prevalence of hypertrophic scars in patients with burns.

Reference	Publication date	Patients n	Scars n	Patients with hypertrophic scars, n (%)
124	2008	705	---*	340 (44)
125	2003	110	---	74 (67)
123	2007	---	127	51 (40)
126	1999	779	---	249 (32)
127	1990	---	---	(50)

*---: no data given

In Figure 2 a number of possible transmission routes are schematized. Colonization of the nose, skin or wound in a given individual can give rise to additional contamination of individuals and environment. In the end this may lead to newly noses and wounds. The risk of acquisition is influenced by the colonization status of other patients (131). This has been demonstrated for methicillin resistant *S. aureus* (MRSA) (132), vancomycin-resistant enterococci (VRE) (133) and *Enterobacteriaceae* (134).

PREVENTION OF COLONIZATION AND INFECTION

Measures to prevent and treat infections are essential for the survival of patients with extensive burns. In patients with less extensive burns, infections may increase morbidity, length of hospital stay and the risk of hypertrophic scarring.

Hygienic measures

Preventing the spread of *S. aureus* at a burn centre can be partly achieved by (i) the layout of the burn centre and the use of a dedicated operating theatre, (ii) the implementation of contact precautions (HCWs wear masks, gowns, and gloves while in contact with the patients; these covers are removed and hands are washed after finishing contact with the patient) (iii) cohort nursing (i.e., grouping patients of a given colonization status, with designated HCWs, which is targeted at a minimum ratio of 1:1 of nursing staff to patients, (iv) using of strict aseptic techniques for changing dressings, (v) hand-disinfection and location of hand-disinfectant (alcohol 70% isopropanol/ethanol) dispensers near all beds, (vi) timely closure of the burn wound.

Laminar airflow techniques have been shown to decrease the infection rate in a number of infection-prone patient populations (135, 136).

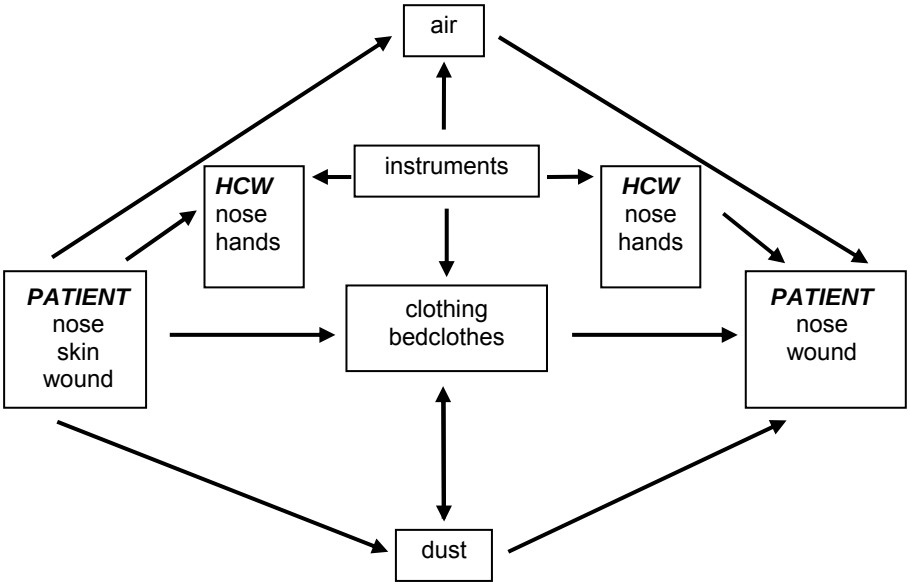

Figure 2. Schematic representation of different routes to acquire S. aureus colonization.

Decolonization strategies

Topical antibacterial treatment: silver and cerium

The gold standard in topical burn treatment is silver-sulfadiazine (Ag-SD), a useful broad spectrum antibacterial agent for burn wounds treatment. Silver has been used to treat wounds for a very long time (137, 139) and has been proven to be very effective in controlling infections. The silver ion is a highly reactive ion, readily binding to negatively charged proteins, RNA, DNA, chloride ions and other moieties. This explains its broad spectrum of antibacterial activity. Silver sulfadiazine was introduced in 1968 (139). Silver sulfadiazine acts on the bacterial wall and binds relatively strongly to DNA (139). It was shown to significantly lower mortality in severely injured patients (140). Later in 1976, cerium (belonging to the Lanthanides or rare earth elements) was added to the ointment as various studies had shown that cerium in combination with silver-sulfadiazine enhanced the antibacterial effect (141-143). Cerium forms a yellow-green, leathery eschar over the wound site providing a barrier against bacterial colonization and infection (142, 144-146). Some studies suggest a major effect of cerium in binding and denaturing the immunosuppressive lipid protein complex generated by burned skin (147-149). Thus,

cerium-based burn wound topical therapies appear to limit local inflammation and systemic immunosuppression. However, topical agents reduce the microbial overgrowth, but they seldom prevent further colonization with other potentially invasive bacteria and fungi.

Although it was originally thought that treatment with silver did not lead to the development of resistance, recently resistance to silver compounds has been described (150). In addition silver has been shown to display cytotoxic effects on cultured keratinocytes, which might hamper the use of this compound in combination with tissue engineered skin substitutes.

Selective decontamination of the digestive tract (SDD)

Selective decontamination of the digestive tract (SDD) is a prophylactic strategy to reduce infectious morbidity and mortality in granulocytopenic patients and in immunocompromised patients (151-153). The efficacy of SDD in burned patients is studied in a number of studies (46, 154-157). Manson *et al*. showed that SDD limits the colonization of burn wounds with micro-organisms originating from the GI tract (46). The efficacy of SDD has been evaluated in patients with severe burns in one prospective and in one historically controlled trial (154, 155). Jarrett *et al*. studied the efficacy of SDD prospectively and found that infections were halved in the SDD group as compared with the control group (154). Mackie *et al*. (155) found that the incidence of respiratory tract infections (27.3% versus 6.5%) and mortality (21.2 versus 3.2%) were reduced in the SDD group. De la Cal *et al*. (156) performed a double blind, placebo controlled study at a single centre; patients with burns >= 20% of TBSA and/or suspected inhalation injury were enrolled and assigned to receive SDD or placebo for the total duration of treatment in the burn intensive care unit (ICU). Treatment with SDD was associated with a significant reduction in mortality in the burn ICU. The incidence of pneumonia was significantly higher in the placebo group: 30.8 and 17.0 pneumonias per 1000 ventilation days (P=0.03) in placebo and SDD group, respectively.

Barrett *et al*. studied the efficacy of SDD to decrease the bacterial colonization of the aerodigestive tract and burn wounds, and the incidence of septic complications in severely burned children in a prospectively randomized double-blind study (157). Colonization rates of the wound, sputum, nasogastric aspirates, and feces were similar. Pneumonia, sepsis and other complications had similar incidences in both groups. They concluded that SDD is not effective in decreasing bacterial colonization and infectious episodes in severely burned pediatric patients. The efficacy of SDD in severely burned patients in decreasing bacterial wound colonization seems controversial.

Elimination of nasal S. aureus carriage.

Mupirocin, (pseudomonic acid) is a topical antibiotic agent produced by *Pseudomonas fluorescens*. It displays a strong activity against most Gram-positive bacteria, including MRSA, although it is also active against some Gram-negative bacteria, including *Neisseria* spp., *Haemophilus* spp., and *Mycoplasma* spp. (158). Mupirocin inhibits bacterial protein synthesis by reversible binding to bacterial isoleucyl-tRNA synthetase (IleS) (159, 160). Mupirocin has been used primarily for skin infections and for the prevention and the topical treatment of infections by MRSA (159, 160). During the last decades mupirocin has been the agent of choice for the eradication of nasal and pericatheter colonization by *S. aureus* in patients undergoing peritoneal dialysis (161-164), hemodialysis (165), cardio-thoracic surgery (166), and HIV-infection (167).

Clinical trials with mupirocin have consistently shown that this agent temporarily eliminates nasal carriage of *S. aureus* in surgical patients and patients undergoing hemodialysis (168, 169). Moreover, nasal mupirocin has proved effective in eliminating nasal carriage of MRSA during outbreaks in a variety of clinical settings, including nursing homes (170) and neonatal units (171). However, some clinical studies found little or no efficacy of mupirocin in preventing nosocomial infections (172-174). Wertheim *et al.* (61) showed that mupirocin is effective in overall decolonization of nasal carriers but less effective in decolonizing extranasal sites.

Decolonization therapy includes a 5 consecutive day course of mupirocin nasal ointment applied twice daily. It has been reported to result in elimination rates of 91% directly after therapy, 87% after 4 weeks, and 48% after 6 months (175).

Mackie *et al*. showed that supplementing the SDD-regimen with intranasal mupirocin for patients with TBSA >30% was effective in elimination of the endogenous bacterial reservoirs (176). This study showed an overall decline in the incidence of bacterial wound colonization in patients treated with SDD and nasal mupirocin. In this group, the incidence in *S. aureus* wound colonization was significantly decreased when compared to the historic control group with patients who were treated with SDD only (24% and 65%, respectively).

A few studies have been published in which the effect of prophylactic antibiotic use in burn centres was described. Ergün *et al.* (2004) observed no reduction in the rate of wound infection in a group of patients who where treated with antibiotic prophylaxis, when compared to the control group (177). Ugburo *et al.* (2006) concluded that systemic antibiotic prophylaxis is of no value in controlling burn wound sepsis, and might even favour the growth of *P. aeruginosa* in the burn wounds (178). Durtschi *et al.* (1982) showed that routine administration of prophylactic penicillin neither protects against cellulites nor burn wound sepsis (179).

NOVEL THERAPIES FOR STAPHYLOCOCCAL INFECTIONS

In the past era, severe staphylococcal infections were primarily treated with antibiotics. This treatment became progressively more difficult because some strains developed or acquired resistance to multiple antibiotics, including vancomycin (180). Staphylococcal infections with multi-drug resistant *S. aureus* will lead to serious clinical problems. These problems require new therapies and prevention strategies. Over the past decade several therapies have been suggested in the literature. First of all, experimental bacteriophage-mediated prophylaxis in mice and rabbits demonstrated a preventive effect of phages against local and systemic staphylococcal infections (181, 182). Ahmad *et al.* proposed a treatment of infections of the burn wound with a cocktail of phages specific for opportunistic pathogens which is sprayed on the burn wound (183). However, before applying phage therapy in humans, its possibly adverse side effects on physiology, biochemistry and immune system must be studied. Also rapid clearance in the spleen, the inability to kill intracellular bacteria and stimulation of neutralizing antibodies are currently still objections to the use of bacteriophage therapy (184, 185, 186). Recently, however, Capparelli *et al.* showed that *S. aureus* A170 phage M^{Sa} lacks these shortcomings and is capable to lyse MRSA-cells in mice (181).

Secondly, nasal carriage of *S. aureus* is a risk factor for subsequent colonization and infection (10, 75). Nasal *S. aureus* eradication therefore, is urgently recommended when the colonizing strain is MRSA or a multi-drug resistant *S. aureus* strain. Nowadays, MRSA carriers are treated with mupirocin to eliminate carriage. However, this can lead to the development of resistance. The nasal cavity can be colonized with various other microorganisms such as *Staphylococcus epidermidis* or species of corynebacteria (187) Uehara *et al.* showed that artificially inoculated strains of *Corynebacterium* spp. eradicated *S. aureus* in the majority of the *S. aureus* carriers (187). Two other recent studies showed that carriage of *Streptococcus pneumoniae* suppressed the *S. aureus* carriage rates in healthy children (188, 189). So, when properly investigated microbial interference could be used as novel prophylactic management of infections.

Finally, another therapy protective against *S. aureus* infections is mucosal vaccination. Narita *et al*. showed recently that intranasal immunization with mutant toxic shock syndrome toxin 1 (TSST-1) could elicit a protective effect against nasal colonization as well as systemic *S. aureus* infection in a mouse model (190).

Each of the above mentioned novel therapies might contribute to the reduction of *S. aureus* carriage and/or *S. aureus* infections in patients in general and in patients with burn wounds in particular. However, additional research is required prior to further development of these therapies. In the future infected burn wounds might be topically treated with one or more of the innovative therapies identified above.

CONCLUSION

This review has summarized the impact of nasal *S. aureus* carriage on burn wound colonization and the effect of several prophylactic measures on *S. aureus* burn wound colonization and infection. Despite a variety of infection control measures, i.e patient cohorting and contact precaution at burn centres, *S. aureus* is still frequently encountered in burn wounds.
Multiple *S. aureus* reservoirs and routes of transmission may exist and each contributes to the epidemiology and pathogenesis of *S. aureus* colonizations and infections in a dedicated Burn Centre. However, little is known about the relative contribution of each of the potential reservoirs and routes of transmission in the colonization and infection of patients with burns. More intervention studies should be performed to elucidate the transmission dynamics of *S. aureus* at burn centres.

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PART II

STAPHYLOCOCCUS AUREUS NASAL CARRIAGE AND BURN WOUND COLONIZATION

CHAPTER 3

MOLECULAR EPIDEMIOLOGY OF *STAPHYLOCOCCUS AUREUS* COLONIZATION IN A BURN CENTRE

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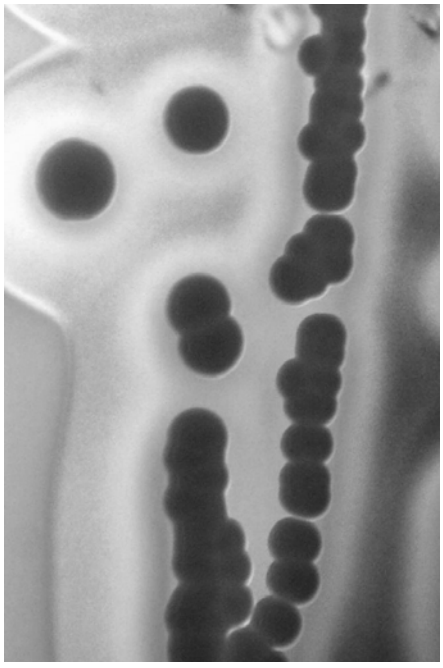

ABSTRACT

The aim of this study was to investigate carriage of *S. aureus* by patients and health care workers and to define the genetic relationship of *S. aureus* strains isolated from burn wounds. At admission, 19/55 (34.5%) patients carried *S. aureus* in their nose and/or throat. Of this group, 95% subsequently colonized their burn wounds with *S. aureus*. Molecular analysis showed that in 78% of these cases the burn-wound colonizing strain was identical to the strain carried at admission. Importantly, 23/36 (64 %) patients who did not carry *S. aureus* at admission also developed burn wound colonization. In this group, three dominant genotypes were identified as colonizing strains of burn wounds. These clones represented also the majority (59 %) of *S. aureus* strains cultured from the nose and/or throat of health care workers and patients. If patients were admitted to one of the Intensive Care rooms burn wounds of non-carriers were not colonized with *S. aureus* as long as they remained in such isolation. Only patients who carried *S. aureus* at admission developed burn-wound colonization with that genotype they carried in the nose or throat. Both carriage in patients and health care workers and auto-infection play a crucial role in (cross-) colonization events.

INTRODUCTION

Thermal injury destroys the physical skin barrier that normally prevents invasion of micro-organisms. This provides novel sites of bacterial colonization, infection and clinical sepsis in burned patients. During the first weeks following thermal trauma the affected sites are colonized with Gram-positive bacteria, including *Staphylococcus aureus*, and as time passes, Gram-negative bacteria become increasingly prevalent and dominant (8, 13, 17, 20, 21). Consequently, patients admitted to burn centres are at increased risk for nosocomial infections, including infections due to *S. aureus*.

Several studies have shown that the rate of burn wound colonization with *S. aureus* varies significantly (14-83%), depending on total body surface area burned, the age of the patient and, more importantly, with the type of care provided by the burn centre health care team (1, 14, 15, 20). Colonization with *S. aureus* is often associated with delayed wound healing, an increase in the need for surgical interventions and prolongation of stay at the centre (8, 15). Transmission of *S. aureus* occurs often and involves both patients and persons in close contact with them (6). Nasal and pharyngeal colonization of patients as well as health care workers in burn centres appear to play an important role in *S. aureus* colonization of burn wounds (1, 19). However, detailed molecular epidemiological analysis of the dynamics of *S. aureus* carriage and transmission in burn units has not yet been presented.

We performed an epidemiological survey on staphylococcal colonization of burn wounds. The study was performed in a single dedicated burn centre. Data on clinical practice were recorded and regular surveillance culture, specific for *S. aureus*, was performed for patients and personnel. Longitudinally collected strains of *S. aureus* were genetically characterized in order to elucidate the dynamics of wound colonization and possible transmission routes.

METHODS

The study was performed in the Burn Centre at the Martini Hospital, Groningen, The Netherlands. The Burn Centre is a closed unit including a dedicated operating theatre. It consists of four rooms with two beds each, and two Intensive Treatment (IT) rooms with one bed each, accommodating a total of 10 patients. In the whole Burn Centre an air-flow with positive air pressure is maintained; each IT-room has its own laminar down-flow with positive air pressure. When the patient is alone in the IT-room the ventilation rate is 30 air changes per hour. If there are Health Care Workers (HCW) or visitors in the IT-room, the ventilation rate is raised to 80 air changes per hour. At the Burn Centre a

dedicated team of HCW (n= 46), which have no other healthcare duties outside the Burn Centre, takes care of the patients. Patients remain at the Burn Centre during the entire hospital stay. However, occasionally patients may be moved from room to room within the Burn Centre.

Patients and Health Care Workers

During a period of 31 weeks, from September 1997 to May 1998, all patients hospitalized at the Burn Centre, were included in this study. At admission, burn wounds were sampled at least once and nose and throat swabs were taken in order to assess staphylococcal carriage. During hospital stay all burns were sampled weekly. At regular two months-intervals, nasal and pharyngeal swabs were taken for *S. aureus* screening from those HCW, who were at that period present at the Burn Centre. To study whether clonal types of *S. aureus* circulate persistently among HCW, a short screening period of one week was implemented approximately 19 months after the study period.

Decontamination regimens

Adults with more than 20 % Total Burned Surface Area (TBSA) and children with more than 15 % TBSA received a regimen of selective decontamination (SDD) of the digestive tract, from 0 days post burn till 5 days after last surgical intervention. The SDD regimen comprised Co-trimoxazole (500 mg, given three times daily), Colistine (200 mg, given four times daily) and Amphothericin B (0 days post burn 500 mg, four times daily and there after 500 mg, two times daily).

Microbiological Methods

Samples of nasal and pharyngeal flora were collected from patients and HCW. The nasal vestibule of both the right and left nares were swabbed with a sterile swab, and the flora of the throat was sampled with another sterile swab. Swabs were inoculated on 5% sheep blood agar (Oxoid, Haarlem, The Netherlands) and incubated overnight at 37^0C. Bacteriological examination of the burn wound was performed at least once a week. Burn wounds were sampled using 10 cm^2 contact plates with blood agar. These contact plates were placed on the burn surface on a spot for a few seconds under aseptic conditions, after which they were incubated overnight at 37^0C. Presumptive *S. aureus* colonies were tested for slide coagulase and DNAse activity (Oxoid). Coagulase and DNase positive strains were identified as *S. aureus* and stored in skim milk (Oxoid, Haarlem, The Netherlands) at - 70^0C.

Pulsed Field Gel Electrophoresis (PFGE)

The protocol for the preparation of chromosomal DNA was modified from that described

by Bannerman *et al*. (2). *S. aureus* isolates were grown overnight at 37°C in 9 ml BHI-broth (Oxoid). The culture was brought to a density of 8 McFarland; cells of 0.7 ml of this suspension were harvested by centrifugation, 6500 rpm for 2 min (MSE Microfuge, Beun de Ronde, Abcoude, The Netherlands). After washing with 1 ml sterile TEN buffer (0.1 M Tris-HCl, 0.1 M EDTA, 0.15 M NaCl) cells were re-suspended in 0.3 ml autoclaved EC-buffer (6 mM Tris-HCl, 1 M NaCl, 0.1 M EDTA, 0.5 % Brij-58, 0.2 % deoxycholate, 0.5 % N-Lauroylsarcosine). Three µl of 1 mg/ml lysostaphin (Sigma Co., St. Louis, USA) dissolved in 20 mM NaAc and 0.3 ml 2 % agarose (Molecular Biological Certified agarose, BioRad, Veenendaal, The Netherlands) were added to the cell mixture. After brief vortexing, 100 µl of this suspension was pipetted immediately in a plug mould and incubated for 1 hour in 3 ml EC-buffer at 37 °C. The EC-buffer was decanted, replaced by 1 ml autoclaved TE-buffer (10 mM Tris-HCl, 5 mM EDTA) and the tube was incubated for 1 hour at 55 °C. The plug was washed four times with 3 ml TE-buffer at room temperature with gentle shaking.

Restriction-endonuclease digestion was performed by placing a small slice (3 by 5 mm) of the plug in 125 µl restriction buffer A (Roche Diagnostics Corporation, Indianapolis, USA) containing 20 U *Sma*I (Roche Diagnostics). After incubation for 2 hours at 25 °C with gentle shaking, the slice was brought into a well of a 1.4 % agarose gel (Molecular Biology Certified Agarose, BioRad). The gel was prepared in 0.5xTBE (45 mM Tris-HCl, 45 mM Borate, 1.0 mM EDTA pH 8.0). Bacteriophage lambda DNA concatemers (BioRad, The Netherlands) were used as molecular size standards and placed in each 6th well in the gel. ATCC-strains 25923 and 29213 were used as controls for the total procedure. The running parameters of the CHEF-DR II system (BioRad) were as follows: block 1 switching from 1 to 10 seconds for 3 hours, followed by block 2 switching from 5 to 40 seconds for 17 hours, all at 6 V/cm and 13 °C. Gels were stained with 0.5 µg/ml ethidium bromide (Sigma-Aldrich, Zwijndrecht, The Netherlands) and photographed.

Interpretation of PFGE banding patterns

The software package Molecular Analyst (Biorad) was used to analyze and group PFGE patterns (0.8 % position tolerance, Dice, UPGMA). Interpretation of the grouped PFGE-patterns was performed visually according the guidelines of Tenover *et al*. (18). A clonal type consists of a group of identical *S. aureus* strains, including closely related subtypes.

Statistical analysis

The Chi-square-test, Fisher exact test and Survival-analysis (Taron-Ware) were used to compare proportions, a p-value of <0.05 indicating statistical significance.

RESULTS

S. aureus carriage among burn wound patients

From September 1997 through May 1998 55 patients were admitted to the Burn centre and all were included in the analysis. The bed occupancy was 20-90 % (mean 54%). All the four rooms with two beds each were occupied 29 weeks.

Twenty-one out of fifty five (38 %) of the patients had been admitted to another health care centre prior to transfer to the burn centre. The remaining patients were admitted without prior treatment in another hospital. Cultures taken at the time of admission revealed that 19 (35%) patients carried *S. aureus* in their nose and/or throats (5 nose and throat, 2 throat only and 12 nose only). In 18/19 (95%) of these carriers their burn wounds became colonized with *S. aureus* with an average interval of 5 (1-19) days after admission. Molecular fingerprinting showed that in 14/18 (78%) of these wound colonization events the colonizing strain was identical to the strain carried in nose or throat at the time of admission (Figure 1).

The majority of patients that did not carry *S. aureus* at the time of admission also developed wound colonization with this bacterial species during their stay in the burn unit. However, their risk of acquiring *S. aureus* burn wound colonization was less when compared to the rate of colonization observed among the nasal *S. aureus* carriers (23/36 [64%] versus 18/19 [95%], p=0.012) (Figure 1). When *S. aureus* strains, isolated from wounds of patients that were non-carriers at admission but nonetheless did acquire *S. aureus* wound colonization during their stay at the centre, were genotyped, three dominant genotypes (A, B and C) were identified. In sixteen out of the entire group of non-carriers (n=36; 44%) burn-wounds became colonized with clones A (n=4), B (n=7) or C (n=5). Five out of 22 patients (23%) who had been hospitalized in another health care centre prior to admission to the Groningen' burn centre, carried genotype B (n=2), C (n=2) or D (n=1) in the nose.

S. aureus carriage among personnel

During the inclusion period the carrier rate among burn centre personnel varied between 35 - 45%. Genotyping of the strains cultured from personnel regularly showed identity with patients' strains. Thus, the same dominant genotypes A, B and C represented the majority of *S. aureus* strains cultured from health care workers, i.e. 17/22 (77%), 15/23 (65%) and 13/18 (72%) of the strains collected at the three sampling times. When re-screened in January 2000, i.e. some 19 months after the last patient had been discharged from the centre, clones A, B and C were found to persist in the noses of many health care workers in the burn centre, although one new clonal type (designated

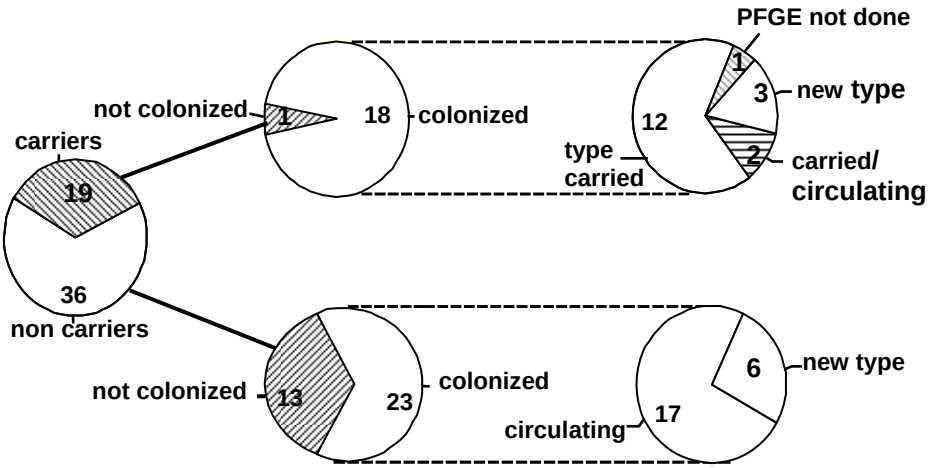

Figure 1. S. aureus burn wound colonization of carriers and non-carriers of S. aureus during their stay at the burn wound centre. Represented are the number of patients who colonized the burn wound with the same genotype carried in the nose or throat (type carried), with genotypes isolated from HCW and/or patients at the burn centre (circulating) or with clonal types which were not isolated from HCW and patients in the burn centre (new type).

Z) of *S. aureus* seemed to have gained a foothold among personnel in the centre at that time (Table 1).

Intensive care versus regular treatment

Nine patients were hospitalized in one of the two single bed IT-rooms. Three of these patients were already carrier at their admission in the IT-room; two of them colonized their burn wounds with the genotype they carried in the nose or throat. One patient didn't colonize the burn wound with *S. aureus*. Six other patients entered the IT-room without *S. aureus* as evidenced by negative cultures from nose, throat and burn wound at the time of admission. These six patients remained free of *S. aureus* burn wound colonization for as long as they remained nursed in the IT-room. However, upon transfer to one of the standard non-IT- rooms in the burn centre, the burn wound of five patients colonized with *S. aureus*, usually with one of the prevalent clones also found among personnel (see Figure 2).

Three of the nine patients received the SDD regimen. Burn wounds of two of these patients were colonized during stay in the IT-room with *S. aureus*. These patients were already carrier at their admission in the IT-room and colonized their burn wounds with the genotype they carried in the nose or throat.

Colonization of the burn wound with *S. aureus* was less for patients treated at IT-rooms when compared to the colonization of the burn wound of patients treated at standard

Table 1. S. aureus carriage rates among healthcare workers (HCW).

Screening period	HCW[a] *n*	Carriers *n* (%)	Clonal type carried					
			A	B	C	Z	AJ	Other types
			n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
October 1997	49	22 (44)	8 (16)	6 (12)	3 (6)			5 (10)
December 1997	66	23 (35)	8 (12)	5 (7)	2 (3)	1 (2)		7 (11)
March 1998	44	18 (41)	7 (16)	3 (7)	3 (7)		1 (2)	4 (9)
January 2000	49	26 (53)	7 (14)	3 (6)	4 (8)	4 (8)	2 (4)	6 (13)

The HCW were repeatedly screened for S. aureus carriage.

[a]Besides the dedicated team at the Groningen= burn centre also another health care workers (therapeutists psychologists and dieticians), may enter the centre to take care of the patients.

rooms (p=0.011). In IT-rooms 50 % of the patients had no burn wound colonization after 21 days, in standard rooms 50 % of the patients had no burn wound colonization after 8 days (p=0.032). Colonization of the burn wound with *S. aureus* by patients who were no nasal *S. aureus* carriers with treatment at IT-rooms was less frequently seen as compared to standard rooms (p=0.009).

DISCUSSION

Burn wounds lack the normal physical barrier provided by intact skin. In burn wounds, molecules such as fibronectin, fibrinogen, collagen and many others are exposed at the wound surface (7). Many bacterial species harbour specific receptors for such molecules and, hence, burn wound surfaces are easily colonized by bacteria. *S. aureus* encodes many proteins that specifically interact with human cellular matrix components. These Microbial Surface Compounds Recognizing Adhesive Matrix Molecules (MSCRAMMS) enable *S. aureus* to be one of the most common microbes found to be colonizing burn

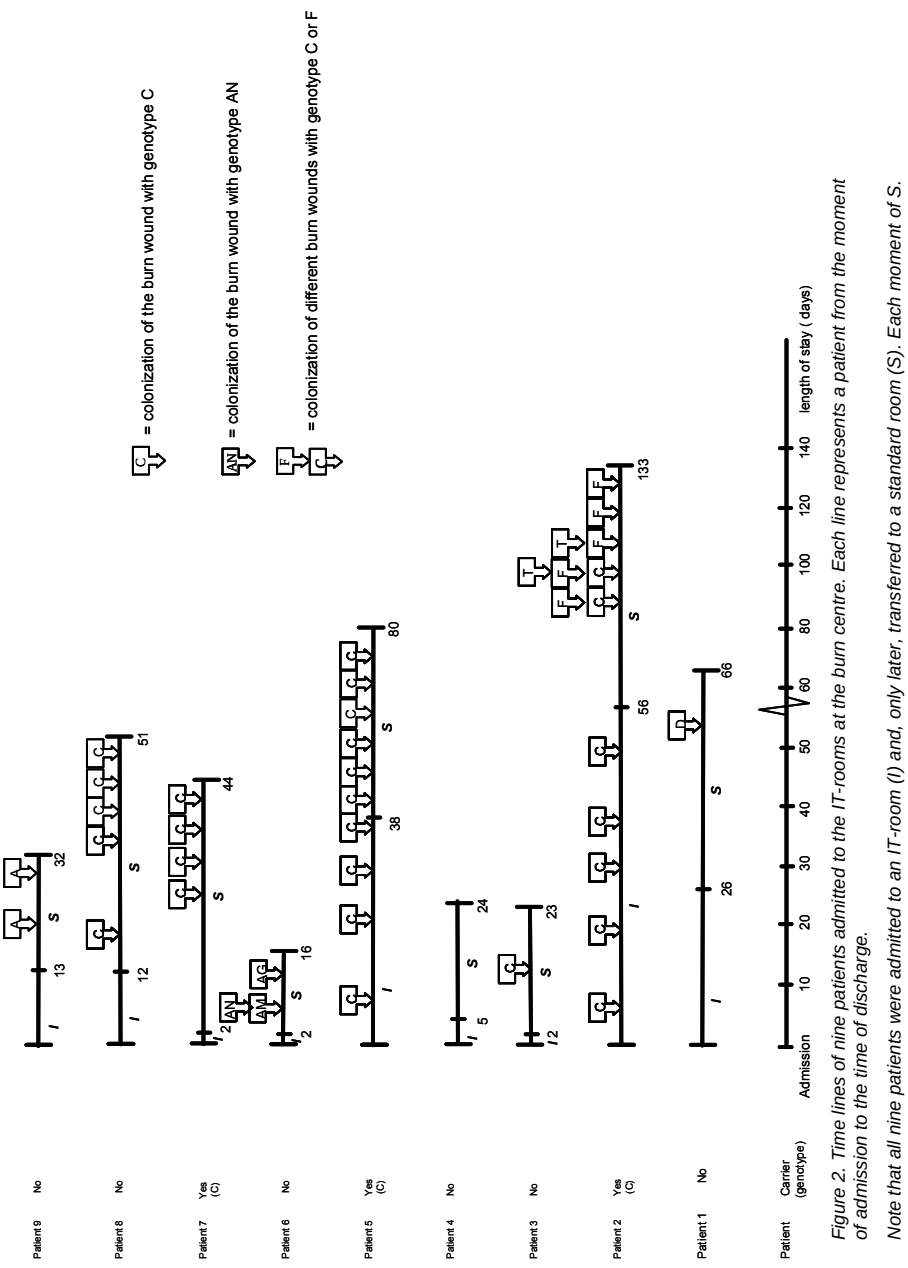

Figure 2. Time lines of nine patients admitted to the IT-rooms at the burn centre. Each line represents a patient from the moment of admission to the time of discharge.

Note that all nine patients were admitted to an IT-room (I) and, only later, transferred to a standard room (S). Each moment of S. aureus burn wound colonization is given as boxes.

wounds (10). Prevention of this colonized state is important and adequate identification of the source of the *S. aureus* strains is mandatory in this respect. Colonization of the nose, skin or wound of an individual can give rise to contamination. In addition, the environment (through air, bandages, clothes, bed linen, dust etc) may become contaminated as well. In the end this may lead to new cases of colonized wounds. Recent studies have demonstrated, that transmission via the hands of contaminated health care workers is a very important determinant of the spread and persistence of pathogens (4, 5, 9). Changing dressings and bed making generates dust and airborne micro-organisms. A recent study showed that 15 minutes after bed making the number of *S. aureus* containing particles is significantly higher than during the resting period (16). These airborne *S. aureus* cells can colonize the burn wound and can be inhaled. Inhalation of such particles is likely to play a role in *S. aureus* colonization of the nares.

Admission *S. aureus* carriage state was a predictor of *S. aureus* colonization: 95% of carriers but only 64% of non-carriers later developed *S. aureus* colonization. Strain typing of paired admission and subsequent clinical isolates in 18 patients with acquired burn wound colonization with *S. aureus* indicated that 14 (78%) became only colonized with a strain identical with their admission isolate. Other studies also show that *S. aureus* carriage state is a predictor of subsequent development of *S. aureus* colonization and infection in burn and non-burn patients (19, 23).

Strain typing of the 'burn centre acquired' isolates (i.e. patient cultured negative on admission who later developed *S. aureus* colonization) showed different strains with three genotypes predominant. An important finding of our study is the predominance of these same 3 genotypes among health care workers at our burn centre. Taylor *et al*. showed in their study no predominating strains in the 'burn centre acquired' isolates and strains isolated from health care workers (19).

Nasal carriage of *S. aureus* plays a key role in the development of *S. aureus* wound colonization. Our current data identify two important common sources for *S. aureus* burn wound colonization in the Groningen' Burn Centre. One important source is the patient him- or herself in case of *S. aureus* carriage prior to the thermal trauma. In these cases colonization can be classified as *endogenous.* The other source of colonization, and quantitatively at least as important, are the health care workers of the burn unit. Especially patients that do not carry *S. aureus* at the time of admission, are prone to become 'infected' with staphylococci through contact with the persons that take care of them. In these cases the colonization can be classified as *exogenous* or as cross-colonization. Environmental cultures were not taken during this study. Airborne transmission only seems important in the acquisition of nasal carriage (22).

In burn units, patients and personnel may continuously present new clones to the centre. Besides the transmission routes also the clonal nature of *S. aureus* populations may play

an important role. Apparently, some strains are much more successful colonizers than others and are well-equipped to induce colonization. Persistent carriers often carry a single strain (3), whereas intermittent carriers can be colonized with unrelated strains over time. This suggests that bacterial factors could be involved (11). In this study, clones A, B, C, and later Z turn out to be most efficient in colonizing patients and healthcare workers. Apparently, these clones have a selective advantage making them colonization prone.

An another important finding of our study is that the Intensive Treatment rooms, as defined in this study, can prevent cross-colonization in a burn centre. This is in agreement with van Rijn *et al.* who showed the effectiveness of bacteria-controlled nursing units in preventing cross-colonization with resistant bacteria in severely burned children (24). Thompson *et al*. evaluated the effect of a closed unit on infection rates. They found a significant decrease in the incidence of infection during treatment in these units (25).

Our study shows that at this dedicated burn centre only treatment in the IT-rooms, as defined in this study, can prevent cross-colonization with *S. aureus*. A constant laminar down-flow, a high ventilation rate and other preventive procedures (including sterile coats, gloves, and gowns), during all contacts with HCW prevent burn wounds from cross-colonization. The SDD-regimen didn't prevent the colonization of burn wounds with *S. aureus.* At standard rooms, the same preventive procedures as used at IT-rooms, are carried out only during changing of dressings of burn wounds of patients with more than 5% total body surface area burned. After all HCW-patient contacts, hand cleansing is carried out using an alcohol-based hand-rub solution. Strict hand hygiene policies may already achieve some success in the battle against the transmission of *S. aureus* (12).

Due to physical and financial constraints, it is not possible to hospitalize all patients at IT-rooms. Until new strategies have been developed to minimize transmission of *S. aureus*, we propose a few useful clinical actions that can be taken at burn centres in order to lower the number of burn wounds colonized with *S. aureus*. The endogenous transmission route could be blocked by nasal mupirocin treatment of all patients at admission. Nasal mupirocin has an important role to play in the prevention of *Staphylococcus aureus* infection by eliminating nasal carriage of this organism. Topical mupirocin has been used widely for the clearance of nasal *S. aureus* carriage particularly during outbreaks (26, 27). It has been shown to reduce the rate of nasal carriage and clinical infection in surgical and dialysis patients and in human immunodeficiency virus (HIV) disease (28-31). Mackie *et al*. found a reduction in *S. aureus* wound colonization using nasal mupirocin and selective decontamination of the digestive tract in extensive burns (32).

Doebbeling *et al*. described the long-term efficacy of intranasal mupirocin ointment (33). They found that a single brief treatment course was effective in reducing nasal *S. aureus* carriage for up to 1 year. Several studies reported the short-term efficacy of 91-100 % (34-36). Dupeyron *et al*. found a re-acquisition rate of 18 % (34). Martin *et al*. noted the recolonization rate in a population of HIV-patients increased over time (27%, 45% and 71% at 2, 6, and 10 weeks respectively) (37). Recolonization is dependent upon epidemiological pressure and mupirocin should not be used as the sole method for infection control (38). Mupirocin resistance has been reported in the literature following lengthy courses or when applied to large wounds of areas (27, 38). To avoid resistance one could decide to avoid repeated applications and to treat patients once. Cross-colonization events can be reduced by strict hand hygiene, by changing of dressings of burn wounds under a laminar down-flow, and finally by disinfection of the inanimate environments after changing dressings and bed linen.

On the basis of our results, we believe the routine practice of taking only one nasal swab at burn centre admission could identify those patients who are at high risk for subsequent development of staphylococcal colonization. We also saw that strains cultured from health care workers regularly showed identity with patients' strains. Finally we can say that Intensive Treatment rooms, as defined in this study, can prevent burn wounds from *S. aureus* cross-colonization. Further studies, including quantification of transmission routes, incidence, risk factors and interventions, are required to fully elucidate the transmission dynamics of *S. aureus* at burn centres.

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CHAPTER 4

PREVENTION OF *STAPHYLOCOCCUS AUREUS* BURN WOUND COLONIZATION BY NASAL MUPIROCIN

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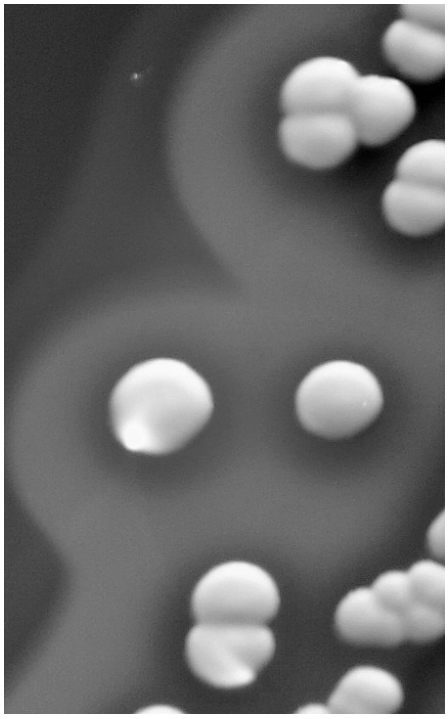

ABSTRACT

There are two important routes for the transmission of *S. aureus* to the burn wound. In the endogenous route, patients naturally carrying *S. aureus* colonize their own wounds, whereas in the exogenous route burn wounds are cross-infected from other sources. In this study we evaluated the effect of blocking the endogenous route on *S. aureus* burn wound colonization by mupirocin application in the nose of patients at the time of admission.

From September 2000 to January 2002 all patients with burns admitted to a single dedicated Burn Centre received nasal mupirocin upon admission. This period was compared to two control periods (C1: July 1999-July 2000 and C2: January 2002-January 2003) for *S. aureus* burn wound colonization. The colonization risk was analysed, adjusting for confounding, with Cox proportional hazard regression.

A total of 98 patients did not have *S. aureus* burn wound colonization at the time of admission and were, thus, considered at risk for *S. aureus* acquisition during their stay. As compared to C1, the relative risk of acquiring *S. aureus* in their wound was 0.48 (95%CI: 0.24-0.97) in the Mupirocin period and 0.55 (95%CI: 0.28-1.1 during the C2 period. *S. aureus* nasal/pharyngeal colonization was a significant independent risk factor for wound colonization (RR: 2.3; 95%CI: 1.2-4.2).

Nasal mupirocin may contribute to reduce the risk of *S. aureus* wound colonization in patients with burns.

INTRODUCTION

Patients with burns are at a high risk of contracting nosocomial pathogens and developing nosocomial infection. Several studies have shown that the rate of burn wound colonization with *Staphylococcus aureus* varies considerably (1-5). The risk of colonization is related to the total burned surface area (TBSA), the age of the patient, nasal and pharyngeal *S. aureus* carriage of patients as well as of their health care workers, and the type of care provided by the burn centre. Colonization with *S. aureus* has been associated with delayed wound healing, increased need for surgical interventions and prolonged length of stay at the centre (3, 6). Transmission of *S. aureus* occurs often, both between patients and between patients and care takers (7). Part of *S. aureus* wound colonizations in patients is of endogenous origin, i.e. their wounds become colonized by the *S. aureus* strain already present in the patients' nose or throat at the time of admission (8, 9, 10, 11). By eliminating nasal carriage, nasal mupirocin could prevent endogenous *S. aureus* wound colonization.

Topical mupirocin has been widely used to treat nasal *S. aureus* carriage, particularly during outbreaks of methicillin resistant strains (MRSA) (12, 13). Mupirocin prophylaxis has been shown to reduce the rate of nasal carriage, and hence, clinical infection in surgical and dialysis patients and in human immunodeficiency virus (HIV) disease (14-17). In a single center study Mackie *et al.* found a significant reduction in *S. aureus* wound colonization when using nasal mupirocin combined with selective decontamination of the digestive tract in patients with more than 30% TBSA (18). Potentially, mupirocin could be used to prevent endogenous colonization of burn wounds. However, the efficacy of standard mupirocin prophylaxis in a Burn Centre has not yet been established.

The aim of the present investigation was to evaluate the effect of a short course of nasal mupirocin on the incidence of *S. aureus* burn wound colonization.

METHODS

Setting

The study was performed at the Burn Centre of the Martini Hospital, Groningen, The Netherlands. The Burn Centre is a closed unit including a dedicated operating theatre. It consists of four rooms with two beds each and two single Intensive Treatment (IT) rooms, potentially accommodating a total of 10 patients. Throughout the centre an air-flow with positive pressure is maintained and, additionally, each IT-room has its own laminar down-flow with positive air pressure.

At the Burn Centre a dedicated team of health care workers (HCW), who have no other healthcare duties outside the Burn Centre, takes care of the patients. In addition to this team, other HCW (e.g. surgeons, psychologists and dieticians) combine their work at the centre with duties outside the Burn Centre. Patients are cohorted and contact precaution is implemented. This includes hand washing procedures, barrier isolation, waste management, equipment and environmental cleansing and disinfection. During wound care, additional contact isolation precautions are used, i.e. use of gowns and gloves by all care givers having physical contact with the patient or the patients' environment. Also, alcohol-based hand disinfectants are used at bedside, following standard guidelines.

Decontamination regimens

All wounds were treated with silver sulphadiazine in combination with cerium nitrate (Flammacerium®, Solvay Pharma, Weesp, The Netherlands) usually for a minimum of 10 days. Adults with more than 20 % TBSA, children with more than 15 % TBSA and patients with burns in the peri-anal area received an antibiotic regimen for selective decontamination of the digestive tract (SDD), from admission till wounds were healed spontaneously or 5 days after the last surgical intervention. The SDD regimen consisted of co-trimoxazole (480 mg, three times daily), colistin (200 mg, four times daily) and amphotericin B (500 mg, four times on the admittance day and, subsequently 500 mg, two times daily).

Study design

This study included one intervention period (September 2000 to January 2002) and 2 control periods of 12 months each; one preceding and one following the intervention period (C1: July 1999-July 2000 and C2: January 2002-January 2003). During the intervention period (Mupirocin period) all patients received nasal mupirocin on admission to eradicate nasal *S. aureus*, directly after sampling nose, throat and burn wounds. Mupirocin ointment (2% mupirocin calcium cream; Bactroban® Nasal, GlaxoSmithKline BV, Zeist, The Netherlands) was administered three times daily for 5 days, according to the manufacturer's guidelines. Therapy failure was defined as a patient having a nasal culture positive for S. aureus within two weeks after the start of the mupirocin course.

During hospital stay burn wounds were sampled weekly and on indication. A patient was labeled nasal carrier if the nasal culture was positive with *S. aureus*. Burn wound colonization was defined upon isolation of *S. aureus* from the wound. Days until first burn wound colonization with *S. aureus* were recorded for all patients.

Burn wound colonization during the Mupirocin period was compared to those in both control periods (C1 and C2).

Exclusion criteria

Patients hospitalized for less than 7 days were excluded from the analyses.

Microbiological Methods

To collect nasal and pharyngeal swabs the vestibula of both right and left nares were swabbed with a sterile swab, and the throat was sampled using another swab. Swabs were plated directly on 5% sheep blood agar (Oxoid, Haarlem, The Netherlands), and subsequently immersed in Tryptone soya broth. All swabs were processed on the day of sampling. All media were incubated at 35°C. After overnight incubation, broths were subcultured on 5% sheep blood agar.

Burn wounds were sampled using 10 cm^2 contact plates containing 5% sheep blood agar. These plates were placed on the surface of the wound for a few seconds, after which they were incubated overnight at 35°C. Contact plates were also processed on the day of sampling.

After incubation media were investigated and presumptive *S. aureus* colonies, based on colony morphology and Gram stain, were tested with the coagulase tube test for free coagulase and for DNase activity (Oxoid). Coagulase and DNase positive strains were considered to be *S. aureus* and stored in skim milk (Oxoid, Haarlem, The Netherlands) at - 70°C.

Statistical Analysis

Crude comparisons were done with Student T test, Chi-square or Fisher's Exact if applicable. Cox proportional hazard regression was used to compare the study periods for wound colonization risk during hospital stay, while adjusting for differences in patient characteristics.

RESULTS

During the Mupirocin period 42 patients were included versus 39 and 32 patients during control periods C1 and C2, respectively. Table1 shows the characteristics of analysed patients. Both TBSA and duration of hospital stay were significantly higher/longer during the Mupirocin period as compared to control periods. These differences are mainly due to the admission of many seriously burned young patients that survived a disastrous fire

Table 1. Characteristics of patients included in the study during the intervention (Mup) and two control periods (C1 and C2).

	C1	Mup	C2	
Period	July 1999 to July 2000	September 2000 to January 2002	January 2002 to January 2003	difference p-value** (ns: non-significant)
Patients (n)	39	42	32	
Age (years), mean (sd)	25.6 (22.2)	30.9 (23.8)	29.6 (25.2)	ns
TBSA* (%), mean (sd)	11.9 (7.8)	19.0 (15.9)	11.0 (9.2)	0.015 (c1 vs mup) 0.013 (mup vs c2)
Patients, adults, TBSA >20%, n (%)	2 (10.0)	6 (24.0)	3 (14.3)	ns
Patients, < 18 years, TBSA > 15%, n (%)	3 (17.6)	10 (58.8)	1 (9.1)	0.032 (c1 vs mup) 0.016 (mup vs c2)
Selective decontamination of the digestive tract, n (%)	8 (20.5)	18 (42.9)	3 (9.4)	0.036 (c1 vs mup) 0.002 (mup vs c2)
Hospitalization (days), mean (sd)	26.5 (17.2)	37.6 (22.4)	25.7 (11.4)	0.015 (c1 vs mup) 0.007 (mup vs c2)
Patients with *S. aureus* nasal/throat colonization on admission, n (%)	8 (21)	12 (29)	10 (31)	ns
Patients with *S. aureus* burn wound colonization on admission, n (%)	5 (13)	9 (21)	1 (3)	0.036 (mup vs c2)

* Total burned surface area (missing in two persons).

** Calculated with Student t-test or Fisher's Exact.

on New Year's eve, 2001, in a café in Volendam (19). Also *S. aureus* burn wound colonization upon admission was significantly higher during the intervention period.

In these cohorts of patients there was no association between age and *S. aureus* nasal/pharyngeal colonization on admission, and age was not associated with wound colonization during hospitalisation. However, the mean TBSA was higher in patients

already colonised in their wounds on admission (20.5%) versus those who were not (13.2%; p=0.031). There was no significant difference in TBSA between those who acquired *S. aureus* in the wound during hospital stay (14.6%) and those who did not (11.3%; p=0.15).

During the Mupirocin period, on admission, nine of 42 (21%) patients were *S. aureus* nasal carriers. In seven of these (78%) mupirocin treatment was successful to eradicate *S. aureus* from their nares. Four of the 33 (12%) patients negative for nasal *S. aureus* on admission, became *S. aureus* nasal carriers within 14 days after admission, in spite of treatment with mupirocin.

Crude analyses revealed no difference between intervention and control periods with respect to the rate and time to *S. aureus* wound colonization, but this may have been due to our finding that patients in the intervention period were more severely injured (see above). Therefore, we performed Cox regression including only patients at risk of newly acquiring *S. aureus* in their wound. In this analysis wound colonization was the outcome and age, TBSA, SDD and nasal/pharyngeal colonization on admission were included as confounders, days until wound colonization or discharge served as time variables. In this regression analysis, the C1 group was the reference. Compared to C1, patients in the MUP period were at significantly reduced risk of *S. aureus* wound colonization (relative risk, RR: 0.48; 95% confidence interval, CI: 0.24 to 0.97 p=0.040). This risk was also lower, though not statistically significant, during C2 (RR: 0.55 95% CI: 0.28 to 1.1; p=0.083). *S. aureus* nasal/pharyngeal colonization on admission appeared to be an independent risk factor for subsequent wound colonization (RR: 2.3; 95% CI: 1.2 to 4.2; p=0.009). The risk of *S. aureus* wound colonization increased with 3.1% per percentage increase in TBSA, although this increase was not significant (95%CI: -0.3 to 6.6; p=0.072). SDD did not influence the risk of *S. aureus* wound colonization (p=0.30).

DISCUSSION

This study showed that the risk of *S. aureus* burn wound colonization was reduced in the period during which a short course of nasal mupirocin was administered to all patients upon admission to a Burn Center. We also confirmed that *S. aureus* nasopharyngeal colonization significantly increased the risk of burn wound colonization, which supports the importance of the endogenous infection route. Interestingly we found that in the 2nd control period following this intervention period, the colonization risk remained lower, albeit not significantly so, when compared to the first, pre-intervention, control period.

Coincidentally, we found a marked difference in patient characteristics during the intervention period where the patients had sustained significantly more severe burn

injuries. For those reasons we elected to adjust for possible confounders, in particular TBSA, which is a good proxy for burn severity. Though wound colonization risk was reduced statistically significantly during the mupirocin period, the risk did not fully return to the pre-treatment level after discontinuation of mupirocin prophylaxis. Infection-prevention awareness among HCW may have been higher during the intervention period and remained so thereafter. The colonization pressure may also have remained lower in part of the C2 period, due to a spill over effect from the intervention period. Therefore, it is probably reasonable to conclude that nasal mupirocin will reduce wound colonization by *S. aureus*, but that there are other determinants of *S .aureus* burn wound colonization involved as well, including cross-contamination from *S. aureus* carriers among hospital personnel and from the environment.

Prophylactic nasal mupirocin application has a variable efficacy in preventing *S. aureus* nosocomial infections (17, 20-26). A recent study showed that nasal mupirocin effectively decolonizes the nose but not the extranasal sites (27). Though the nose is the most important *S. aureus* reservoir, in patients with burns these extra-nasal sites may also be an additional, equally important source of *S. aureus* burn wound colonization. Some studies have shown rectal carriage as source of infection (7, 22).

In our study the efficacy of mupirocin treatment on nasal colonization was similar to that reported in previous studies (27, 28). We found an *S. aureus* elimination rate of 78%. Therapy failure due to high-level mupirocin resistance is rare in the Netherlands (29). In the Mupirocin period we treated all patients, both carriers for *S. aureus* and those proven culture negative on admission. We choose to do so in order to exclude the possibility of not treating intermittently colonized patients, that per chance were culture negative on the day of admission, but would be positive at a later phase (30).

Health care workers play a role in burn wound colonization. Data from our Burn Centre showed that 34% of the patients who developed burn wound colonization were colonized by the endogenous route, and 66% by the exogenous route (31). As the exogenous route seems to be the dominant one, one cannot expect a major effect from eradication of just the source of endogenous *S. aureus* in the patient. However, even a limited reduction in the proportion of positive patients may significantly reduce the colonization pressure on other patients and health care workers (HCW) in the burn centre.

Our results suggest that a short course of prophylactic intranasal mupirocin to all patients admitted to a Burn Centre may help to decrease the overall rate of *S. aureus* burn wound colonization. Also, combining eradication of endogenous sources of *S. aureus* with the elimination of exogenous sources of *S. aureus* among health care personnel and from innate objects and surfaces in the environment may be needed to reliably prevent *S. aureus* burn wound colonizations in the majority of these patients. A randomized

concurrent-and-placebo-controlled trial of nasal mupirocin is indicated to fully elucidate the effect of nasal mupirocin in patients on the colonization risk of burn wounds.

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CHAPTER 5

ELIMINATION OF *STAPHYLOCOCCUS AUREUS* NASAL CARRIAGE IN HEALTH CARE WORKERS OF A BURN CENTRE AND ITS EFFECT ON BURN WOUND COLONIZATION

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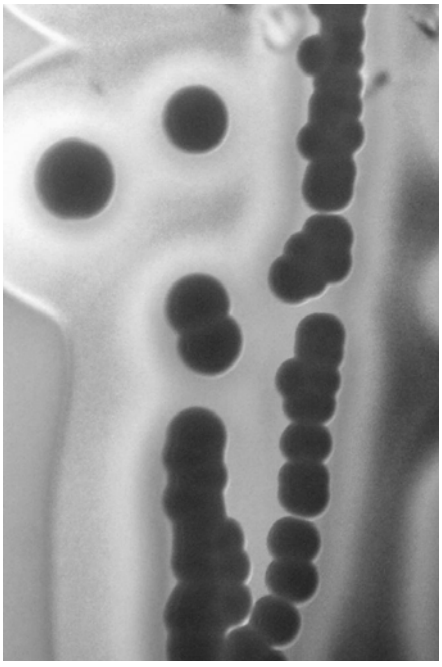
Submitted



ABSTRACT

S. aureus burn wound colonization and infection occurs frequently and may be due to cross infection from health care workers (HCWs) carrying *S. aureus*.

HCWs *S. aureus* carriage was eradicated with nasal mupirocin treatment in a single dedicated burn centre. The study covered a one-year pre-intervention period (preMup group), a 5 day mupirocin treatment period, and a one-year post-intervention period (postMup group). HCWs were regularly screened for *S. aureus* carriage. The effect of mupirocin on burn wound colonization was assessed.

43/46 (93%) HCWs self-administered nasal mupirocin. Eradication was successful in 16/18 (89%) carriers. HCW carriage rate decreased from 44% to 10% within one week, and slowly increased to 24% after 6 months and to 39% after one year. In the preMup and postMup groups 65/101 (64%) and 81/120 (68%) patients, respectively, were at-risk for *S. aureus* burn wound colonization. Among these patients the *S. aureus* acquisition rates were 1.24 and 1.38 per 100 patients' days at-risk, respectively.

In preMup *S. aureus* cross-infections of wounds were epidemiologically linked to HCWs in 7/11(64%) patients. In contrast, during the first six months after mupirocin only 1/7(14%) patients cross-infected were linked to HCWs (p=0.027). At the same time, the intervention caused a significant shift in the distribution of *S. aureus* genotypes colonizing the burn wounds.

A single course of mupirocin significantly reduced *S. aureus* carriage among HCWs. Although this intervention did not reduce the overall incidence of *S. aureus* burn wound colonization it did cause a temporary shift in sources and genotypes of *S. aureus* colonizing the patients' wounds.

INTRODUCTION

Burn patients are highly vulnerable to burn wound colonization by *Staphylococcus aureus.* Several studies have shown that the incidence of burn wound colonization with *S. aureus* varies considerably (14-83%), and that colonization risk is related to the total body surface area (TBSA) that is burned, the age of the patient, the type of care provided by the burn centre health care team and nasal and pharyngeal *S. aureus* carriage of patients as well as health care workers (HCWs) (1, 32, 33, 35). The anterior cavity of the nose forms the main reservoir of *S. aureus* from which it spreads to the skin and transmits from one individual to another. Among healthy adults, approximately 10-35% is persistent *S. aureus* nasal carrier, 20-75% is intermittent carrier and up to 50% never carries *S. aureus* in the nose (2, 13, 15, 16, 17, 27, 38). Transmission of *S. aureus* from patient to patient is thought to be mediated by transiently colonized HCWs, possibly via HCWs' hands contamination (12, 25, 28, 30, 36, 37, 40). Reagan et al evaluated the effect of intranasal mupirocin on hand carriage. Hand cultures were more likely to be negative in mupirocin versus placebo treated individuals (31). This finding is consistent with the hypothesis that the nose is a major reservoir of *S. aureus* but that the hands may be the prime "vectors" (17, 23).

To eradicate nasal *S. aureus* carriage in patients, topical mupirocin treatment has been widely used, particularly during outbreaks of methicillin resistant strains (MRSA) (3, 7). Mupirocin prophylaxis and treatment has been shown to reduce nasal carriage, and, hence, infection among surgical, dialysis patients and HIV infected patients (9, 11, 18, 26). In patients with extensive burns Mackie *et al*. found a significant reduction in *S. aureus* wound colonization using nasal mupirocin and selective decontamination of the digestive tract (24). In a previous study at the Groningen Burn Centre, data suggested that eradication of nasal *S. aureus* with mupirocin may reduce the risk of *S. aureus* wound colonization (20). The eradicated state after a five day course of mupirocin treatment persists from weeks to months (3, 8, 14, 36).

A few studies have shown the controlling effect of mupirocin treatment of HCWs on *S. aureus* outbreaks (4, 22). Furthermore, it was shown that treatment of HCWs with nasal mupirocin led to decolonization that lasted for up to six months (10, 14, 39).

Despite a variety of infection control measures, i.e patient cohorting and contact precaution, *S. aureus* is still frequently encountered in burn wounds. The aim of the present investigation was to evaluate the effect of mupirocin on nasal *S. aureus* carriage (by means of a short course of nasal mupirocin) in all HCWs in our burn centre, and its consequences on the incidence and routes of *S. aureus* burn wound colonization.

METHODS

Setting

The study was performed at the Burn Centre of the Martini Hospital, Groningen, The Netherlands. The Burn Centre is a closed unit including a dedicated operating theatre. At the time of the study it consisted of four rooms with two beds each and two one-bed Intensive Treatment (IT) rooms, accommodating a total of 10 patients. A dedicated team of HCW (n=28), who have no healthcare duties outside the Burn Centre, take care of the patients. In addition to this dedicated team, other HCWs (e.g. psychologists and dieticians) (n=18) visit the centre daily to tend to patients. Burn patients were thus cohorted, and strict contact precautions were implemented. Hand washing procedures, barrier isolation, waste management, equipment and environmental cleansing and disinfection were daily routines. During wound dressing changes, precautions were even stricter. Additional measures involved the use of gowns, surgical masks and gloves by all HCWs in contact with the patients or the patients' environment.

Decontamination regimens

All wounds were treated once daily with silversulphadiazine in combination with ceriumnitrate (Flammacerium®, Solvay Pharma, Weesp, The Netherlands) for at least 10 days. Patients aged 16 years and older, with more than 20 % TBSA, patients younger than 16 years or older than 60 with more than 15 % TBSA and patients with burns in the perianal area received an antibiotic regimen for selective decontamination of the digestive tract (SDD). SDD was given from admission until wounds were healed naturally or 5 days after the last surgical intervention. For adults the SDD regimen consisted of co-trimoxazole (400 mg, four times daily), colistine (100 mg, four times daily) and amphotericin B (500 mg, four times on the day of admission and two times daily thereafter).

Study design

This was a prospective study consisting of a one-year pre-intervention observational period, a 5 day mupirocin treatment period, and a one-year post-intervention period. The study was approved by the local Medical Ethics Committee (METC-MZH 2004-08). Included were all HCWs who were on the ward at least once a week for more than 1 hour. All HCWs were informed about the study and had to give informed consent to partake in the study. HCWs who were pregnant or were giving breast-feeding and HCWs who were allergic for mupirocin were excluded.

All included HCWs were asked to self-administer the mupirocin (2% mupirocin calcium cream; Bactroban® Nasal, GlaxoSmithKline BV, Zeist, The Netherlands), three times

daily for 5 days, according to the manufacturer's guidelines, starting July 12th 2004. Nasal and pharyngeal swabs were taken directly before the first application of mupirocin and 48-96 hours after the last application of mupirocin. After that, all HCWs were sampled monthly both in the nose and throat for 6 months and after 9 and 12 months to assess the HCWs' carriage rate.

All patients with burn wounds were screened directly upon admission for *S. aureus* nasal and throat carriage. Burn wounds were sampled on admission and also during hospital stay, weekly and on indication, according to a standardized protocol. The following data were recorded for each patient: age, gender, TBSA, length of hospital stay, colonization status on admission, dates and results of burn wound cultures and, in case of burn wound colonization with *S. aureus*, the number of days until the first positive burn wound culture.

Microbiological methods

Swabs of both the right and left nares were obtained using a sterile cotton swab (Transwab® For Aerobes and Anaerobes, Medical Wire & Equipment Co. Ltd., Corsham, Wiltshire, England). The throat was sampled using another sterile swab. Swabs were plated directly on 5% sheep blood agar (Oxoid, Haarlem, The Netherlands), and subsequently placed in Brain Heart Infusion enrichment broth. All swabs of a single individual were processed the same day. After overnight incubation at 35°C, all enrichment broths were subcultured onto 5% sheep blood agar (Oxoid, Haarlem, The Netherlands).

Burn wounds were sampled using 10 cm^2 contact plates containing 5% sheep blood agar. These contact plates were placed on the surface of the wound for a few seconds under aseptic conditions, after which they were incubated overnight at 35°C. All contact plates of a single patient were processed the same day. After incubation, presumptive *S. aureus* colonies were tested with the coagulase tube test for free coagulase and for DNase activity (Oxoid). Coagulase and DNase positive strains were considered to be *S. aureus* and stored in sterile skim milk (Oxoid) at - 70°C.

Genotyping

All *S. aureus* strains were genotyped by Pulsed Field Gel Electrophoresis (PFGE) to define their clonal relationships. For PFGE the method described by Kooistra-Smid *et al.* was used with minor modifications (19).

The software package BioNumerics (Applied Maths NV, Sint-Martens-Latem, Belgium) was used to analyze and group PFGE-patterns (0.8% position tolerance, Dice, UPGMA).

Interpretation of the relatedness between grouped PFGE-patterns was performed visually according to guidelines of Tenover et al. (34).

Definitions

Nasal mupirocin treatment in a previously colonized HCW was considered successful as long as nasal swabs remained negative after the end of treatment. A patient or HCW was labeled *S. aureus* carrier if the nasal and/or pharyngeal culture was positive for *S. aureus*. *S. aureus* burn wound colonization was defined upon isolation of *S. aureus* from the wound by culture.

Colonization was classified as 'present on admission' when *S. aureus* was demonstrated in cultures obtained less than 48 hours after admission and as 'acquired' when demonstrated only in cultures taken more than 48 hours after admission.

We used the following definitions: (i) a patient was defined 'at-risk' when no burn wound *S. aureus* colonization was detected by culture on admission; (ii) *S. aureus* burn wound colonization can be acquired exogenously by transmission (cross infection) or endogenously when the patient is already nasal/pharyngeal carrier at the time of admission (self infection); (iii) strains were genetically similar if their PFGE-patterns did not differ more than three DNA-fragments; (iiii) patients with genetically similar strains were considered to be epidemiologically linked if these patients had an overlapping period of stay or when the time between discharge of one and admission of the other patient was at most 7 days.

Cross transmission was defined when a patient acquired a *S. aureus* strain genetically similar to one isolated before in an epidemiologically linked patient. Cross transmission was also inferred when a patient became colonized with a strain that was genetically similar to a strain carried by HCWs in the period from 1 month before admission until his/her discharge date.

Statistical Analysis

The difference in carriage rates or proportions was tested for significance with exact binomial. The difference between group means was tested with the Student t-test. For rates we calculated exact binomial confidence intervals.

RESULTS

Of all included HCWs, 93% (43/46) self-administered a 5-day course of nasal mupirocin between July 12th 2004 and July 19th 2004. Just before the mupirocin course, 41 HCWs

were screened for *S. aureus* carriage. Of those 18 (44%) were carrier: 10 nasally, 4 pharyngeally and 4 naso-pharyngeally. Three of the 4 naso-pharyngeal carriers carried the same type in the nose and throat. Prevalent clones among HCWs were genotype A (n=4), B (n=4), C (n=2), D (n=2), and E (n=2). After finishing their mupirocin courses 39 HCWs were screened for *S. aureus* carriage. Eradication was successful in 10/10 (100%), 2/4 (50%) and 4/4 (100%) of the nasal, pharyngeal and naso-pharyngeal carriers, respectively. The mupirocin significantly reduced *S. aureus* carriage ($p<0.001$). After the course, 4 (10%) HCWs carried *S. aureus* (genotypes B, P, U, V), but only in the throat.

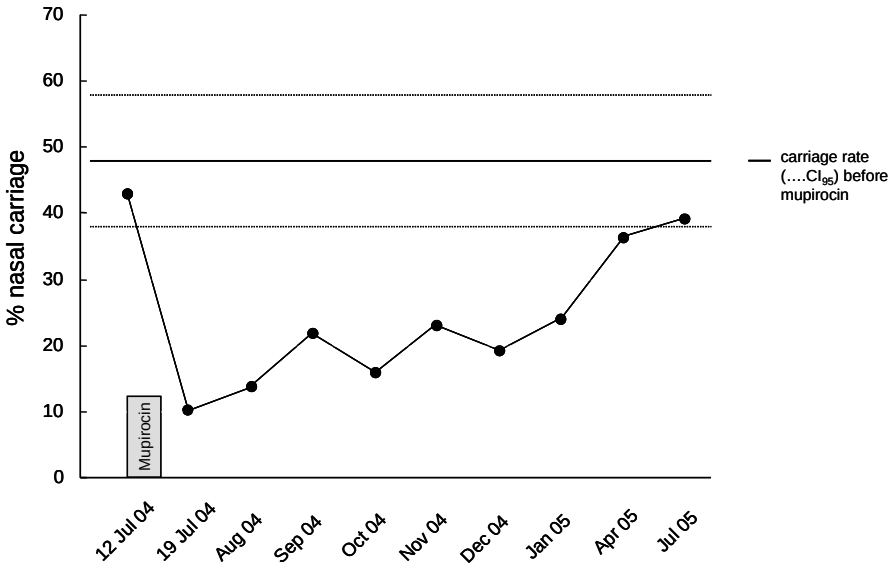


Figure 1. Effect of nasal mupirocin on S. aureus carriage among health care workers in a dedicated burn centre.

Figure 1 shows the dynamics in the carriage rate among HCWs from just prior to the start of the mupirocin course (July 12th, 2004) until 12 months after. In the year preceding the intervention the average rate of *S. aureus* carriage among HCWs was 48% (CI_{95}: 38%-58%). HCWs' carriage rate dropped significantly from 44% immediately prior to mupirocin treatment 10% within one week after mupirocin($p<0.001$), and, thereafter, gradually increased again to 24% after 6 months and to 39% after one year, when it reached the range again normally observed before the intervention (Figure 1).

Table 1 shows the characteristics of patients admitted during the preMup and postMup period. None of the characteristics differed significantly between pre- and postMup group.

In total 221 patients were admitted during the study period; 101 during the preMup and 120 during the postMup period. Of these groups, 65 (64%) and 81 (68%) patients had no *S. aureus* burn wound colonization on admission and were considered at-risk. During the preMup and postMup period 15 (23%) and 19 (23%) patients at-risk acquired *S. aureus* burn wound colonization during their stay at the centre, resulting in acquisition rates of 1.24 and 1.38 per 100 patients' days at-risk, respectively. The mean time to acquiring colonization was 14 days in the preMup group and 15 days in the postMup group. Hence, susceptibility to wound colonization does not seem to be affected by successful mupirocin treatment of HCWs.

Table 1. Characteristics of the study population.

Period	**PreMup group**	**PostMup group**
Patients (n)	101	120
Male/female	69/32	80/40
Age (years), mean (SD)	27 (24)	30 (25)
TBSA * (%), mean (SD)	9 (10)	8 (9)
Patients, adults, TBSA >20%, n (%)	11 (18)	9 (12)
Patients, < 18 years, TBSA > 15%, n (%)	3 (8)	1 (2)
Patients at-risk, n (%)	65 (64)	81 (68)
Total patient-days at-risk	1205	1372
Days at-risk/patient, mean (sd)	12 (18)	11 (15)
Hospitalization (days), mean (sd)	20 (20)	19 (20)

Table 2. S. aureus colonization of burn wounds of patients-at-risk and the transmission routes.

	TBSA* (%)	LOS** days	Number of days until first wound colonization	*S. aureus* carriage at admission, genotype	Genotype *S. aureus* isolate burn wound	Transmission route*	Cross transmission from	
							HCWs	patients
PreMup group								
916	10	25	25	Yes,B	B	endo	no	no
922	51	70	26	no	C	exo	yes	yes
924	8	26	11	Yes,E	B	exo	yes	yes
925	5	27	5	Yes,Q	Q	endo	no	no
957	3	6	3	Yes,ND±	ND	NA°	NA	NA
958	3	15	7	no	B	exo	yes	yes
965	8	17	9	no	M	exo	no	no
1001	33	37	21 21	no	P B	exo	yes yes	no yes
1011	30	53	7	no	A	exo	Yes	yes
1047	13	27	6	no	K	exo	yes	no
1055	8	28	7	no	I	exo	no	no
1028	6	20	10	no	B	exo	yes	yes
1064	37	76	10	no	K	exo	no	yes
1099	24	44	44	No	H	exo	no	yes
1120	22	25	6	Yes,F	F	endo	no	no
PostMup Group *1-6 months following the mupirocin intervention*								
1075	7	14	13	no	I	exo	no	no
1122	9	15	3	no	K	exo	no	no
1134	11	27	11	no	L	exo	no	no
1069	9	21	8	Yes,N	N	endo	no	no
1090	38	73	71	no	N	exo	no	yes
1094	5	29	4	no	A	exo	yes	yes
1093	32	102	36	no	H	exo	no	yes
1144	24	89	31	no	O	exo	no	yes

* endo: endogenous; exo:exogenous
** LOS: length of stay
± ND: not done
° NA; not assessed

= continued =

Table 2. S. aureus colonization of burn wounds of patients-at-risk and the transmission routes.

Patients no.	TBSA* (%)	LOS** days	Number of days until first wound colonization	*S. aureus* carriage at admission, genotype	Genotype *S. aureus* isolate burn wound	Transmission route*	Cross transmission from HCWs	Cross transmission from patients
PostMup Group *6-12 months following the mupirocin intervention*								
1195	5	19	6	no	G	exo	no	No
1216	5	8	2	no	B	exo	yes	yes
1222	13	21	2	yes,K	E	Exo	no	yes
1225	7	19	8	yes,B;P	B	Endo	no	no
1214	4	42	31	no	K	exo	yes	yes
1241	9	18	8	no	J	exo	no	no
1245	18	49	4	no	S	exo	no	no
1247	5	26	23	yes,B	K	exo	yes	yes
1294	4	34	9	no	H	exo	yes	yes
1281	4	11	4	no	A	exo	yes	yes
1305	10	20	9	no	T R	exo exo	No no	no no

* endo: endogenous; exo:exogenous
** LOS: length of stay
± ND: not done
° NA; not assessed

Table 2 lists for all patients at-risk who acquired burn wound colonization during hospitalization the percentage TBSA burned, length of stay and the number of days until first *S. aureus* wound colonization. This table also specifies the PFGE-type of the first *S. aureus* isolate of the burn wound and whether or not cross transmission was a possible explanation for the wound colonization.

Of the 15 patients at-risk who acquired burn wound colonization in the preMup group, 11 (73%) appeared to have been colonized via the exogenous route. In 7/11 (64%) of these patients, burn wounds were colonized with *S. aureus* strains which were epidemiologically and genetically linked to strains carried by HCWs. In 8/11 (73%) patients, burn wounds were colonized with strains similar to the ones of other patients in the burn centre, who were already colonized. In two patients an exogenous source was deemed most likely, but was not identified among health care workers nor among other patients. Since environmental cultures were not done contaminated innate objects could have been the sources in these cases.

Table 3. Sources of S. aureus burn wound colonization before and after mupirocin treatment of health care workers.

	before mupirocin	**1-6 months after mupirocin**	**6-12 months after mupirocin**	***P*-value*, 1-6 months after mupirocin versus before and 6-12 months after**
Patients with nosocomially colonized burns	15	8	11	
Source of *S. aureus*				
endogenous source, n	3	1	1	1.000
exogenous sources[#], n	11	7	10	0.713
health care workers, n (%)	*7 (64)*	*1 (14)*	*5 (50)*	0.027
other patients, n (%)	*8 (73)*	*4 (57)*	*6 (60)*	0.693
unknown environmental n (%)	*2 (18)*	*3 (43)*	*4 (40)*	0.416
not assessible**	1	0	0	

*Calculated exact binomial

for each patient all possible exogenous sources were scored; per patient more than 1 source

** strain not available for typing

In the postMup group, 17/19 (89%) patients at-risk who acquired burn wound colonization, were most probably colonized via the exogenous route. During the first 6 months after the mupirocin intervention only 1 out of 7 (14%) strains from cross-infected patients was epidemiologically and genetically linked to a strain of a HCW (p=0.027 compared to preMup and to 6-12 months postMup (Table 3). 4/7 (57%) patient' strains were linked with strains from already colonized patients and in 3 patients the exogenous sources were not identified (possibly environmental). Thereafter, 5/10 (50%) and 6/10 (60%) cross-infected patients were linked to HCWs or already colonized patients, respectively , and in 5 patients the exogenous source of their colonizing strain of *S. aureus* remained hidden.(possibly environmental) (Table 3).

During the whole study period 19 different PFGE types were identified as first burn wound colonizers: types B, K, H, A and I were more prevalent and colonized burn wounds of 19/34 (56%) patients (6, 5, 3, 3 and 2 patients, respectively). Burn wounds of a single patient were typically colonized by a single type. Interestingly, type B which caused most of the burn wound colorizations acquired from HCWs in the preMup Group did not colonize burn wounds in the first six months after the HCWs had been treated with mupirocin.

DISCUSSION

The transmission dynamics of *S. aureus* in burn centres is complex. Factors influencing the prevalence and incidence of burn wound colonization include admission and discharge of colonized and non-colonized patients, colonized and non-colonized HCWs and cross transmission, usually via temporarily contaminated hands of HCWs (5). Also, innate objects and surface in the burn unit environment may act as temporary sources of *S. aureus*. In this single-centre study we investigated the incidence and acquisition routes of *S. aureus* burn wound colonization and the effect of a 5-day nasal mupirocin course in all HCWs.

Our results show that mupirocin indeed significantly reduced *S. aureus* carriage among HCWs, with a 100% elimination rate in the nose. Furthermore, the data suggest a long-term effect of mupirocin with reduced carriage rates for approximately 10 months, a finding that is similar to that reported elsewhere (10, 39). We treated both carriers and non-carriers because the nasal-pharyngeal cavity can be persistently or intermittently colonized with *S. aureus* (29), and we wanted to be sure to treat the latter category of HCWs as well.

Eradication of *S. aureus* nasal carriage in HCWs, however, did not significantly reduce the incidence of *S. aureus* burn wound colonization and burn wounds of patients at-risk were still being colonized by cross transmission from exogenous sources. Interestingly, the present study suggests that *S. aureus* eradication among HCWs resulted in a shift in sources. Before eradication of *S. aureus* in HCWs, isolates colonizing burn wounds were epidemiologically and genetically linked to isolates from HCWs and/or *S. aureus* isolates from the group of already colonized patients. These linkages were equally strong and explained the sources of burn wound colonization in the majority of patients since no linkage with HCWs or other patients were found in only 18% of the cases. During the first 6 months following the mupirocin intervention, burn wounds were mainly colonized with PFGE types not carried by HCWs. In approximately 50% of the colonized patients at-risk, *S. aureus* types were linked with those from already colonized patients or from unidentified sources in the burn unit environment. After these initial six months following mupirocin, strains colonizing patients at-risk were again epidemiologically linked with those of HCWs or those of other patients in the majority of cases. Thus, eradicating *S. aureus* nasal carriage among HCWs resulted in a biological replacement. This finding suggests that nasal carriage of HCWs plays a significant role in the epidemiology of *S. aureus* at the burn centre.

Obviously, our clinical study has limitations. Firstly, several potential reservoirs of *S. aureus* including environmental ones were not screened in this study. When two epidemiologically linked patients are colonized by the same bacterial genotype, this does not necessarily mean that one patient acquired colonization from the other. A highly prevalent clone in the extramural population could be introduced by other routes. Also, contaminated environmental surfaces may contribute to the transmission of *S. aureus.* Health care-associated pathogens such as MRSA can survive for days to weeks on environmental surfaces and can be spread through hand (or glove) contamination from HCWs to susceptible patients (6, 21).

We conclude that nasal mupirocin is highly effective in reducing carriage in HCWs, especially nasal carriage, an effect that lasted for approximately 10 months. However, the patients' burn wound colonization rate was not significantly affected by this intervention. Instead, we observed a clear shift in sources and in the genotypes of *S. aureus* colonizing the burn wounds, away from HCWs and toward other patients and unidentified (environmental) sources of *S. aureus* present in the burn centre. Eradicating nasal *S. aureus* from HCWs should be considered as part of a *S. aureus* control strategy, especially when a particular virulent or endemic strain needs to be managed.

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CHAPTER 6

CLONAL ENRICHMENT OF INTEGRATED RESISTANCE PLASMID CONTAINING *STAPHYLOCOCCUS AUREUS* IN A BURN CENTRE ASSOCIATED WITH PERSISTENT CARRIAGE AMONG HEALTH CARE WORKERS

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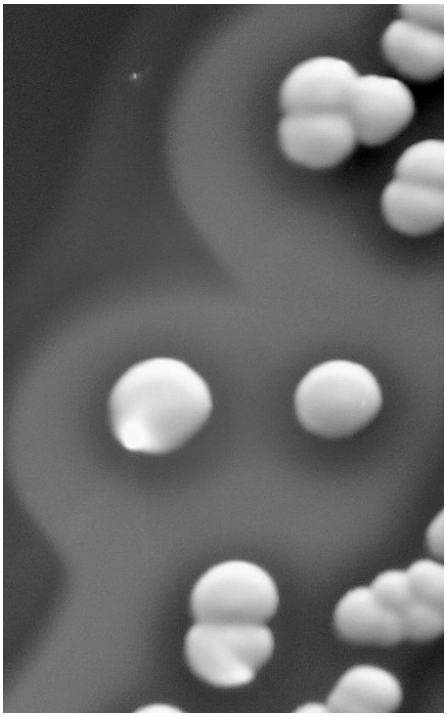
Submitted



ABSTRACT

The genetic population structure of serial *S. aureus* isolates obtained from patients and healthcare workers (HCWs) in a burn centre was investigated. We assessed the frequency of auto- versus exo-infection and established a model describing import and local persistence of *S. aureus* clones.

Three populations of *S. aureus* isolates were collected (2001-2005) and typed by PFGE. Population I comprised 375 strains from HCWs, Population II harboured 586 nosocomially acquired strains from burn wounds. Population III involved 202 strains from patients at admission. Comparative genome hybridisation (CGH) was performed for endemic versus incidental *S. aureus* strains.

The diversity index for Population III was significantly higher than those for Populations I and II. Three PFGE-types were clearly endemic among HCWs and nosocomially acquired *S. aureus* strains. CGH revealed that endemic strains possessed an integrated plasmid encoding resistance to heavy metals.

Genetic diversity for *S. aureus* strains circulating in the burn centre was lower than that of strains in the open community. Apparently, endemic *S. aureus* clones have a superior potential to colonize burns which may be associated with their heavy metal resistance in an environment where silver and cerium containing antibiotics are the most used.

INTRODUCTION

Immediately following thermal injury, burn wound surfaces are sterile. However, the burn wound surface is a nutrient-rich environment consisting of avascular necrotic tissue that provides a favourable niche for microbial colonization and proliferation (1-5). In burn wounds, molecules such as fibrinogen, fibronectin, collagen and many others are exposed at the surface (6). *Staphylococcus aureus* encodes many proteins that specifically interact with such human cellular matrix components. These microbial surface compounds recognizing adhesive matrix molecules (MSCRAMMS) render *S. aureus* one of the most common microbes found in burn wounds (7).

S. aureus burn wound colonization can be acquired exogenously by transmission or endogenously when the patient is already a natural carrier at the time of admission (3, 4, 8-10). In burn centres, patients as well as health care workers (HCWs) may continuously introduce new staphylococcal strains to the centre, but it is not know whether each of these strains has similar capabilities to colonise burn wounds, spread and become endemic. Such epidemiological knowledge is important for planning effective infection control measures. The aim of this study, therefore, was to investigate the population structure of *S. aureus* isolates systematically obtained from patients and HCWs of a burn centre over a period of 5 years.

METHODS

Burn Centre

The study was performed at the Burn Centre of the Martini Hospital, Groningen, The Netherlands. This dedicated Burn Centre is a closed unit including a operating theatre. At the time of the study it consisted of four separate rooms with two beds each and two one-bed Intensive Treatment (IT) rooms, accommodating a total of 10 patients. Patients remained in their room during the entire hospital stay, except for bathing and for surgical interventions.

A team of HCWs, who did not have healthcare duties outside the Burn Centre, took care of the patients. In addition to this team, other HCWs (e.g. psychologists and dieticians) visited the centre daily to tend to patients. Patients with burns were cohorted, and strict contact precautions were implemented. During wound dressing changes, routine contact precautions were supplemented with additional measures including the use of gowns, surgical masks and gloves by all HCWs in contact with the patients or the patients' environment.

Decontamination regimens

All wounds were treated once daily with silversulphadiazine in combination with ceriumnitrate (Flammacerium®, Solvay Pharma, Weesp, The Netherlands) usually for at least 10 days. In addition, adults with more than 20% total body surface area (TBSA) burned received an oral antibiotic regimen for selective decontamination of the digestive tract (SDD). SDD was also given to children (<16 yrs) and elderly (>60 yrs) with burns of >15% TBSA, and patients with perianal burns; SDD was given from admission until wounds were healed spontaneously or 5 days after the last surgical intervention. For adults the SDD regimen consisted of co-trimoxazole (400 mg, four times daily), colistine (100 mg, four times daily) and amphotericin B (500 mg, four times on the day of admission and two times daily thereafter).

Study design

S. aureus strains were collected from routine clinical specimens and from admission screening of patients, as well as from screening of HCWs during 3 different time-periods covering a period of 5 years in total (October 2000- August 2005).

We investigated the genetic population structure of three sets of *S. aureus* strains. Population set I (n=375) consisted of all *S. aureus* strains isolated from swabs of the vestibulum nasi of both the right and left nares and of the throat of HCWs. Population set II (n=586) included all *S. aureus* strains which were isolated from burn wounds that became nosocomially colonized, i.e. more than 48 hours after admission. These strains were classified as 'nosocomially acquired strains'. Finally, Population set III (n= 168) included all *S. aureus* strains from patients, isolated within 48 hours after admission from routine screening swabs taken of the vestibulum nasi of both the right and left nares, of the throat and/or of the burn wound at the time of admission. These strains were classified as 'strains present on admission'.

Culture and PFGE

All patients with burn wounds were screened directly upon admission for *S. aureus* carriage. Burn wounds were routinely sampled on admission and weekly thereafter during hospital stay, and when clinically indicated, according to a standard protocol. The samples from burn wounds, noses and throats from patients and HCWs were obtained and cultured as described previously (11). Presumptive *S. aureus* strains were tested for free coagulase production and DNase activity and *S. aureus* strains were stored in sterile skim milk (Oxoid) at - 70°C. Finally, *S. aureus* strains were genotyped with PFGE as described previously (11). The software package BioNumerics (Applied Maths NV, Sint-Martens-Latem, Belgium) was used to analyze and group PFGE-patterns (0.8% position tolerance, Dice, UPGMA). Interpretation of the relatedness between grouped PFGE-

patterns was performed visually according to guidelines of Tenover *et al.* (12). The results were represented as PFGE-types (each type included closely related PFGE patterns that differed by ≤ 3 bands).

To describe the genetic population structure for *S. aureus*, we used a simple diversity index as described by Gastmeier *et al*. (13). In a population of N isolates of *S. aureus* in a defined environment, a given isolate will belong to one of the D distinguishable types. The diversity index can be computed as (D/N)x100.
For HCWs who where screened for *S. aureus* nasal carriage at least 3 times during the study period a carriage index was defined as the number of nasal swab specimen cultures that grew *S. aureus* divided by the total number of nasal swab specimen cultures performed for that person. Persistent nasal carriers were those persons with carriage indices of 0.9 or higher, intermittent carriers were categorized as 'intermittent, regular' or 'intermittent, occasional' when carriage indices fell between 0.5-0.8 and 0.1-0.4, respectively. Noncarriers were those persons with indices of zero.

Microarray

To isolate chromosomal DNA from *S. aureus*, bacteria were grown overnight in BHI. The bacteria were harvested and mechanically lysed using a Fastprep (speed 6.0, 45 sec, X2 with incubation on ice for 1 min in between). The mix was centrifuged (10000g) and the supernatant was isolated. Ethanol was added to a final concentration of 33.3% and the DNA was isolated using the Minikit (Qiagen, Venlo, The Netherlands). Hybridization probes were generated from 3 µg DNA according to the protocol from BµG@s (Bacterial Microarray Group). DNA was mixed with 3 µg random primers (Invitrogen, Paisley, United Kingdom), heat denatured (5 min. 95°C) and snap cooled on ice. DNA was labeled by adding 5 µl of 10 x REact 2 buffer(Invitrogen, Paisley , United Kingdom) , 1 µl dNTPs (5 mM A/G/T/TP, 2 mM dCTP(Amersham Biosciences, Chalfont St.Giles, United Kingdom), 1.5 µl Cy3 or Cy5 dCTP (Amersham Biosciences, Chalfont St.Giles, United Kingdom), and 1 µl Klenow (3-9 U/µl; Invitrogen, Paisley , United Kingdom). Samples were incubated at 37°C in the dark for 90 min. Tester DNA was always labeled with Cy3, reference DNA (MRSA252) with Cy5.
The two samples were pooled, purified using a MinElut kit (Qiagen, Venlo, The Netherlands) and hybridized to an *S. aureus* microarray overnight, before washing and scanning (14). *S. aureus* microarrays were used with PCR amplicons printed on Ultragaps (Corning) glass slides (Bacterial Microarray Group (BµG@S), St. George's Hospital Medical School, London, UK) (14).

Data analysis microarray

Scanned images were converted to raw data by Imagene 6.0 (Biodiscovery, El Segundo, CA). Raw data were normalized as described by Lindsay *et al.* 2006 (15). A one-way ANOVA analysis was performed to find genes which were distributed significantly differently among endemic and non-endemic strains. Genes of which no data were obtained in one or more strains were taken out of the analyses. Furthermore, we restricted the analyses to the MRSA252 genes on the array. As defined by Lindsay *et al.* (15) genes with a fluorescence intensity ratio (test isolate/reference isolate) between 0.5 and 2 were considered to belong to the core genome. A strain with a gene-specific fluorescence intensity ratio of < 0.5 was considered to be negative, if a fluorescence intensity ratio of > 2 was found genes were presumed to be available in more than one copy. Finally, in a Fisher's exact analysis we scored a gene absent when its fluorescence intensity ratio was <0.5, and present when its fluorescence intensity was ≥0.5.

Statistical analysis

The difference in numbers of cultures positive for *S. aureus* in population set III versus population set II was tested for significance with Pearson Uncorrected chi-square.
Odds ratios with 95% confidence intervals were calculated for the *S. aureus* burn wound colonization rates in *S. aureus* carriers and noncarriers.
The binomial exact calculation was used to determine the 95% confidence intervals of Simple Diversity indices.

RESULTS

Patients

In total, 316 patients were admitted to the burn centre during this study. The average length of stay was 20 days (SD 21 days); the median length of stay was 15 days. At admission, 248/316 (78%) patients were cultured for *S. aureus* carriage in nose, throat and burn wound, according to protocol. Of 22 patients not all swabs were taken because patients were admitted for reconstructive surgery (n=5), for observation (n=5), or patients were already intubated (n=5), or were admitted at another ward before admission to the Burn Centre (n=7). Of 46 (15%) patients not all data were recorded.
Cultures taken at the time of admission revealed that 74/248 (29.8%) patients were *S. aureus* carrier; 44 (17.7%) were nasal carrier, 19 (7.7%) were nasal and pharyngeal and 11 (4.4%) were pharyngeal carrier only. Of these 74 carriers 20 had already colonized their burn wounds at the time of admission. 24/248 (9.7%) patients were nasopharyngeal

S. aureus noncarriers at the time of admission but already had *S. aureus* colonized burn wounds. In total, thus, 98 patients carried *S. aureus* at the time of admission in nose, throat and/or burn wound. Of these patients, burn wounds of 74 (75%) patients were sampled during their stay at the centre. The remaining 24 patients were discharged within 7 days. In 49/74 (66%) of the patients *S. aureus* was isolated in at least one of the cultures of the burn wounds. Molecular fingerprinting showed that in 13/23 (57%) patients who were nasopharyngeal *S. aureus* carrier at admission the colonizing strain of the burn wound during hospital stay was identical to the strain carried at the time of admission. These patients' burn wounds were most probably colonized via the endogenous route.

At the time of admission, 150/248 (60%) patients did not carry *S. aureus* in nose, throat or burn wound. Of this group 119 (79%) patients were also recultured during hospital stay; 42/119 (35%) of these patients developed wound colonization with this bacterial species during their stay at the burn centre. Cross infection was the most probable explanation for the wound colonization of these patients. However, the risk of acquiring *S. aureus* burn wound colonization was less for patients who were noncarrier at admission when compared to patients who were *S. aureus* carrier at admission (42/119 [35%] versus 49/74 [66%], odds ratio: 3.54 [95% CI: 1.87 - 6.95]).

S. aureus strains

Overall, 5,750 cultures were performed. In 1,163 cultures (20%) *S. aureus* was isolated. 985/1163 (85%) strains were submitted to molecular typing. PFGE following *SmaI* digestion resolved 58 PFGE-types. Most prevalent PFGE-types were designated A, B and C (Figure 1). These three groups represented 178 (18%), 179 (18%), and 105 (11%) isolates, respectively. Table 1 shows the diversity index for each of the three Population sets of *S. aureus.* The population structure for *S. aureus* isolated from patients at admission (Population III) was significantly more heterogeneous (diversity: 20 [CI95: 14-27]) when compared to the population of *S. aureus* isolated from burn wounds (Population II) and from HCWs (Population I), with diversity indices of 9 (CI95: 6-11) and 9 (CI 6-12), respectively. These differences in the genetic diversity between *S. aureus* populations isolated from patients on admission versus those isolated from HCWs and nosocomially colonized burn wounds remained significant throughout intervals of the 5 year observation period (data not shown). Table 2 shows for all three *S. aureus* Population sets the quantitative distribution of the PFGE-types. The *S. aureus* Population set I (strains isolated from HCWs) was clearly dominated by only three PFGE-types (A, B and C). In contrast, no such dominance of only a few strains was observed among

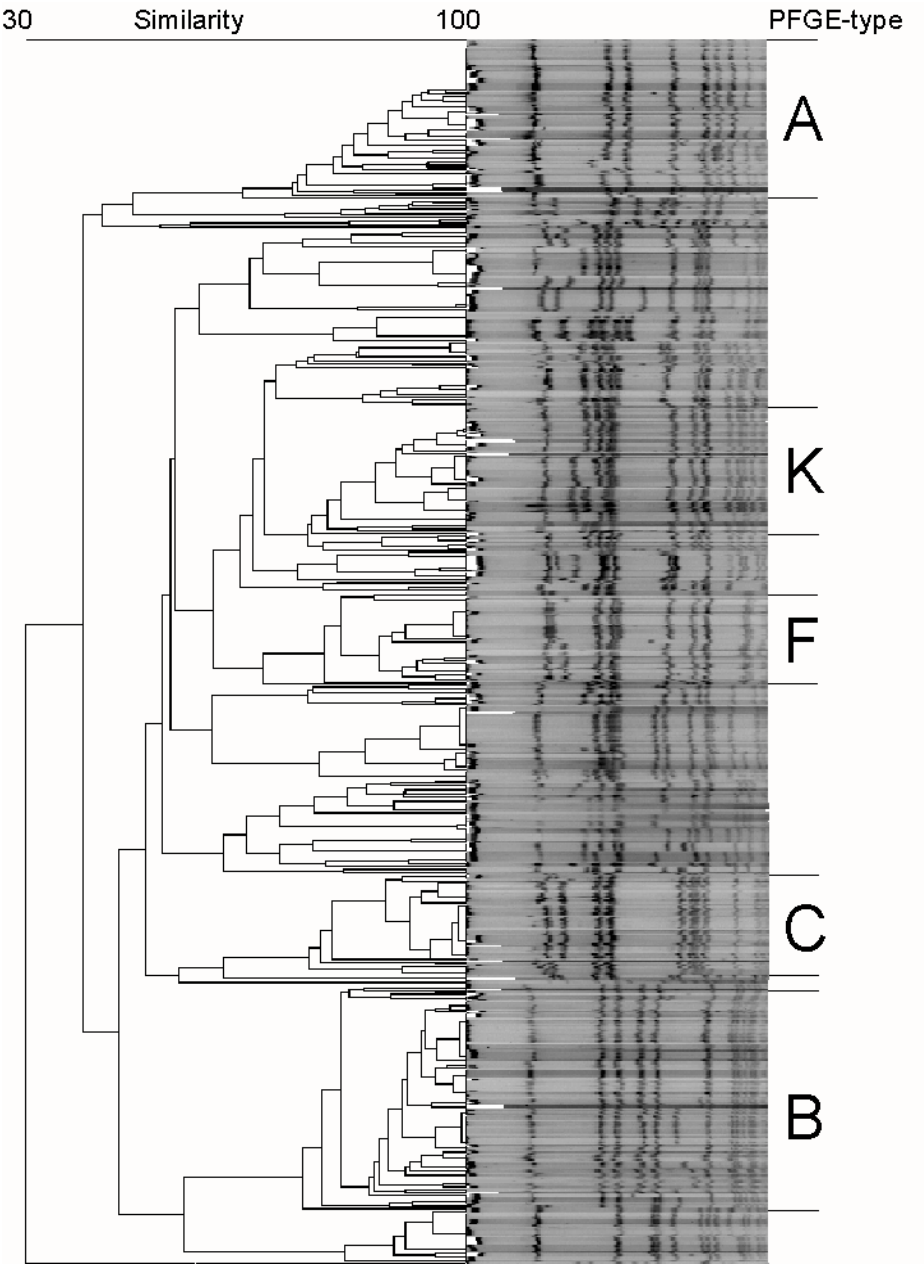


Figure 1. Dendrogram of PFGE patterns showing the genetic relatedness of 985 S. aureus strains isolated from patients at the Groningen Burn Centre over a 5-year period. The capital letters on the right of the figure indicate the predominant PFGE types.

Table 1. Characteristics of three sets of S. aureus strains isolated from health care workers and patients.

Set	I	II	III
	Health Care Workers	**Patients' acquired strains in burn wounds**	**Patients' strains present on admission**
Cultures, n	1628	2518	1604
Individuals, n	130	250	311
Cultures positive for *S. aureus*, n (%)	375 (23.0)	586 (23.3)	202 (12.6)
S. aureus isolates genotyped, n (%)	336 (89.6)	481 (82.1)	168 (83.2)
PFGE-types, n[a]	29	41	33
Simple diversity index[b] (95% CI)	8.6 (5.9-12.2)	8.5 (6.2-11.4)	19.6 (13.9-26.5)

[a] *PFGE-type: PFGE-patterns that differed at ≤ 3 band positions*
[b] *In a population of N isolates of S. aureus in a defined environment, a given isolate will belong to one of the D distinguishable types, the Simple Diversity Index= (D/N)x100.*

S. aureus isolated from patients at the time of their admission to the centre even though PFGE types A and B were still quite prevalent (Table 2). The population of *S. aureus* nosocomially acquired in patients' burn wounds (Population set II) was dominated by two PFGE types (A and B).

HCWs

In total, 1628 cultures were taken from 130 HCWs during the observation period; 57/130 HCWs were in the dedicated Burn Centre team at the Martini Hospital. 59 of the HCWs were screened for nasal *S. aureus* carriage at least 3 times during the observation period. Among this group of HCWs 18/59 (31%) HCWs were identified as noncarriers, 17/59 (29%) as intermittent, occasional carriers, 11/59 (19%) as intermittently, regular carriers and 13/59 (22%) as persistent carriers (Table 3). Table 3 also shows the distribution of PFGE-types among the 3 categories of carriers and the group of

noncarriers. This table shows clearly that PFGE-types A, B and C were dominant in all categories of carriers.

Table 2. Quantitative distribution of clones within three sets of S. aureus strains isolated in a Burn Centre.

Set	**I**		**II**		**III**	
	Health Care Workers		**Patients' acquired strains in burn wounds**		**Patients' strains present on admission**	
isolates (n)	**number of PFGE-types**	**PFGE-type**	**number of PFGE-types**	**PFGE-type**	**number of PFGE-types**	**PFGE-type**
>50	3	*A,B,C*	2	*A,B*	0	
40-50	0		2	*C,K*	0	
30-40	0		1	*F*	1	*B*
20-30	0		1	*H*	1	*A*
10-20	2	*E,F*	8	*ns*	2	*U,K*
0-10	24	*ns*[a]	27	*ns*	29	*ns*
total	29		41		33	

[a]ns=not further specified

However, among persistent carriers, these three endemic clones represented 70% of all typed isolates. The diversity among the strains isolated from persistent carriers was clearly less when compared with the diversity in the populations of *S. aureus* isolated from the intermittent carrier categories. 8/13 (62%) persistent carrier HCWs were persistently colonized with a single PFGE-type of *S. aureus* throughout the 5 year observation period.

Microarray

In order to identify genes associated with the endemic character of PFGE-types A, B and C, we selected a representative set of 5 endemic and 8 non-endemic strains of *S. aureus* and performed a comparative genomics analyses. Based on a one-way ANOVA analysis 184 genes were obtained showing significant ($P<0.05$) differences between endemic and non-endemic. 29 genes were taken out of the analyses for these genes showed no data in one or more strains. From the remaining 155 genes, 139 genes were part of the genome of MRSA252 and used for further comparative analysis.

Based on the definition of core genome (fluorescence intensity ratio between 0.5 and 2 as core genome) defined by Lindsay *et al*. (15), another 108 genes were taken out of the

Table 3. Quantitative distribution of PFGE-types among health care workers in a burn centre..

	carriage -index	HCWs n (%)	PFGE-type																	n types	SD (95% CI)[b]
			A	B	C	D	F	K	E	G	U	L	J	H	I	O	M	P	N	?		
persistent carrier	0,9-1,0	13 (22)	30	18	18	1	9	3	5	2										12	8	9.3 (4.1 - 17.5)
intermittent, regular	0,5-0,8	11 (19)	4	11	4	3			4		3	2	2		2	2	1	1		8	12	30.8 (17.0 - 47.6)
intermittent, occasionally	0,1-0,4	17 (29)	10	4	2			1				2	1	1			1	2	1	5	10	40.0 (21.1 - 61.3)
noncarrier	0	18 (31)																				

[a] *Simple Diversity index, based on typed isolates only*

Table 4. S. aureus genes which are significantly associated with endemic strains.

	endemic strains					non-endemic strains							
Gene annotation	1567	1578	1692	728	868	1625	1711	1751	1771	1844	1874	734	827
SaMRSA252-0702 (1N14)	1,265	0,337	1,661	4,136	4,056	0,28	0,0577	3,382	0,244	0,0576	0,171	0,109	0,536
SaMRSA252-0707 (1K15)	1,228	0,43	1,226	3,102	3,723	0,4	0,127	3,462	0,192	0,262	0,0476	0,347	1,7
SaMRSA252-0714 (1O15)	0,904	0,856	1,463	0,695	0,919	0,446	0,0703	0,757	0,333	0,221	0,0802	0,262	0,63
SaMRSA252-0715 (1P15)	0,91	0,741	1,068	0,632	0,673	0,317	0,0854	0,725	0,627	0,366	0,0645	0,193	0,64
SaMRSA252-0717 (1J16)	1,15	0,588	1,091	0,506	0,796	0,225	0,0668	0,483	0,443	0,127	0,0273	0,112	0,368
SaMRSA252-0718 (1K16)	1,112	0,378	1,017	0,261	0,405	0,104	0,0494	0,348	0,297	0,0575	0,0512	0,106	0,197
SaMRSA252-0719 (1L16)	1,015	1,092	0,719	1,607	2,002	0,186	0,101	1,287	0,969	0,107	0,182	0,118	0,295
SaMRSA252-0720 (1M16)	0,925	0,202	0,968	0,18	0,276	0,0808	0,0913	0,18	0,141	0,0397	0,125	0,0504	0,163

gene present
gene not present
gene probably available in more than one copy

analyses for in these genes the fluorescence intensity ratios in both endemic and non-endemic strains fall in between. For all remaining 31 genes in one or more of the strains the genes were found to be absent (a fluorescence intensity ratio of < 0.5). Absence of genes (compared to MRSA252) was predominantly found in the non-endemic stains with a mean of 21.4 when compared to the endemic strains with a mean of 6.0. All genes in which fluorescence intensity ratio >2 were found to be part an integrated plasmid in MRSA252. Interestingly, the only gene showing a complete association with endemic strains and is missing in non-endemic strains is SaMRSA252-0717; this gene is part of this plasmid as well and it encodes a LysR regulatory protein. Apart from SaMRSA252-0717 several other genes are part of this plasmid and have a significant association with endemic behavior; these are SaMRSA-0702, 0707, 0714, 0715, 0718, 0719, 0720. These data indicate that in several of the investigated strains the plasmid is either absent, integrated, or present in multiple copies (Table 4). When we use the criteria fluorescence intensity ratio <0.5 for absence and ≥0.5 for presence and performed a Fisher's exact analysis four genes were significantly associated with endemic strains (Table 5).

Table 5. S. aureus genes which are significantly associated with endemic strains.

Gene annotation	Gene	Characteristics	P value
SaMRSA252-0284v	essC	Required for secretion of esxA (By similarity). Cell membrane; Multi-pass membrane protein (Potential). This strain lacks esaC and esxB. EsaC is probably involved in the establishment of infection in the host; esxB is an exoprotein that may interact with esxA and it is important for the virulence of some *S. aureus* strains. Contains 2 FtsK domains.	0.0319
SaMRSA252-0714	SAR0714	Hypothetical protein [*Staphylococcus aureus* subsp. *aureus* MRSA252]	0.0210
SaMRSA252-0717	SAR0717	Plasmid, LysR family regulatory protein [*Staphylococcus aureus* subsp. *aureus* MRSA252]	0.0008
SaMRSA252-2787	SAR2787	Hpothetical protein [*Staphylococcus aureus* subsp. *aureus* MRSA252]	0.007

727954..728670		SAR0680 conserved hypothetical protein
728670..729143		SAR0681 conserved hypothetical protein
729496..730140		SAR0682 hypothetical protein
730258..731124		SAR0683 LysR family regulatory protein
731256..732476		SAR0684 putative sugar efflux transporter
733183..734391complex	SAR0686	putative transposase (pseudogene
734448..735122		SAR0687 sequence IS257(818C) putative transposase
com(735157..735369)	SAR0688	IS element inverted repeat
735689..736024		SAR0689 recombinase (partial)
736085..736399		SAR0690 arsenical resistance operon repressor 1
736399..737688		SAR0691 arsenical pump membrane protein 1
737706..738101		SAR0692 arsenate reductase
738308..738466		SAR0693 putative membrane protein
738481..738774		SAR0694 putative exported protein
738849..740783		SAR0695 putative membrane protein
740787..741098		SAR0696 putative exported protein
741110..741736		SAR0697 ABC transporter ATP-binding protein
742098..743744		SAR0698 putative transposase"
743899..744192complex	SAR0699	plasmid recombination enzyme (pseudogene)"
com(745289..745432)	SAR0702	hypothetical protein
745474..746334		SAR0703 plasmid replication initiation protein
746421..746762		SAR0704 putative DNA-binding protein
746836..746967		SAR0705 putative membrane protein
complex(747622..748152)	SAR0707	putative replication-associated protein
748482..748598		SAR0710 hypothetical protein
748651..748653complex	SAR0711	putative replication initiation protein
749717..749902		SAR0713 putative replication protein
750428..750637		SAR0714 putative membrane protein
com750819..751142)	SAR0715	hypothetical protein
751371..751463		SAR0716 putative membrane protein
com751765..752586)	SAR0717	LysR family regulatory protein
752711..753706		SAR0718 putative membrane protein
com754041..754616)	SAR0719	putative resolvase
754883..756928		SAR0720 putative cation exporting ATPase protein
756943..758376		SAR0721 multicopper oxidase protein
com758443..760089)	SAR0722	putative transposase
com760452..762632)	SAR0723	probable cadmium-transporting ATPase
com762625..762990)	SAR0724	putative cadmium efflux system accessory
com763228..763605)	SAR0725	Tn554 transposase C
763700..764374		SAR0726 putative transposase
com765037..765726)	SAR0728	putative membrane protein
765853..766296		SAR0729 putative acetyltransferase
766363..766752		SAR0730 putative lipoprotein
766890..767189		SAR0731 putative acetyltransferase

Significant in ANOVA
Significant in ANOVA and Fisher Exact
Heavy metal ion involvement

Figure 2. Gene content (boxed region) of the integrated plasmid that serves as a molecular marker for distinguishing endemic and non-endemic burn wound-associated S. aureus strains.

Three of these genes are located on a plasmid (Figure 2). Interestingly, this plasmid is coding for multi heavy metal resistance traits. This plasmid could serve as a molecular marker for distinguishing endemic and non-endemic burn wound associated strains.

DISCUSSION

We define a significant difference in the genetic diversity of *S. aureus* strains isolated from patients at admission versus the lesser diversity of *S. aureus* strains circulating among HCWs and those colonising burn wounds of patients during their stay. While strains from patients at the time of admission were rather diverse, probably reflecting natural diversity of *S. aureus* circulating in the open community, strains circulating within the burn centre were much less diverse and clearly showed 'enrichment ' of only a few clones. We identified three endemic *S. aureus* types which were found among patients as well as HCWs. Apparently, these endemic *S. aureus* clones were better equipped for colonizing burn wounds than other, non-endemic PFGE-types.

This finding suggests that this burn centre can be classified as a special ecological niche favouring some clones of *S. aureus* over others. It is not yet known what makes these *S. aureus* clones more capable to persist and circulate at the burn centre. Koning *et al.* observed clonal expansion of a certain *S. aureus* genotype associated with skin disease (impetigo) (16). Skråmm *et al*. also identified three clonal complexes which dominated in an orthopaedic unit in Norway (17). In addition, outbreaks of MRSA in hospitals are reported as clonal suggesting that selective advantage of certain strains of *S. aureus* occurs regularly in various clinical settings (18-20).

Some studies showed that several bacterial factors which were associated with invasive disease may directly reflect their involvement in pathogenesis of severe disease (21, 22). In our study, comparative genomics analysis showed that most of the genes which were significantly discriminating endemic strains from non-endemic strains are located on a plasmid. This plasmid could serve as a molecular marker to distinguish endemic and non-endemic burn wound associated strains. In the 13 tested strains (5 endemic and 8 non-endemic) the regulatory protein SaMRSA252-0717 and the cell membrane associated protein SaMRSA252-0284v were reliably discriminating endemic from non-endemic strains. Whether indeed these genes play a role in the endemic behavior of these strains needs to be investigated further.

At the Groningen Burn Centre all wounds were treated once daily with silversulphadiazine in combination with ceriumnitrate (Flammacerium®, Solvay Pharma, Weesp, The Netherlands) usually for at least 10 days. An environment in which such antimicrobials agents are routinely used could select for bacteria that are (inherently)

resistant to this agent. Silver resistant bacteria have been found in burn settings (23-27). Whether the above mentioned plasmid plays a role in silver resistance needs to be investigated further.

Our data suggest that spread and persistence of a few related strains occurred at this burn centre. This occurred despite cohorting of patients and implementation of strict contact precautions. The long term sharing of a few clones of *S. aureus* between the same persistently colonized HCWs and subsequent cohorts of burn patients may be taken as evidence that HCWs can serve not only as vectors but also as important reservoirs in the transmission of *S. aureus* to patients with burns.

S. aureus carriage state during admission, was a predictor of subsequent *S. aureus* colonization: 66% of carriers but only 35% of non-carriers developed *S. aureus* wound colonization during their stay at the centre. Strain typing of paired admission and subsequent clinical isolates indicated that 57% of the carriers became colonized with a strain identical to their admission isolate. Previous studies also show that the *S. aureus* carriage state is a predictor of subsequent development of *S. aureus* colonization and infection (28, 29).

At the Groningen Burn Centre 22% of the HCWs were identified as persistent carrier, 48% as intermittent carrier of *S. aureus* and 31% as noncarrier. Several longitudinal studies reported that *S. aureus* nasal carriage rates patterns differ between individuals; 10-35% of individuals carry *S. aureus* persistently, 20-75% carries *S. aureus* intermittently, and 5-50% never carry *S. aureus* in the nose (30-32). Furthermore, the number of *S. aureus* cells in the nose is significantly higher in persistent carriers than in intermittent carriers (33, 34) resulting in an increased risk of spread of *S. aureus* (35). We found that 62% of the persistent carriers were colonized with a single PFGE-type. This finding is supported by other studies which showed that persistent carriers are often colonized by only one selected single strain over long periods, while intermittent carriers carry many different strains over time (30, 32, 36, 37).

We conclude that a Burn Centre represents a special ecological setting for *S. aureus* in which some clones may flourish over others and become enriched, and where HCWs play an important part in the maintenance of the epidemiological network. Microanalysis showed that genes discriminating endemic strains from non-endemic strains were located on an integrated plasmid. Whether these genes play a role in the endemic behavior of these strains needs to be investigated.

Infection control in burn centres should address the issue of HCWs as a potentially important reservoir of endemic nosocomial *S. aureus.*

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CHAPTER 7

A MODEL FOR THE DYNAMICS OF *STAPHYLOCOCCUS AUREUS* IN A BURN CENTRE

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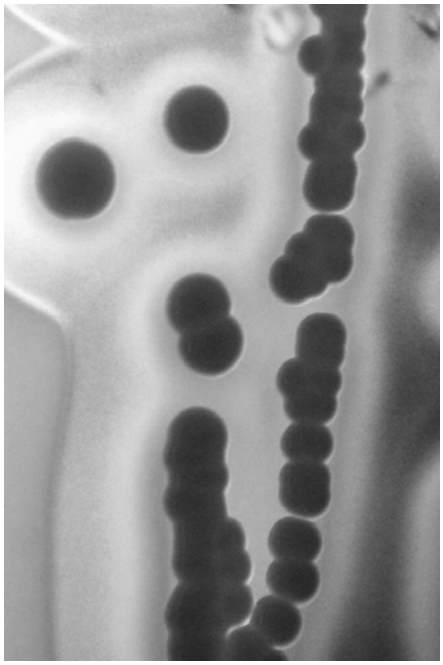

ABSTRACT

Wound colonization by *Staphylococcus aureus* usually precedes *S. aureus* infection in burn wound patients. To assess which measures are effective in the prevention of colonization, knowledge of the colonization dynamics is required. Here we apply a transmission model to data on length of stay and wound colonization to estimate whether the importance of different transmission routes changed during two interventions: eradication by application of mupirocin of nasal *S. aureus* carriage in patients (1) and health care workers (2).

Eradication of *S. aureus* in patients reduced the acquisition risk of *S. aureus* wound colonization in patients without nasal *S. aureus* colonization on admission, but not in patients with nasal colonization. A protective effect on wound colonization of nasal application of mupirocin in health care workers could not be demonstrated. During the first study, cross transmission between patients, possibly mediated by the hands of health care workers, seemed absent, while it was present during the second intervention. Despite the small patient population and their low turnover time, the model showed a protective effect of nasal application of mupirocin in burn wound patients.

INTRODUCTION

Infections are common complications in burn wound patients as the skin, a natural barrier against pathogens, is damaged. The burn wound provides an attractive niche for microbial colonization and proliferation (13, 21). One of the most common burn wound pathogens is *Staphylococcus aureus* (*S. aureus*) (3, 29). *S. aureus* is a commensal microorganism in 10-35% of the human population, and preferentially resides in the anterior nares. Several studies showed that the risk of *S. aureus* burn wound colonization varies considerably (14-83%) (1, 23, 24, 27).

Colonization with *S. aureus* usually precedes an infection. Patients who are nasal carriers of *S. aureus* have a significantly higher risk of burn wound colonization (16). Several studies showed that burn patients regularly (10%-28%) develop septicaemia (2, 14, 25) in which *S. aureus* is one of the most common pathogens. Interventions, which could prevent colonization and infection with *S. aureus* would, therefore, be useful. However, to predict what type of interventions will be successful as preventive measure(s), we need to know the different routes via which patients acquire *S. aureus* colonization. Several mechanisms can be identified as factors which influence the prevalence of *S. aureus* colonization in burn centres.

1. Admission of patients. Admission of patients who are carriers of *S. aureus* colonization will increase the prevalence, while admission of uncolonized patients will reduce the prevalence.
2. Discharge of patients. Discharge of colonized patients reduces the prevalence while discharge of uncolonized patients increases the prevalence in the centre.
3. Transmission between patients. With this route, two mechanisms are incorporated, either transmission due to direct patient-patient contact and, probably more important, transmission from patient to patient via temporarily colonized HCWs. This last mechanism can be foremost when hand decolonization of HCWs after a patient contact is not optimal (6, 8).
4. Transmission between patients and persistently colonized health care workers (HCWs). HCWs may be persistent nasal/throat carriers of *S. aureus*. These persistent carriers may spread their *S. aureus* strain to patients (20). Also uncolonized HCWs may become nasal/throat carriers, albeit temporarily, due to contact with persistently colonized HCWs and with colonized patients.
5. Environmental contamination (7, 11, 27). Colonized patients and HCWs may contaminate their local environment. When this contamination is persistent, even after the patient is discharged or decolonized, this environmental contamination can be a source of colonization and infection for patients and HCWs, especially for patients in the room of the discharged patient. Apart from environmental colonization of surfaces, also

colonization of medical instruments (12) or colonization of the air conditioning (19, 18, 28) can occur.
6. Visitors (20), including health care workers who visit the centre infrequently, may be colonized with *S. aureus* and, hence, may contribute to the force of infection.
7. Contaminated foodstuffs (15) may also be a source of *S. aureus*.

Apart from the different dynamics of colonization in a Burn Centre, patient factors may also be important. 1. The site of colonization. The infectivity towards other patients may depend on whether the nares of a patient are colonized and/or whether their burn wounds are colonized. Moreover, also the size of the colonized wound may influence the infectivity of a patient. 2. The susceptibility of an uncolonized patient. If a large proportion of the body surface is burned, patients are more susceptible to colonization of the wounds. Also, the severity of the burn wounds may influence the susceptibility. Apart from that, several clinical and behavioral characteristics of patients may expose patients to significant risks of colonization.

Genotyping combined with epidemiological linkage of patients is an important tool to assess the importance of different colonization routes. Based on this approach, we performed two clinical studies at one of the three dedicated Burn Centers in the Netherlands. In the first study we evaluated the effect of *S. aureus* nasal eradication in patients on admission, by a short course of nasal mupirocin, on *S. aureus* burn wound colonization (MUP1) (for further details see 16). In the second study we evaluated the effect of *S. aureus* nasal eradication in HCWs, also with a short course of mupirocin, on *S. aureus* burn wound colonization in patients (MUP2) (17). The first study aimed to interfere in the within patient transmission (from nose to wound) and the second study aimed to interfere in the HCW to patient route.
In the current study, we have used the data from both studies to quantify different transmission routes, using a recently published mathematical model (5, 9). The underlying observation of the model is that different transmission routes lead to different natures of fluctuation in the prevalence. For instance, if cross transmission between patients is the dominant route, there will be strong autocorrelation in the longitudinal prevalence data, i.e., periods of a low or high prevalence will last relatively long. This is in contrast with the situation where cross transmission is not important and, for instance, patients already colonized upon admission are most relevant for an increased prevalence. In such a scenario there is no dependency in the colonization status between patients, and the fluctuations in the prevalence will be smaller and occur more rapidly. The research question was whether the two interventions changed the colonization dynamics of *S. aureus* in the burn wound centre.

METHODS

We consider the following simplified model for transmission in a centre of N beds. Each patient has a risk to acquire colonization in the burn wounds, which depends on (1) a constant colonization pressure (α), e.g., due to visitors or persistently colonized HCWs, (2) the colonization pressure due to wound colonization of other patients, which we call λ. In our baseline scenario, this force of colonization is proportional to the fraction of the colonized patients in the centre ($\lambda=\beta I/N$), (3) the colonization status of the nose/throat of a patient. The force of colonization is γn, where n, equals 0 or 1, when the nose/throat is uncolonized and colonized, respectively, and (4) the fraction of burned body surface ($0 \leq b \leq 1$).
The probability for a patient to acquire colonization on a given day is given by $1-e^{-b(\alpha+\beta\lambda+\gamma n)}$. For small probabilities per day, this expression can be approximated by: $b(\alpha+\beta\lambda+\gamma n)$. In these expressions, α, β and γ are parameters which determine how important routes 1, 2 and 3 are, respectively. Another assumption here is that the force of colonization is proportional to the fraction burned body surface, i.e., the risk for a patient to acquire colonization is twice as high when the burned body surface is twice as high. We will also consider the scenario when the burned body surface does not affect the acquisition risk, i.e., the probability for a patient to acquire colonization is $1-e^{-(\alpha+\beta\lambda+\gamma n)}$.

Unfortunately, the exact colonization pressure in the center is unknown, as patients are not cultured on a daily basis but on admission and once a week only (and on indication of course). So, even if we assume that culture results are 100% reliable, we only know that a patient acquired colonization after his/her last negative culture and before his/her first positive culture. However, we can assume that wound colonization will persist during the stay in the centre and we assume that the result of the first culture performed represents the colonization status on admission. By using the methodology described by Bootsma *et al*. (4) we can determine estimates (and confidence intervals) for the values of the parameters α, β and γ, (see Bootsma 2007 (5) for a more detailed explanation of how the likelihood function of the parameters is calculated).
Confidence regions for the acquisition parameters were determined by using the standard likelihood ratio method (10) and a χ^2-test the difference of the log-likelihood with the maximum log-likelihood. Confidence intervals for single acquisition parameters or functions of the acquisition parameters were determined by the profile likelihood method. A χ^2-test was used to determine goodness of fit and likelihood ratio test were used to compare hierarchical models.

RESULTS

Setting

The study was performed at the Burn Centre of the Martini Hospital, Groningen, The Netherlands, a specialised unit dedicated to burn care. At the time of the study it consisted of four separate rooms with two beds each and two one-bed Intensive Treatment (IT) rooms, accommodating a total of 10 patients. Patients remained at the Burn Centre, and as a rule in their room during their entire stay. There was a dedicated team of HCWs, who had no healthcare duties outside the Burn Centre, while other HCWs (e.g. psychologists and dieticians) visited the centre daily. Patients were cohorted with strict contact precautions for all patients. Hand hygiene procedures, barrier isolation, waste management, equipment and environmental cleansing and disinfection were daily routines. During wound dressing changes, routine contact precautions were supplemented with additional measures including the use of gowns, surgical masks and gloves by all HCWs in contact with the patients or the patients' environment.

Elimination of *S. aureus* carriage among patients by a short course of intranasal mupirocin treatment at admission (MUP1).

In our analysis, we compare the MUP1 period (01-07-2000 to 31-12-2001) with a baseline period before MUP1 of the same duration (01-01-1999 to 30-06-2000). We have only included patients in the analysis for whom at least one culture result was available.

The point estimates of the parameters suggest a decrease in the acquisition risk via cross transmission (β) and external sources (α). Mupirocin seems to increase the risk that wounds become colonized with *S. aureus* from their own nose (γ). The parameter estimates with 95% confidence intervals for both periods are shown in Table 1. The difference in log likelihood of the model with (β≥0) and without (β=0) cross transmission was small (0.012 and 0 for the baseline and MUP1, respectively), and there was, therefore, no reason to include cross transmission.

The parameters for both periods without the influence of parameter *β* are shown in Table 2 and Figure 1. Not surprisingly given the almost non-overlapping 95% confidence intervals for the parameter *α,* a likelihood ratio test of two nested models (one in which *α* and *γ* were allowed to be different in both periods and one in which *α* and *γ* were constant over the combined period), preferred the model with different parameter values in the two periods. As *α* is lower in the MUP1 period, the intervention seems to protect

Table 1: Parameter estimates with 95% confidence intervals for the Baseline period and the MUP 1 period.

	α (95% CI)	β (95% CI)	γ (95% CI)
Baseline	0.054 (0;0.084)	0.012 (0;0.15)	0.035 (0-0.13)
MUP1	0.032 (0.013;0.046)	0 (0;0.041)	0.093 (0.026;0.197)

Table 2: Parameter estimates with 95% confidence intervals for the Baseline period and the MUP 1 period assuming there is no cross transmission.

	α (95% CI)	γ (95% CI)
Baseline	0.061 (0.042;0.083)	0.035 (0;0.121)
MUP1	0.032 (0.021;0.043)	0.093 (0.026;0.197)

patients who were uncolonized (both in the nose and in the wounds) from acquiring wound colonization. For patients with nasal colonization on admission, the acquisition risk per day ($\alpha+\gamma$) increases slightly from 0.096 to 0.125, although this increase is not statistically significant.

When we tested whether the acquisition risk was proportional to the fraction burned body surface (i.e., the risk to acquire colonization is $1-e^{-b(\alpha+\gamma n)}$), a lower likelihood was observed as compared to the model without such a correlation. This was also the case when we allowed for cross transmission. Thus a higher fraction burned body surface does not seem to increase the acquisition risk.

Finally, there was no reason to reject the model based on a χ^2-test (p=0.12 and 0.28 and 0.36 for the baseline, and the MUP1).

Elimination of *S. aureus* carriage among HCWs by one short course of intranasal mupirocin treatment (MUP2).

We have performed a similar analysis for the period preceding (pre-MUP2 (17-7-2004 to 16-7-2005) and following the short course of nasal mupirocin in health care workers (post-MUP2 (15-7-2004 to 9-7-2005). For the model in which we ignore the nasal/throat colonization status of the patients, the maximum likelihood estimates and the 95% confidence intervals are shown in Table 3 and Figure 2.

There were no statistically significant changes in parameters due to the MUP2 intervention (Table 3). This is supported by the comparison of the two types of nested models (one in which α and β were allowed to be different in both periods, and one in which α and β were constant over the combined period) that failed to yield evidence for a difference between both periods (p=0.13).

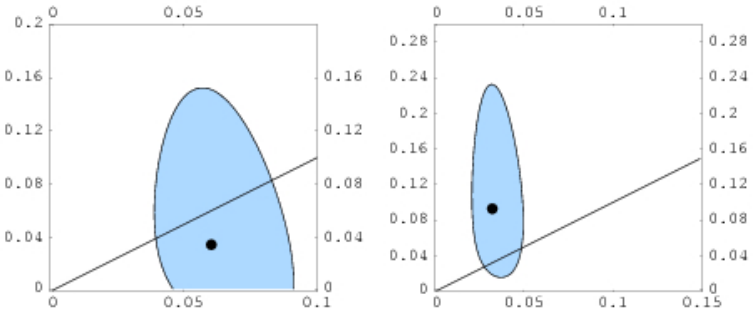

Figure 1: Maximum likelihood estimates (dots) and 95% confidence regions for α and γ. Vertical axis: γ, horizontal axis: α. Left figure baseline, right figure MUP1. The line corresponds to the combination of parameters for which the infectious pressure from the nose/throat and from other sources are equal for patients with nasal/throat colonization.

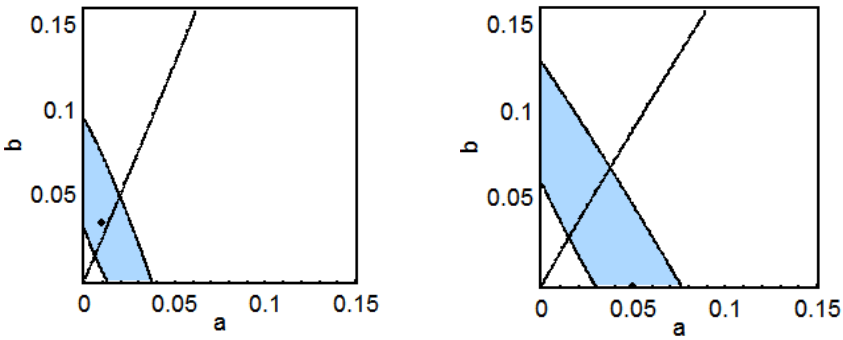

Figure 2: Maximum likelihood estimates (dots) and 95% confidence regions for the parameter of the endogenous route (horizontal axis) and the parameter for cross transmission (vertical axis). Left: Pre-MUP2, Right: Post-MUP2. The line corresponds to equal importance of both the endogenous and the exogenous route.

Table 3: Parameter estimates with 95% confidence intervals for all S. aureus strains for the Pre-MUP2 and the Post-MUP2 period.

	Pre-MUP2	Post-MUP2
α	0.010 (0-0.033)	0.050 (0-0.070)
β	0.036 (0-0.085)	0 (0-0.116)
# acquisitions	21 (20-24)	35 (33-37)
# endogenous acquisitions	9 (1-23)	35 (1-37)
# exogenous acquisitions	13 (0-22)	0 (0-36)
contribution endogenous route (%)	41.7 (3.4-98.7)	100 (1.6-100)
Mean prevalence (%)	54.91 (54.68-55.18)	67.35% (67.00-67.81)

DISCUSSION

We used the mathematical model published by Bootsma *et al*. (5, 7) to study the effect of two different nasal mupirocin interventions on transmission routes of *S. aureus* at a dedicated burn center. In our setting, interference in the within patient transmission (from nose to wound) has led to a decrease in the acquisition risk via external sources. An important observation is that the MUP1-intervention seems to protect patients who were uncolonized (both in the nose and in the wounds) from acquiring wound colonization, while nasal carriers had no reduced risk of acquisition of wound colonization. A possible explanation is that the nares serve as an intermediate step in the transmission process in the sense that colonization of the nares precedes colonization of the wounds. This also coincides well with the observation that the percentage of burned body surface is not correlated with an increased risk of wound acquisition. These results are in concordance with those of the preceding study at the Groningen Burn Centre (16).

Interference in the route from HCWs to patients by timely eradication of *S. aureus* nasal carriage among HCWs, did not lead to a significant effect on the transmission dynamics. This is in concordance with the study results of Kooistra-Smid *et al*. (17). However, they did observed a clear shift in sources and in *S. aureus* genotypes which were colonizing the burn wounds before and after the intervention. After the intervention burn wound colonizing strains were more genetically and epidemiologically related with other patients' strains than with HCWs' strains.

This study showed clearly that mathematical modeling of data could give insight in the importance of different transmission routes of *S. aureus*. Furthermore, effects of an intervention of these transmission routes can be quantified.
The Burn Centre where our studies were performed is characterized, as most other burn centres, by a small patient population with a long length of stay. This implies that the number of acquisitions of *S. aureus* per unit of time is limited. Furthermore, when cross transmission is important, acquisitions will not occur independently from each other (they will cluster in time) (22). These characteristics imply that Burn Centres belong to the most difficult study settings to obtain statistically significant differences between study periods. Even in settings with a high turnover of patients, long study periods may be needed to demonstrate a statistically significant effect of interventions unless the intervention is very effective (4). For Burn Centres, it may, therefore, be worthwhile to increase the α-error, e.g., to accept differences between study periods as statistically significant when the p-value is 0.1 or less, instead of the commonly used value of 0.05. This will increase the probability to detect an effect of a useful intervention, at the cost, of course, of a higher frequency of studies with false-positive outcomes. One could argue to accept this risk as even for false positive studies, it is more likely that the intervention has no effect than that the intervention will have a negative effect.
Finally, special attention should be paid to interventions with antibiotics, as selection of antibiotic resistance may be a long-term effect.
In conclusion, the application of mathematical modeling in burn centres is limited by the small patient population with a long length of stay.

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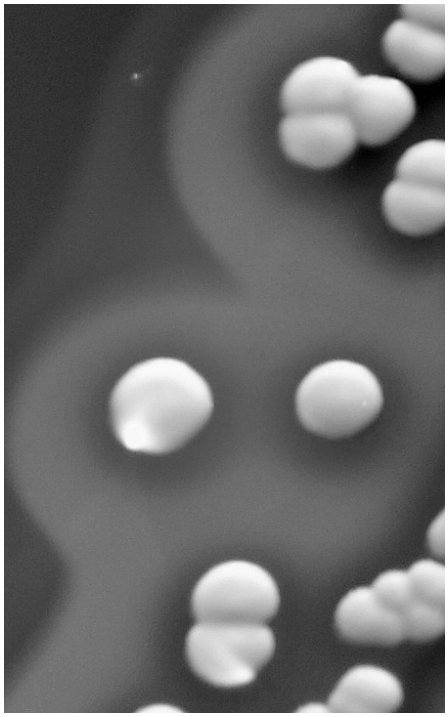
PART III

DISCUSSION

CHAPTER 8

SUMMARY



SUMMARY

General

Patients hospitalized with burn wounds are susceptible to microbial colonization and infection. The risk of colonization and infection is influenced by factors of the host (e.g. age, percentage total burned surface area, underlying diseases), the environment (e.g. close presence of other patients, health care workers (HCWs), invasive devices, improper burn care), and the infecting micro-organisms (e.g. type of species, virulence and anti-microbial resistance profiles). Infection of burn wounds will prolong the hospitalization period of the patient with burns and will increase the need for surgical interventions (1-5). Furthermore, colonization and infection of the burn wound may increase the risk on hypertrophic scarring (6). Despite major advances in burn care, infection remains a major cause of morbidity and mortality in patients with burns.

One of the most common pathogens in burn wounds is S*taphylococcus aureus* (7). The anterior nares are the primary ecological reservoir of *S. aureus* in humans (8, 9). Several studies have shown that approximately 10-35% of al human individuals are persistent carriers, about 20-75% carries this species intermittently and over 50% of the individuals appear to be persistent noncarriers (10-15). *S. aureus* carriage has been identified as a risk factor for the development of infections in various settings (8, 16). The frequency of *S. aureus* burn wound colonization varies considerably and is associated with the total burned surface area, the age of the patient and *S. aureus* carriage of patients and health care workers (1-4).

Important sources of *S. aureus* in burn centres are carriage sites of patients and HCWs. The principal route of transmission of *S. aureus* is thought to be from patient-to-patient via transiently colonized hands of the HCWs. HCWs could acquire *S. aureus* after direct patient contact or after handling contaminated material. Autologous strains of the HCWs themselves may be spread via the same mechanism. Furthermore, locations in the inanimate environment can become colonized with *S. aureus* and this species is known to survive for weeks or months on various surfaces. Patients as well as HCWs may continuously introduce new staphylococcal clones to a burn centre. However, it is not kown whether each of these clones has similar capabilities to colonize burn wounds and spread through the burn centre.

Extensive and detailed molecular epidemiological analyses of *S. aureus* carriage and transmission dynamics in burn centres have not yet been described in the literature. Elucidation of the transmission dynamics of *S. aureus* at a burn centre is essential for planning measures for preventing *S. aureus* burn wound colonization. In this chapter the overall results described in this thesis will be briefly summarized and discussed, and at the end recommendations will be given.

Prevention of *S. aureus* colonization and infection of burn wounds

In the past decades a variety of measures has been proposed to prevent *S. aureus* infections in patients with burns. Preventing the spread of *S. aureus* can be partly achieved by the implementation of hygienic measures such as contact precautions, cohort nursing and strict aseptic techniques when performing wound dressing changes. Furthermore, anti-microbial decolonization strategies have been applied in order to reduce the risk on *S. aureus* burn wound colonization. The current gold standard in topical burn treatment is silver-sulfadiazine which acts on the bacterial cell wall and binds relatively strongly to DNA (17). Selective decontamination of the digestive tract (18-20) and elimination of nasal *S. aureus* carriage in patients (21) have also been described as an attempt to reduce the risk of *S. aureus* colonization. Eradication of nasal *S. aureus* cells can be achieved temporarily by a single course of intranasal mupirocin (22).

The effect of nasal mupirocin in eliminating nasal *S. aureus* has been reported to last from a few weeks up to 12 months (23-26). However, prophylactic nasal mupirocin application has shown variable efficacy in preventing *S. aureus* infections (25, 27-33).

We studied the effect of mupirocin eradication of nasal *S. aureus* carriage on *S. aureus* burn wound colonization in two different interventions at the Groningen Burn Centre. During the first intervention we treated all patients directly after admission with one course of nasal mupirocin (**Chapter 4**). Our findings confirmed the efficacy of nasal mupirocin on the eradication of nasal *S. aureus* carriage. Furthermore, we found that *S. aureus* nasopharyngeal colonization significantly increased the risk on burn wound colonization, and that the overall rate of *S. aureus* burn wound colonization was reduced during the study period. This supports the importance of the endogenous transmission route. The second intervention was carried out among HCW after an interval of two years. In **Chapter 5** the effect of a single simultaneous course of nasal mupirocin in all HCWs on *S. aureus* burn wound colonization was studied. We found that nasal mupirocin is highly effective in reducing nasal carriage in HCWs with its effect extending up to 10 months. However, this did not result in a significant reduction of the incidence of *S. aureus* burn wound colonization. Interestingly, data of molecular analysis of all *S. aureus* isolates showed a significant shift in genotypes and sources of the burn wounds colonizing *S. aureus* strains. This finding suggests that (i) HCWs play a significant role in the transmission dynamics of *S. aureus*, not only as a vector but also as a source or (ii) that there are other sources that are currently overlooked. **Chapter 6** clearly showed that *S. aureus* carriage was a predictor of subsequent *S. aureus* burn wound colonization during hospital stay. This is in concordance with previous studies (34, 35).

The overall conclusions of above mentioned intervention studies are: (i) nasal mupirocin is highly effective in eradication of *S. aureus* nasal carriage from patients and health care workers, (ii) for patients at admission, *S. aureus* carriage is a predictor of subsequent *S.*

aureus burn wound colonization during hospital stay, (iii) acquisition of exogenous strains by noncarriers during hospital stay is common, and finally (iv) blocking a single nosocomial *S. aureus* transmission route does not necessarily result in a significant reduction of the overall incidence of acquired *S. aureus* burn wound colonization. Other sources and spreading routes of *S. aureus* must be present and may replace or substitute the one that was blocked by a given intervention. Also, mupirocin is not effective in decolonizing extra-nasal sites which was shown recently by Wertheim *et al.* (36). These extra-nasal sites may be additional, equally important sources of *S. aureus* burn wound colonization. Rectal *S. aureus* carriage has been shown as a source of infection (30, 37, 38). Secondly, other potential reservoirs such as contaminated surfaces in the inanimate environment may influence the prevalence of *S. aureus* burn wound colonization. Thirdly, visitors may also introduce *S. aureus* strains in to the burn centre.

Genetic characterization of *S. aureus* isolates

S. aureus burn wound colonization can be acquired exogenously by transmission (cross-infection) or endogenously when the patient is already a natural carrier at the time of admission (self-infection or auto-infection) (5, 26, 39, 40). To identify inter strain relatedness and structure the population of *S. aureus* isolates, DNA fingerprinting techniques have been used. In the studies described in this thesis we used pulsed-field gel electrophoresis (PFGE) for the large scale genotyping of *S. aureus* isolates. The first extensive bacterial genotyping study carried out at the Groningen Burn Centre (**Chapter 3**) revealed that among the majority of patients who were *S. aureus* carrier on admission and who did acquire burn wound colonization, the wound colonizing strain was identical to the strain carried at the time of admission. However, in a sizeable minority of cases nosocomial *S. aureus* strains were apparently acquired from other sources. Thus, both endogenous and exogenous sources are important. Interestingly, genotyping of *S. aureus* strains isolated from wounds of patients who at admission did not carry *S. aureus* revealed three dominant PFGE-types (A, B, C). Furthermore, these PFGE-types also represented the majority of the *S. aureus* strains isolated from HCWs (**Chapter 3**). In **Chapter 6** we described the population structure of *S. aureus* isolates systemically obtained from patients and HCWs over a period of 5 years. Most important conclusions were that (i) this burn centre represents a separate ecological niche in which some clones (A, B, C) behave become entrenched, i.e. well-adapted and endemic, (ii) a significant proportion of nosocomially acquired burn wound colonization is due to *S. aureus* strains acquired from other sources than the patient him- or herself, and (iii) persistently colonized HCWs play an important role in the maintenance of the *S. aureus* epidemiological network. In **Chapter 5** we found that timely eradication of *S. aureus*

nasal carriage among all HCWs resulted in a significant shift in genotypes and sources of *S. aureus* colonizing the patients' wounds. Burn wounds were mainly colonized with *S. aureus* types which were not carried by HCWs.

The overall conclusions of studies mentioned above are: (i) despite cohorting of patients and strict contact precautions, both persistence and the emergence and spread of a few well adapted clones may occur at the burn centre, (ii) HCWs can serve not only as a vector but also as an important reservoir in the dynamics of *S. aureus* transmissions, and, finally, (iii) that the *S. aureus* carriage state at admission is a predictor of subsequent *S. aureus* burn wound colonization. The spread and persistence of a few clones during a long period of time may be maintained by persistent *S. aureus* carriers among HCWs who continuously spread their specific strains in the environment. These strains are probably encountered regurlarly by the noncarriers among the health care workers such that they are at risk to become intermittent carriers of *S. aureus*. Also, surfaces in the inanimate environment could be repeatedly contaminated with these same endemic strains shed from the persistently positive HCWs and their patients.

Comparative genomics analyses

Characterization of *S. aureus* strains showed that three PFGE-types (A, B, C) were more able to colonize burn wounds than other PFGE-types and showed an endemic behavior at the burn centre (**Chapter 6**). Comparative genomics carried out on a small set of endemic and non-endemic strains revealed that compared to the genome of the sequenced *S. aureus* type strain MRSA252 absence of genes was more prevalent among nonendemic than among endemic strains. Interestingly, the discriminating genes were part of a plasmid that codes for multi-heavy metal resistance. A possible explanation for this observation could be the routine use of silver-sulfadiazine and cerium in the treatment of burn wounds. This could be selecting for bacteria which are resistant to this agent. Silver resistant strains have been found in other burn settings as well (41-45).

Mathematical modelling

In **Chapters 3 to 6** we combined genotyping (PFGE) with epidemiological data of patients to elucidate the precise transmission dynamics of *S. aureus*. In **Chapter 7** we developed a mathematical model to try and explain the importance of the endogenous and exogenous transmission routes of *S. aureus*. Despite the relatively small patient population and the relatively long length of stay of patients the model confirmed the positive effect of application of nasal mupirocin in patients with burn wounds.

Probably due to the relatively low numbers of patients which resulted in wide confidence intervals, the effect of nasal mupirocin given to health care workers did not reach statistical significance.

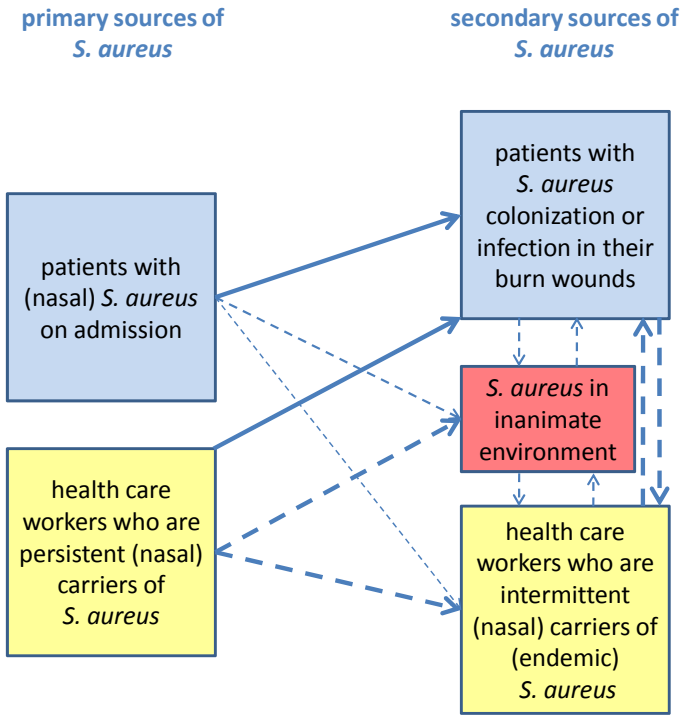


Figure: Primary and secondary sources of S. aureus dissemination in a burn centre. Solid arrows indicate major transmission routes, dotted arrows represent minor or hypothetical routes of S. aureus transmission in this setting.

Recommendations for clinical practice

In an attempt to decrease the risk on *S. aureus* burn wound colonization at the burn centre several measures may be taken. To guide such interventions a clear understanding of the primary and secondary sources of *S. aureus* as well as of the possible transmission routes is needed (Figure).

Clearly, the major transmission routes of *S. aureus* should be blocked in an efficient and systematic manner. The main options are:

- Screening of all patients at the time of admission for nasal, pharyngeal and rectal *S. aureus* carriage; most preferable method is an accurate and rapid diagnostic technique such as PCR (i.e. with a turn-around-time of two hours).
- Treatment of identified nasal carriers starting at the day of admission with one course of mupirocin during five days, two times daily; follow-up nasal cultures are necessary to monitor unlikely but possible recolonization with *S. aureus.* The simultaneous use of other measures, such as chloorhexidine medicated soap, should be considered to rapidly eradicate non-nasal body carriage sites of *S. aureus* colonization as well.
- Similarly, in theory, eradicating *S. aureus* from HCWs should be considered as part of an overall *S. aureus* control strategy, especially from those HCWs who are shown to be persistent carriers of a particular virulent or endemic strain. Therefore, regular monitoring of HCWs for *S. aureus* carriage in nose and throat is recommended.
- The infection control committee should address the issue of HCWs as a potentially important reservoir of endemic nosocomial *S. aureus.*
- Infection control should also address and identify sources of *S. aureus* other than patients and HCWs. The possible role of the environment (air-, floor-, bed-linnen-, dust-sampling) needs to be studied further as are visitors and family members.
- To reliably prevent *S. aureus* burn wound colonization, eradication of endogenous sources of *S. aureus* and the elimination of exogenous sources of *S. aureus* among health care workers and from objects and surfaces in the inanimate environment should be combined.

Other recommendations for further research are listed below:

- Additional comparative genomics analyses should be performed to study which bacterial genes distinguish endemic strains from non-endemic strains.
- Since silver sulfadiazine is used as a standard therapeutic medication in burn centres and because silver resistance have been reported in burn settings, future studies should be focused on developing a susceptibility test for silver and cerium.
- Similarly, the susceptibility to mupirocin of *S. aureus* strains isolated in the burn centre should be closely monitored; it is likely that increased usage of this agent will bring low- and high-level resistance to *S. aurues*. Alternative eradication

agents, either topical or systemic, should be tested in these patients and HCWs as well.

- The *S. aureus* population structure at the Groningen Burn Centre has been analyzed in detail over the past five years. To study the bacterial dynamics in burn centres in The Netherlands, and possibly even larger geographic regions, other clinical isolates from additional burn centres should be collected and studied. Natural isolates from the same geographically areas should also be included. Furthermore, the influence of the lay-out of the burn centre and the protocols on the bacterial dynamics should be studied.

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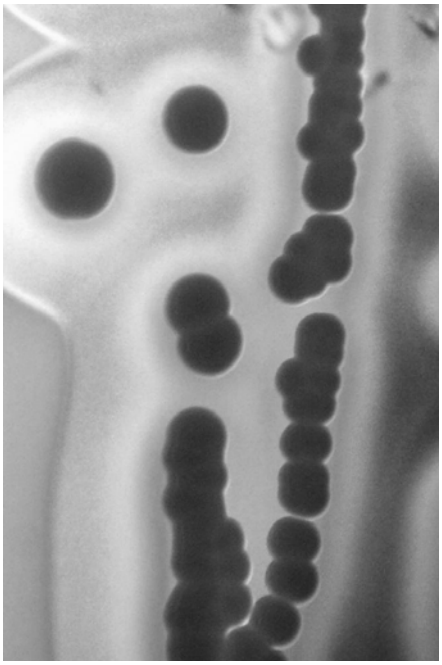
CHAPTER 9

SAMENVATTING



SAMENVATTING

Algemeen

Patiënten met brandwonden die opgenomen zijn in Brandwondencentra zijn vatbaar voor bacteriële kolonisatie en infectie. Het risico op kolonisatie en infectie van de brandwond wordt beïnvloed door factoren die gerelateerd zijn aan de patiënt zelf (bv. de leeftijd, het percentage verbrand lichaamsoppervlak en onderliggende ziektes), aan de omgeving (bv. medepatiënten, ziekenhuismedewerkers, invasieve instrumenten en de kwaliteit van de geleverde brandwondenzorg) en aan het infecterende micro-organisme (bv. species en antimicrobiële virulentie- en resistentieprofielen). Infectie van brandwonden kan resulteren in een verlenging van de opnameduur van patiënten en in een toename van noodzakelijke chirurgische ingrepen (1-5). Bovendien kunnen kolonisatie en infectie van de brandwond het risico op hypertrophische littekenvorming verhogen (6). Ondanks een grote verbetering in de brandwondenzorg, blijft infectie een belangrijke oorzaak van morbiditeit en mortaliteit bij patiënten met brandwonden.

Eén van de meest voorkomende pathogene micro-organismen in brandwonden is *Staphylococcus aureus* (7). De binnenkant van de punt van de neus (het vestibulum nasi) is het primaire ecologische reservoir van *S. aureus* bij mensen (8, 9). In verschillende studies is aangetoond dat ongeveer 10-35% van de mensen persisterend (continu) drager is, ongeveer 20-75% van de mensen soms drager is en ongeveer 5-50% nooit drager is van dit micro-organisme (10-15). *S. aureus* dragerschap blijkt een risicofactor te zijn voor het ontstaan van infecties in bepaalde patiëntengroepen (8, 16). Het percentage brandwonden dat gekoloniseerd raakt met *S. aureus* varieert aanzienlijk en is geassocieerd met het percentage totaal verbrand lichaamsoppervlak, de leeftijd van de patiënt en *S. aureus* dragerschap onder patiënten en ziekenhuismedewerkers (1-4).

In brandwondencentra is dragerschap onder patiënten en medewerkers een belangrijke *S. aureus* bron. Als voornaamste transmissieroute van patiënt naar patiënt, worden de tijdelijke gekoloniseerde handen van ziekenhuismedewerkers gezien. Ziekenhuismedewerkers kunnen *S. aureus* verwerven na direct contact met een patiënt of met gecontamineerde objecten. Bovendien kunnen oppervlakten in de omgeving gecontamineerd worden met *S. aureus*, een bacteriesoort die in staat is om hierop weken tot maanden te overleven. Patiënten en ziekenhuismedewerkers kunnen continu nieuwe stafylokokken klonen introduceren op een brandwondencentrum. Echter, het is niet bekend of elk van deze klonen over een identieke capaciteit beschikt om brandwonden te koloniseren en zich te verspreiden over een brandwondencentrum.

Uitgebreide gedetailleerde moleculaire epidemiologische analyse van *S. aureus* dragerschap en transmissiedynamiek in een brandwondencentrum is tot op heden niet in

de literatuur beschreven. Voor het inzetten van effectieve preventieve maatregelen ter voorkoming van kolonisatie van brandwonden met *S. aureus* is opheldering van de transmissiedynamiek noodzakelijk. In dit hoofdstuk worden de resultaten die in dit manuscript zijn beschreven in het kort genoemd en bediscussieerd en zal er worden afgesloten met het geven van een aantal klinische aanbevelingen.

Preventie van *S. aureus* kolonisatie en infectie bij brandwonden

De laatste decennia zijn er meerdere maatregelen voorgesteld ter voorkoming van *S. aureus* infecties bij patiënten met brandwonden. De verspreiding van *S. aureus* kan deels voorkomen worden door de implementatie van hygiënische maatregelen zoals contactisolatie, cohort verpleging en het onder strict aseptische omstandigheden verrichten van verbandwisselingen. Daarnaast worden dekolonisatie strategieën toegepast met als doel het risico op *S. aureus* kolonisatie van de brandwond te reduceren. De huidige gouden standaard in de topicale behandeling van brandwonden is zilver-sulfadiazine, dat aangrijpt op de bacteriële cel en relatief sterk aan het DNA bindt (17). Selectieve decontaminatie van het maagdarmkanaal (18-20) en eliminatie van *S. aureus* neusdragerschap bij patiënten (21) zijn ook beschreven als mogelijkheid om het risico op *S. aureus* kolonisatie van brandwonden te reduceren. Eradicatie van *S. aureus* neusdragerschap kan tijdelijk bereikt worden door een eenmalige behandeling met nasale mupirocine, een veelgebruikt antibioticum (22).

Een eenmalige behandeling met mupirocine resulteert in een eliminatie van nasale *S. aureus* gedurende enkele weken tot 12 maanden (23-26). Echter, profylactische applicatie van mupirocine heeft een wisselend effect op de preventie van *S. aureus* infecties (25, 27-33).

We bestudeerden het effect van eradicatie van nasale *S. aureus* met mupirocine op *S. aureus* brandwond kolonisaties, door het verrichten van twee interventies op het Brandwondencentrum in Groningen. Bij de eerste interventie behandelden we alle patiënten direct na het moment van opname met mupirocine (**hoofdstuk 4**). Onze bevindingen bevestigden het positieve effect van nasale applicatie van mupirocine op de eradicatie van *S. aureus* neusdragerschap. Tevens werd waargenomen dat de frequentie van kolonisatie van brandwonden met *S. aureus* tijdens de studieperiode gereduceerd was en dat *S. aureus* dragerschap het risico op *S. aureus* kolonisatie van de brandwond significant verhoogde. De laatstgenoemde bevinding ondersteunt het belang van de endogene transmissieroute. Na een periode van twee jaren werd de tweede interventie verricht onder medewerkers van het brandwondencentrum. In **hoofdstuk 5** werd het effect van een eenmalige nasale mupirocine behandeling, gedurende één week, onder alle medewerkers, op de *S. aureus* kolonisatie van brandwonden onderzocht. We vonden dat nasale applicatie van mupirocine zeer effectief

is in het reduceren van *S. aureus* neusdragerschap onder medewerkers, dat tot ca. 10 maanden aantoonbaar was. Echter, dit heeft niet geresulteerd in een significante reductie van de incidentie van *S. aureus* brandwond kolonisatie. Een interessante bevinding was dat moleculaire analyse van alle *S. aureus* isolaten een significante verschuiving liet zien in het genotype en de bron van *S. aureus* stammen die de brandwonden koloniseerden. Deze bevinding suggereert dat medewerkers van het Brandwondencentrum een significante rol spelen in de transmissiedynamiek van *S. aureus*, niet alleen als vector maar ook als een bron. **Hoofdstuk 6** liet duidelijk zien dat *S. aureus* dragerschap voorspellend is ten aanzien van de daaropvolgende kolonisatie van brandwonden met *S. aureus* gedurende het verblijf op het brandwondencentrum. Dit is in overeenstemming met bevindingen uit voorgaande studies (34, 35).
De conclusies uit de bovengenoemde interventiestudies zijn: (i) nasale mupirocine behandeling is zeer effectief bij de eradicatie van *S. aureus* neusdragerschap bij patiënten en medewerkers, (ii) bij patiënten is *S. aureus* dragerschap bij opname voorspellend ten aanzien van brandwond kolonisatie op een later moment tijdens het verblijf op het brandwondencentrum, (iii) onder patiënten die bij opname niet-drager zijn, vindt regelmatig acquisitie van exogene stammen plaats, (iv) het blokkeren van één transmissieroute van het epidemiologische netwerk van *S. aureus* hoeft niet noodzakelijk te leiden tot een significante reductie van de overall incidentie van verworven *S. aureus* kolonisatie van de brandwond. Andere bronnen en routes van *S. aureus* zouden de, door een interventie geblokkeerde, route kunnen invullen of vervangen. Bovendien is mupirocine niet effectief in het dekoloniseren van lichaamsoppervlakken anders dan de neus, wat recent ook aangetoond is door Wertheim *et al*. (36). Deze lichaamsoppervlakken kunnen van additioneel belang zijn en even belangrijke bronnen vormen van *S. aureus* brandwond kolonisatie. Rectaal *S. aureus* dragerschap is als bron van infectie beschreven (30, 37, 38). Ten tweede kunnen gecontamineerde oppervlakken in de omgeving de prevalentie van *S. aureus* brandwond kolonisatie beïnvloeden. Ten slotte kunnen bezoekers ook *S. aureus* stammen introduceren op het brandwondencentrum.

Genetische karakterisatie van *S. aureus* isolaten.
Kolonisatie van de brandwond met *S. aureus* kan verworven worden langs de exogene route (kruisinfectie) en langs de endogene route, wanneer de patiënt van nature drager is van *S. aureus* (auto-infectie) (5, 26, 39, 40). Voor het identificeren van overeenkomsten tussen stammen onderling en voor het structureren van de populatie van *S. aureus* isolaten kan gebruik worden gemaakt van DNA-fingerprints (‘streepjescode’). In de studies die in dit manuscript beschreven zijn hebben we voor de genotypering van *S. aureus* isolaten gebruik gemaakt van pulsed field gel electrophorese

(PFGE). De eerste uitgebreide bacteriële genotyperingsstudie die verricht werd op het Brandwondencentrum in Groningen (**hoofdstuk 3**), wees uit dat onder de meerderheid van patiënten die bij opname *S. aureus* drager was en tijdens het verblijf kolonisatie van brandwonden verwierf, de koloniserende stam identiek was aan de stam die door de patiënt bij opname gedragen werd. Echter, in een fors gedeelte van de nosocomiaal verworven kolonisaties was de stam afkomstig van andere bronnen. Dus, zowel endogene al exogene bronnen zijn belangrijk. Een interessante bevinding was dat de genotypering van *S. aureus* stammen geïsoleerd uit brandwonden van patiënten die bij opname geen drager waren, drie dominante PFGE-types (A, B, C) opleverde. Bovendien vertegenwoordigden deze drie types ook de meerderheid van de *S. aureus* stammen die geïsoleerd werden bij medewerkers (**hoofdstuk 3**). In **hoofdstuk 6** beschreven we de populatiestructuur van *S. aureus* stammen die op een systematische manier geïsoleerd waren bij patiënten en medewerkers over een periode van 5 jaren. De meest belangrijke conclusies waren dat (i) dit brandwondencentrum een specifieke ecologische niche vertegenwoordigt waarin enkele klonen (A, B, C) zich genesteld hebben, die 'well-adapted' (goed uitgerust) zijn en zich endemisch gedragen, (ii) een significant aantal van de nosocomiaal verworven brandwond kolonisaties veroorzaakt worden door een *S. aureus* stam die afkomstig is van andere bronnen dan de patiënt zelf, en (iii) dat persistent gekoloniseerde medewerkers een belangrijke rol vervullen bij het in stand houden van het *S. aureus* epidemiologische netwerk. In **hoofdstuk 5** vonden we dat tijdelijke eradicatie van *S. aureus* dragerschap bij alle medewerkers resulteerde in een significante verschuiving in genotypes en bronnen van *S. aureus* stammen die brandwonden koloniseerden. De verschuiving ging van medewerkers en richting patiënten en andere (niet geïdentificeerde) bronnen.

De belangrijkste conclusies uit voornoemde studies zijn dat (i) ondanks cohort verpleging van patiënten en strikte hygiënische maatregelen kunnen een paar 'well-adapted' klonen zich handhaven, opkomen en verspreiden op het brandwondencentrum, (ii) medewerkers niet alleen als een vector maar ook als een belangrijke bron gezien mogen worden in de transmissiedynamiek van *S. aureus* en tenslotte (iii) dat *S. aureus* dragerschap op het moment van opname voorspellend is ten aanzien van het verwerven van daaropvolgende kolonisatie van brandwonden. De verspreiding en de continue aanwezigheid van een aantal klonen gedurende een lange tijdsperiode zou door persisterende dragers onder medewerkers in stand kunnen worden gehouden, doordat deze medewerkers continu hun specifieke *S. aureus* stammen verspreiden in de omgeving. Deze stammen kunnen opgepikt worden door medewerkers die niet-drager zijn, waardoor deze medewerkers het risico lopen om intermitterend drager te worden. Ook kunnen oppervlakken in de omgeving herhaaldelijk gecontamineerd worden door

dezelfde endemische stammen die door persisterende dragers onder medewerkers en patiënten verspreid kunnen worden.

Comparative genomics analyses (Vergelijkende analyse van genen)

Karakterisatie van *S. aureus* stammen liet zien dat drie PFGE-types (A, B, C) meer dan andere PFGE-types, in staat waren om brandwonden te koloniseren en dat deze drie types endemisch gedrag vertoonden op het brandwondencentrum (**hoofdstuk 6**). Comparative genomics analyses die verricht werden op een kleine set van endemische en niet-endemische stammen liet zien dat, vergeleken met de MRSA252 stam van *S. aureus,* afwezigheid van genen vaker voorkwam bij niet-endemische stammen dan bij endemische stammen. Een interessante bevinding is dat de discriminerende genen deel uit maakten van een geïntegreerd plasmide dat codeert voor meervoudige zware metalen resistentie. Een mogelijke verklaring voor deze observatie zou het routinematige gebruik van zilver-sulfadiazine en cerium kunnen zijn bij de behandeling van brandwonden. Dit zou bacteriën kunnen selecteren die resistent zijn voor dit middel. Zilver resistente stammen zijn eerder aangetoond in andere brandwondencentra (41-45).

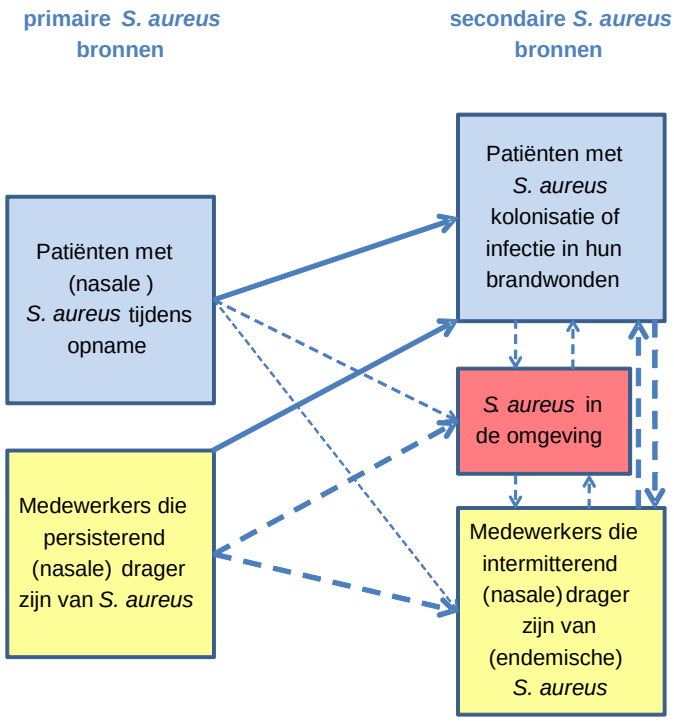
Mathematisch modelleerwerk

In hoofdstukken 3 tot en met 6 combineerden we genotypering (PFGE) met epidemiologische links van patiënten om de *S. aureus* transmissiedynamiek op te helderen. In **hoofdstuk 7** gebruikten we een mathematisch model om de mate van invloed van endogene en exogene transmissieroutes te bepalen. Ondanks de relatief kleine patiëntenpopulatie en de relatief lange opnameduur van patiënten liet het model een positief effect van de applicatie van nasale mupirocine bij patiënten met brandwonden zien.

Mogelijk ten gevolge van de relatief lage aantallen, en dus brede betrouwbaarheidsintervallen, bereikte het effect van toediening van nasale mupirocine aan medewerkers geen statistische significantie.

Aanbevelingen voor de klinische praktijk.

Om het risico op *S. aureus* brandwond kolonisatie op een brandwondencentrum te verlagen zouden verschillende maatregelen genomen kunnen worden. Om dergelijke interventies te sturen is het van belang om een goed beeld te hebben van de primaire en secondaire bronnen van *S. aureus* als ook van de mogelijke transmissieroutes (Figuur).



Figuur: Primaire en secondaire S. aureus bronnen in een brandwondencentrum. Vette pijlen geven belangrijke transmissieroutes weer, gestippelde pijlen vertegenwoordigen minder belangrijke of hypothetische transmissieroutes van S. aureus in deze setting.

Het is duidelijk dat de belangrijkste transmissieroutes van *S. aureus* op een efficiënte en systematische manier onderbroken dienen te worden. De opties hiervoor zijn:

- Screening van alle patiënten op het moment van opname op *S. aureus* neus-, keel- en rectaaldragerschap; de methode van voorkeur is een accurate en snelle techniek, zoals PCR (dat wil zeggen met een turn-around-time van twee uren).
- Behandeling van geïdentificeerde dragers startend op de dag van opname met mupirocine, gedurende 5 dagen, twee keer per dag; follow-up neuskweken zullen afgenomen moeten worden om rekolonisatie met *S. aureus* te kunnen monitoren. Het gelijktijdige gebruik andere maatregelen, zoals het gebruik van chloorhexidine medicinale zeep, kan overwogen worden om *S. aureus* van andere delen van het lichaam snel te eradiceren.

- Theoretisch gezien zal als onderdeel van een *S. aureus* preventiestrategie op een vergelijkende manier eradicatie van *S. aureus* dragerschap onder medewerkers overwogen kunnen worden, met name die medewerkers die aantoonbaar persisterend drager zijn van een bijzondere virulente of endemische stam. Hiervoor zal reguliere monitoring van *S. aureus* dragerschap onder medewerkers noodzakelijk zijn.
- De afdeling Infectiepreventie zal onder de aandacht dienen te brengen dat medewerkers een belangrijk potentieel reservoir kunnen vormen van endemische nosocomiale *S. aureus* stammen.
- Ook zal de afdeling Infectiepreventie andere *S. aureus* bronnen dan patiënten en medewerkers moeten identificeren en benadrukken. De mogelijke invloed van de omgeving (lucht, vloer, bedlinnen, en stof) maar ook van bezoekers en familieleden, zullen onderzocht dienen te worden.
- Om daadwerkelijk tot preventie van *S. aureus* brandwond kolonisatie te kunnen komen, zal eradicatie van endogene *S. aureus* bronnen en de eliminatie van exogene *S. aureus* bronnen onder medewerkers en van objecten en oppervlakken in de omgeving gecombineerd dienen te worden. Omdat andere lichaamsoppervlakken ook potentiële bronnen kunnen zijn voor *S. aureus* kolonisatie, zal het wassen van het lichaam met een desinfecterende zeep van additionele waarde kunnen zijn in de interventiestrategie.

Andere aanbevelingen voor nader onderzoek zijn:

- Meer comparative genomics analyses zullen verricht moeten worden om te onderzoeken welke genen endemische stammen van niet-endemische stammen kunnen onderscheiden. Ook is het noodzakelijk om het mogelijke verband tussen aanwezigheid van genen en het endemisch gedrag van enkele klonen te onderzoeken.
- Omdat zilver-sulfadiazine gebruikt wordt als een routinematige interventie in brandwondencentra en omdat het voorkomen van zilver resistentie beschreven is in deze settings, zullen toekomstige studies gericht moeten worden op het ontwikkelen van gevoeligheidstesten voor zilver en cerium.
- Het is aannemelijk dat een toename in gebruik van mupirocine kan leiden tot een ‘low- en high-level’ resistentie voor dit middel. Van *S. aureus* stammen, geïsoleerd op een brandwondencentrum, zal de gevoeligheid voor mupirocine dan ook nauwkeurig gemonitored dienen te worden.

  Lokale als systemische alternatieve middelen ter eradicatie van *S. aureus* zullen getest dienen te worden bij zowel patiënten met brandwonden als bij medewerkers.

- De *S. aureus* populatiestructuur van het Brandwondencentrum in Groningen over een periode van 5 jaar is geanalyseerd. Om de bacteriële dynamiek in Nederlandse brandwondencentra te onderzoeken zal het zinvol zijn om klinische isolaten van de twee ander brandwondencentra in Rotterdam en Beverwijk te analyseren. Daarnaast kunnen ook niet-klinische isolaten geincludeerd worden uit geografisch verschillende gebieden in Nederland, om in detail de populatie van klinische versus niet-klinische *S. aureus* stammen te onderzoeken. Internationale extrapolatie van zulke studies verdienen aanbeveling. Daarnaast zal het zinvol zijn om te onderzoeken wat de invloed van de lay-out van een brandwondencentrum en de toegepaste protocollen zijn op de bacteriële dynamiek.

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DANKWOORD

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S.R. van Dijk: Beste Sjoukje, bedankt voor je bijdrage tijdens de eerste fase van mijn onderzoek.

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CURRICULUM VITAE

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Anna Maria Dominica Smid werd geboren op 15 februari 1964 te 's-Gravenhage. Na het doorlopen van het VWO aan het St. Maartenscollege te Haren (Gr) volgde zij van 1982 tot 1986 het Hoger Laboratorium Onderwijs te Groningen, waar ze afstudeerde in de richting Medische Microbiologie. In 1986 begon zij als analist op de afdeling Immunochemie op het Laboratorium voor Infectieziekten (LvI) te Groningen (voorheen Streeklaboratorium voor de Volksgezondheid voor Groningen en Drenthe). In 1992 kreeg ze hier een aanstelling als researchanalist op de afdeling Research. Na 3 jaren werd ze benoemd tot hoofdanalist van de afdeling Research & Development. Eind 2006 volgde de benoeming tot hoofd van deze afdeling; deze functie wordt tot op heden door haar ingevuld. Per juli 2008 maakt zij tevens deel uit van de staf van de afdeling Medische Microbiologie. Per 1 oktober 2008 vervult ze de functie van moleculair bioloog op het LvI.

Haar interesse voor de toepassingsmogelijkheden van moleculaire technieken binnen de diagnostiek en epidemiologie van infectieziekten heeft er toe geleid dat ze eind 2001 haar promotieonderzoek "Molecular epidemiology of *Staphylococcus aureus* nasal carriage and wound colonization in a burn centre" startte onder begeleiding van prof.dr H.A. Verbrugh en prof.dr. A. van Belkum (Erasmus Universiteit Rotterdam). Na het verkrijgen van de registratie als Medisch Microbiologisch Onderzoeker zal ze zich aanmelden bij de opleiding tot Medisch Moleculair Microbioloog.
Sinds 1986 is ze gehuwd met André Kooistra; zij zijn de trotse ouders van Jeroen, Arjen en Paula.

PhD Portfolio Summary

PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student: A.M.D. Kooistra-Smid Erasmus MC Department: Medical Microbiology and Infectious Diseases Research School:	PhD period: 2001-2008 Promotor(s): Prof.dr. H.A. Verbrugh Supervisor: Co-promotor: Dr. M.K. Nieuwenhuis	
1. PhD training		
	Year	**Workload (Hours/ECTS)**
General academic skills - Biomedical English Writing and Communication - Research Integrity - Laboratory animal science		
Research skills - Statistics - Methodology		
In-depth courses (e.g. Research school, Medical Training)		
Presentations <u>European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)</u> Mupirocin prophylaxis to prevent *Staphylococcus aureus* wound colonisation in patients admitted to a burn centre. (poster) Dressing changes under laminar flow conditions to prevent *Staphylococcus aureus* wound colonization in patients admitted to a Burn centre. (poster)	 2005 2005	

Nederlandse Vereniging voor Medische Microbiologie (NVMM)		
Mupirocin prophylaxis to prevent *Staphylococcus aureus* wound colonisation in patients admitted to a burn center. (oral)	2003	
Staphylococcus aureus Wound Colonization in Patients admitted to a Burn Centre: an Overview (poster)	2005	
Elimination of *Staphylococcus aureus* Nasal Carriage in Health Care Workers of a Burn Unit: Effect on S. aureus Burn Wound Colonization (poster)	2007	
International Society for Burn Injuries (ISBI)		
Dressing changes under laminar flow conditions to prevent *Staphylococcus aureus* wound colonization in patients admitted to a Burn centre. (oral)	2004	
Mupirocin prophylaxis to prevent *Staphylococcus aureus* wound colonisation in patients admitted to a burn centre. (oral)	2004	
European Burns Association (EBA)		
Intranasal mupirocin does not prevent *Staphylococcus aureus* burn wound colonization (poster)	2004	
Nederlandse Vereniging voor Brandwondenzorg (NVBZ)		
Verbandwisselingen onder laminaire flow condities ter preventie van wondkolonisatie met *Staphylococcus aureus* bij brandwondenpatiënten.(voordracht)	2005	
Wetenschapsdag UMCG		
Mupirocin prophylaxis to prevent *Staphylococcus aureus* wound colonisation in patients admitted to a burn center. (voordracht)	2003	

Vereniging Samenwerkende Brandwondencentra Nederland (VSBN) Molecular epidemiology and clinical impact of Staphylococcus aureus of nasal carriage and infection in a dedicated burn wound centre. (voordracht) Klonale verrijking van *S. aureus* op een brandwondencentrum geassocieerd met een geïntegreerd plasmide en persisterend *S. aureus* dragerschap onder medewerkers.	2004 2008	
International conferences - ECCMID - ISBI - EBA - NVMM	2005, 2007, 2008 2004 2004 2001-2008	
Seminars and workshops -		
Didactic skills		
2. Teaching activities		
	Year	**Workload (Hours/ECTS)**
Lecturing - Laboratory for Infectious Diseases, Dept. Research & Development (Maatschap artsen-microbioloog Brabant) - Molecular screening of stool for the detection of gastro-intestinal pathogens is feasible in a routine clinical microbiology laboratory with multiplex real-time PCR. (general practitioners and medical doctors, Meppel)	2007 2008	

Supervising practicals and excursions Laboratory for Infectious Diseases, Groningen: - molecular biologist - head department of Research & Development, - leading technician, department of Research & Development	 oct 2008- this moment nov 2006-this moment 1995-nov 2006	
Supervising Master's theses		
Other - Oral presentations: Laboratory for Infectious Diseases, Dept. Research & Development (bioMérieux) Laboratory for Infectious Diseases, Dept. Research & Development (Statens Serum Institut, Kopenhagen)	 2007 2007	