STAPHYLOCOCCUS AUREUS INFECTIONS

LEAD BY THE NOSE

Most of the research presented in this thesis was made possible by funding from The Netherlands Organization for Health Research and Development (ZonMw), and The Netherlands Organization for Scientific Research (NWO).

Printing of this thesis was made possible by kind donations from:
WRC2 fonds, GlaxoSmithKline, 3M, Merck Sharp & Dohme, Becton Dickinson, Roche diagnostics, Sanofi-Aventis, Sanyo, Wyeth, bioMérieux, Theranostics

Staphylococcus aureus infections : Lead by the nose

Dissertation Erasmus University Rotterdam.

Cover: *Alexander Ogston, 1880*

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ISBN: 90-8559-061-2

Lay-out by Sigrid Wertheim-Heck

Printed by Optima Grafische Communicatie, Rotterdam

STAPHYLOCOCCUS AUREUS INFECTIONS LEAD BY THE NOSE

Staphylococcus aureus infecties
Bij de neus genomen

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. S.W.J. Lamberts

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
vrijdag 17 juni 2005 om 11:00 uur

door

Heiman Frank Louis Wertheim
geboren te 's-Gravenhage

Promotiecommissie

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Aan mijn ouders

Voor mijn liefjes Sigrid, Lara en Peer

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PART I

INTRODUCTION

CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

"The excursions of the staphylococcus into disease production seem to be aberrant activities outside the main stream of its existence."

R. Williams 1963

INTRODUCTION

Staphylococcus aureus very commonly causes infections in humans: virtually every person will have one or more *Staphylococcus aureus* infections in his or her lifetime. An achievement most microbes do not have on their resume. Most infections occur after an abrasion or cut of the skin due to (non-) accidental trauma, like a child that falls on the street. A lesion of the skin, especially when it has not been cleansed thoroughly, can eventually become painful, red, swollen, and warm, after a day or two. These signs are usually accompanied by a creamy discharge from the wound, known as purulence. This describes the symptoms of an ordinary *S. aureus* wound infection. If such a wound infection occurs, and is cleaned and kept clean, the infection usually subsides and antibiotics are not necessary.

One of the reasons that *S. aureus* is a frequent cause of infections, is that it can survive for months on any type of surface.[1] *S. aureus* cells also possess a wide armamentarium of virulence factors. These virulence factors include factors for adherence, for cell internalization, for evasion of host defense mechanisms, and for invasion of host tissue.[1] With the help of these virulence factors, *S. aureus* is able to colonize the skin and mucous membranes of more than 30% of the human population.[2] It can also colonize the skin and mucous membranes of several animals. This happens on a global scale. Being surrounded or colonized by *S. aureus* is, however, harmless in most cases for a healthy (immune competent) human.

Occasionally such a simple wound infection can become complicated by invasion of the bacteria, where they can cause deep tissue infection and enter the blood stream.[3] Once *S. aureus* cells have entered the blood stream, they will be transported to internal organs, skin and bone, where they can cause new infections, known as metastatic abcesses.[3] This is a serious infection with a high mortality rate, and needs prompt antibiotic treatment.[3] If these infections in healthy humans develop outside the hospital, they are known as community acquired infections. In case these infections develop during hospitalization, they are called nosocomial infections.

S. aureus ranks second as the cause of nosocomial blood stream infections, that leads to increased morbidity, mortality, hospital stay, and costs.[4-7] Patients admitted to the hospital are, in general, at increased risk for infection. They are ill and, therefore, moderately to severely immune compromised. Hospital treatment usually requires that first line barriers for pathogens, of which the skin is an important one, are intentionally breached, as occurs during surgery or placing of indwelling devices, such as bladder and intravascular catheters. Surgery can result in postoperative wound infections, urine catheterization in urinary tract infections,

and intravascular catheters in blood stream infections. Therefore, prevention of these infections is important.

Most of these nosocomial *S. aureus* infections are caused by the patient's own *S. aureus* cells, which were already present on the skin or mucosal membranes prior to hospital admission.[8] The nose, or rather, the anterior nares are the most consistent site from which *S. aureus* can be cultured.[2] Studies, so far, have shown that eradicating *S. aureus* from the nose can eradicate or reduce the load of *S. aureus* from other body sites.[9] Nasal carriers of *S. aureus* are also at increased risk of developing a *S. aureus* infection.[2] Therefore, eradicating *S. aureus* from the nose may prevent these infections, as has been shown for certain patient categories, including dialysis-, dermatological-, and surgical patients.[10-14] Mupirocin nasal ointment is currently the treatment of choice for eradicating *S. aureus* from the nose. This thesis focuses on *S. aureus* nasal carriage as a source for subsequent nosocomial *S. aureus* infections.

An overview and the latest insights regarding *S. aureus* nasal carriage, associated risks of developing infections and possible preventive measures, will be given in **Chapter 2**. Since mupirocin efficacy studies in preventing nosocomial infections have only been performed in surgical and dialysis patients, we decided to design and perform a mupirocin efficacy study in non-surgical patients. These patients are also responsible for a great burden in *S. aureus* hospital infections. This randomized, placebo-controlled trial is described in **Chapter 3**.

This trial lead to four new research questions:

1. What is the risk of nosocomial *S. aureus* bacteremia for *S. aureus* carriers versus non-carriers?
2. Is there a difference in risk of mortality for carriers versus non-carriers once bacteremic with *S. aureus*?
3. What is the efficacy of mupirocin on reducing *S. aureus* carriage at extra-nasal sites?
4. Can invasive *S. aureus* strains be identified by genotyping?

The first two research questions are addressed in **Chapter 4**. **Chapter 5** describes a study in which the effect of mupirocin on nasal, pharyngeal and perineal carriage of *S. aureus* is investigated (question 3). **Chapter 6** describes a nested-case control study where genotyping data of invasive *S. aureus* strains are compared to non-invasive strains (question 4).

Development of prophylactic strategies are always based on the understanding of the pathogenesis of the specific disease. The mechanisms underlying *S. aureus* nasal carriage and how nasal carriage results in disease are still incompletely understood. We decided to study whether nose picking is a determinant of *S. aureus* nasal carriage. Nose picking behaviour seems to be an obvious determinant, but was never studied before. In collaboration with the

department of otolaryngology, we performed a study on nose picking behaviour and *S. aureus* nasal carriage, which we describe in **Chapter 7**.

When we study *S. aureus*, we can extrapolate these findings to methicillin resistant *S. aureus* (MRSA). This is essentially the same micro-organism, the only difference is that the latter is more difficult to treat with antibiotics. The Netherlands are well known for their low prevalence rate of MRSA in the hospitals. In the U.S.A. more than 40% of the *S. aureus* strains cultured from hospitalized patients are methicillin-resistant, as compared to less than 1% in the Netherlands. In the Netherlands, MRSA could usually be related to a hospital admission in a foreign country, indicating that most MRSA strains were imported into the country. But in the last few years there were reports that many MRSA strains could not be related to sources abroad. Therefore, we wanted to know the prevalence of MRSA carriage in patients admitted to the hospital with no relation to a foreign admission. We performed such a prevalence study with an improved detection technique, as described in **Chapter 8** and **Chapter 9**.

All studies included in this thesis are based on the assumption that the anterior nares are the main reservoir for *S. aureus* in humans. All studies, their results and conclusions are, therefore, ***'lead by the nose'***.

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CHAPTER 2

STAPHYLOCOCCUS AUREUS NASAL CARRIAGE: RISKS AND PREVENTION

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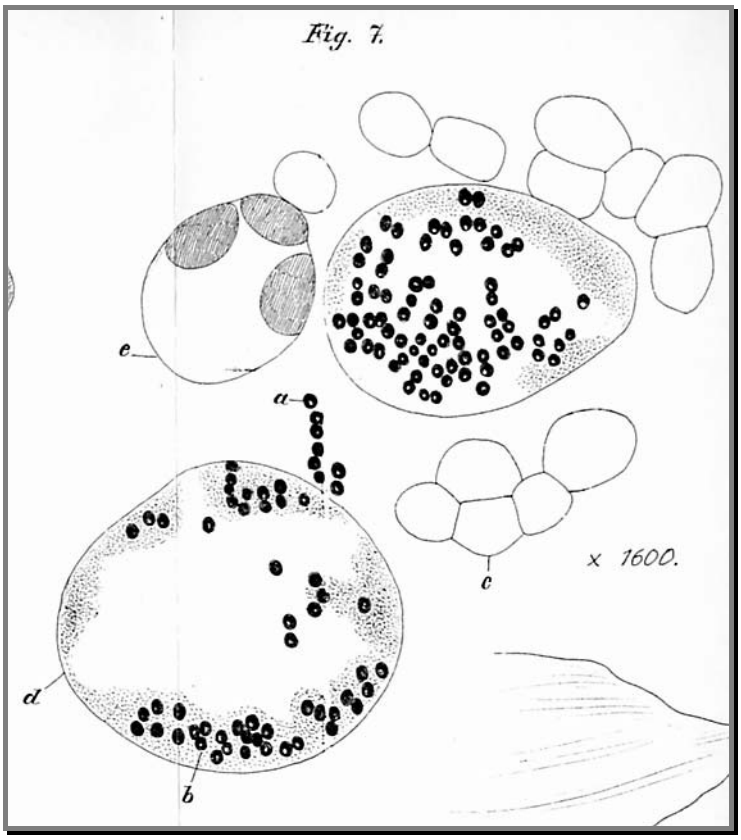
Netherlands Journal for Critical Care Medicine 2004; 8: 457-463



A.Ogston, 'Micrococci in extravasated blood', 1880

ABSTRACT

Staphylococcus aureus causes 25 percent of all nosocomial infections and contributes substantially to the complications and costs of hospitalization. Nasal carriage of *S. aureus* is an important risk factor for these nosocomial *S. aureus* infections. This chapter addresses the determinants of *S. aureus* nasal carriage, the risks of *S. aureus* nasal carriage for subsequent infection with this micro-organism, and strategies to prevent these infections.

INTRODUCTION

Staphylococcus aureus is both a human commensal and a frequent cause of clinically important infections, including bacteremia, metastatic abscesses, septic arthritis, pneumonia, osteomyelitis and wound infections.[1] *S. aureus* infections are frequently nosocomial and lead to increased hospital stay, antibiotic use, costs, and mortality.[2] Though in the Netherlands the prevalence of methicillin resistant *S. aureus* (MRSA) is still very low, the worldwide increasing number of infections caused by MRSA, therapy has become problematic. Since 2002, the first three vancomycin-resistant MRSA strains have been cultured in the United States.[3-5] Therefore, the prevention of staphylococcal infections and emergence of MRSA is essential.

Nasal carriage of *S. aureus* plays a key role in the development of *S. aureus* infections and is a major reservoir for MRSA.[6] Since there are already some excellent reviews on this subject available, this chapter will mostly focus on the latest insights on determinants of *S. aureus* nasal carriage, the risks for infection associated with *S. aureus* nasal carriage, and strategies for prevention.[7-9]

GENERAL

What is *S. aureus* nasal carriage?

S. aureus colonizes the skin and mucosal surfaces of humans and also of several animal species. The mechanisms that lead to *S. aureus* nasal carriage are multi-factorial (Table 1). Conceptually, carriage is the net result of repellent and attracting forces that decide whether an individual is a carrier at a certain time point. Only *S. aureus* strains that are capable of withstanding host defenses and that can reach the site to which it can adhere and propagate from there, will establish a carrier state.

Table 1. *Overview of mechanisms leading to S. aureus nasal carriage.*

Mechanism	Host	*S. aureus*
General	Age, sex, race Antibiotic use Underlying disease (IDDM*, HIV, liver disease, eczema, nasal abnormalities, and others)	Virulence Antibiotic resistance
Exposure	(heavily) colonized partner Hospital environment	
Adherence	Available adhesins Keratin type 10 Epithelial membrane Collagen Vitronectin Fibronectin Fibrinogen Laminin Mucins (Extracellular) matrix proteins Charge Hydrophobicity	Bacterial interference Clumping factor B (Lipo)teichoic acid Capsule Collagen binding protein Vitronectin binding protein Fibronectin binding protein Fibrinogen binding protein Laminin binding protein Capsular polysaccharides MSCRAMM's[#] Charge Hydrophobicity
(Evading) immune response	Mucosal/skin barrier Clearance in mucus by microvilli Immunoglobulins Lysozyme, lactoferrin, antimicrobial peptides Opsonization Immune status HLA type	Proteases, lipases Host cell internalization Protein A (binds Fc of IgG) Resistance to antimicrobial peptides Capsule

* insulin dependent diabetes mellitus
# microbial surface components recognizing adhesive matrix molecules

Studies, as reviewed by Kluytmans *et al.*, have shown that the anterior nares are the most consistent site from which this organism can be cultured [8]. In longitudinal studies, three types of *S. aureus* nasal carriers can be distinguished: persistent carriers, intermittent carriers and non-carriers.[8] Between 10 and 35 percent of healthy individuals almost always carry one strain and are called persistent carriers. A larger proportion (20 to 75 percent) harbors *S. aureus* intermittently, and is called intermittent carriers. Finally, between 5 and 50 percent almost never carry *S. aureus* and are called non-carriers.[8]

Genotyping data reveal that persistent carriers usually carry only one identical *S. aureus* strain over time and that intermittent carriers commonly carry different strains over time.[8,10,11]

The load of *S. aureus* is higher in persistent carriers compared to intermittent carriers, resulting in more dispersal and higher risk of infection.[8,12,13] Persistent carriage is more common in children than in adults and many people shift from persistent carriage to intermittent or non-carriage

between the age of 10 and 20 years.[8] Cross-sectional studies yield a prevalence of approximately 35 percent in the general population, which is actually a mix of persistent and intermittent carriers at that time point.[8,9]

The anterior nares consist of fully keratinized epidermis with hairs, sebaceous glands and sweat glands, squamous epithelium and ciliated mucosal membrane. *S. aureus* predominantly colonizes the moist squamous epithelium on the septum adjacent to the nasal ostium.[14] This area is devoid of cilia and relatively absent of nasal mucous secretions, which contain antimicrobial peptides.[14]

It has been suggested that *S. aureus* nasal carriers have deficiencies in their innate immune response, but recent data do not support this.[15] These data show that *S. aureus* nasal colonization induces a neutrophil mediated inflammatory response, which fails to clear the colonizing bacteria.[14]

Twin studies and family studies are not conclusive in whether there is evidence for genetic determinants for *S. aureus* nasal carriage.[7] However, host determinants play an important role in the pathogenesis of *S. aureus* nasal carriage. This is illustrated in a study where persistent carriers and non-carriers are artificially inoculated with a mix of different *S. aureus* strains, after decolonization, including the resident strain of carriers.[16] This study showed that most non-carriers expel the *S. aureus* strains and that persistent carriers become carrier again and usually select their resident strain out of the mix.[16] A contradicting study, by the same author, shows that the repeated exposure to *S. aureus* cells (e.g. a colonized partner) is probably more crucial than host factors.[17]

S. aureus adherence may be non-specifically mediated via physicochemical forces including hydrophobic interactions.[8] Alternatively, adherence may more specifically be accomplished through binding of certain bacterial cell surface moieties (adhesins) to defined structural receptors in the membrane of the host cell.[8] Recent experiments have shown that clumping factor B, a *S. aureus* virulence factor, is capable of adhering to human cytokeratin type 10.[18] Another study finds that cell wall teichoic acid is essential for *S. aureus* nasal colonization.[19] Differences in the expression of genes coding for these factors, depending on the ecological niche, and other putative adhesins and receptors may provide clues to the 'true' determinants of carriership of *S. aureus*.

Increased carriage rates are found in hospitalized patients. Subgroups of patients with significantly increased carriage rates include those with insulin dependent diabetes mellitus, those on hemodialysis or continuous ambulatory peritoneal dialysis (CAPD), intravenous drug use, *S. aureus* skin infections, liver dysfunction, and human immunodeficiency virus (HIV), as reviewed recently.[8,9] Until now it was believed that repeated punctures in drug users and diabetes patients

were the source for *S. aureus* carriage, but recent studies do not support this. Intravenous drug users actually had a lower prevalence of *S. aureus* nasal colonization when compared to drug users on an oral methadone program.[20] However, confounding variables can not be excluded in this study. Additionally, an increase in fasting glucose levels was significantly associated with *S. aureus* persistent carriage in a recent study.[17]. Both studies indicate that repeated punctures may not play a crucial role in the pathogenesis to *S. aureus* nasal carriage. Another novel determinant is smoking status. Current smoking was shown to be negatively associated with *S. aureus* carriage status.[17] Unfortunately, a full understanding of the determinants of the various carriage states remain elusive.

What are the risks of *S. aureus* nasal carriage?

The nose is regarded as the ecological niche from where *S. aureus* can spread to other parts of the body. Elimination of nasal carriage by using topical mupirocin also eliminates hand carriage.[21] These observations suggest that from the nose, the skin becomes colonized with *S. aureus*, and eventually skin lesions, including surgical wounds and catheter exit sites. Whether colonization of a skin lesion with *S. aureus* leads to infection and whether the infectious process is contained or spreads from there, depends on a complex interplay between *S. aureus* virulence factors and host defense mechanisms.[1] The risk of infection is increased by the presence of foreign material. This can be explained by the impaired function of host phagocytes in the presence of foreign material and by the coating of these materials with human serum proteins to which *S. aureus* can readily adhere and grow.[1]

In 1959, several reports were published that investigated the relation between nasal carriage of *S. aureus* and the development of surgical wound infections. A clonal relation between nasal strains and infectious strains was often found, as determined in those days by phage typing. Further studies showed a significantly increased risk for development of a wound infection by nasal carriers. The causal relationship is emphasized by a correlation between the colonization density of *S. aureus* at the carriage site and the risk for the development of infection.[8,12]

Since then, carriage of *S. aureus* has been identified as a risk factor for the development of infections in various settings. This has been studied extensively in surgical patients (general, orthopedic, and thoracic surgery), in patients on hemodialysis, in patients on CAPD, HIV-infected patients, and in patients in intensive care units. Von Eiff et al. have elegantly illustrated in a prospective study that nasal strains and subsequent bacteremic strains have the same genotype in more than 80 percent of the cases, as determined by pulsed field gel electrophoresis.[22]

One study found that nasal carriage of *S. aureus* was not an independent risk factor for nosocomial *S. aureus* bacteremia, but the design of this study was not suitable to study this association. Nasal carriers in a sub-group of surgical patients did have a higher risk (OR: 4.0) for nosocomial *S. aureus* bacteremia compared to controls. In this study, the presence of a central venous catheter (OR: 6.9), anemia (OR: 3.3), and hyponatremia (OR: 3.3) were associated with hospital acquired *S. aureus* bacteremia.[23] Anemia and hyponatremia may be indicators for severe disease and should not be considered as risk factors.

In hemodialysis patients, *S. aureus* is the most frequently found pathogen in infections at the vascular access site and in bacteremia. The infection rate is higher in carriers on hemodialysis, with relative risks varying from 1.8 to 4.7.[8,24-27] *S. aureus* isolates are usually identical to the one previously isolated from the patient's nares.[22,25] In patients treated with CAPD, *S. aureus* is the leading cause of exit site- and tunnel-infection, often leading to catheter loss. Only CAPD patients who are persistent *S. aureus* nasal carriers are at increased risk of acquiring *S. aureus* infections.[17] Intermittent nasal carriers of *S. aureus* have the same risk of *S. aureus* infection as non-carriers.[17] The observed relative risks for carriage are even higher than those in hemodialysis patients (range: 1.8 to 14.0).[8,28-32] Also in CAPD patients, the nasal strain and the infectious strain are clonally related in most cases.[8,29]

In HIV positive patients, increased rates of *S. aureus* bacteremia and deep soft tissue infections have been observed, which frequently recur. Even higher infection rates are found in patients with AIDS, as compared to HIV-positive asymptomatic patients. Nguyen and others found that nasal carriage is an important risk factor in this patient population (OR: 5.1).[33] Other risk factors for infection in this study were presence of a vascular catheter (OR: 4.9), low CD4 cell count (< 100 cells/mm3; odds ratio 3.5) and neutropenia. The risk for developing an *S. aureus* infection was approximately 10% for every six months in patients who were nasal carriers of *S. aureus* and had CD4 cell counts of less than 100/cells/mm3. It should be noted that nasal carriage was more common in patients who were not receiving cotrimoxazole prophylaxis for prevention of *Pneumocystis jiroveci* pneumonia. The latter is confirmed in another study.[34]

After coagulase-negative staphylococci, *S. aureus* is the second most prevalent organism causing intravascular device-associated bacteremia.[8,35,36] However, no study has been performed with the primary aim of establishing the role of *S. aureus* nasal carriage in this setting. Pujol et al looked at bacteremia in an intensive care unit. Most of the *S. aureus* bacteremias had an intravascular device as a source. In this study carriers of *S. aureus* had a relative risk of 12.4 for the development of *S. aureus* bacteremia.[37]

Methicillin-resistant *S. aureus* (MRSA)

Carriage of MRSA constitutes a special problem with regard to prevention and treatment of infection. Studies show that nasal MRSA carriers have a higher risk of nosocomial infection with this micro-organism. Furthermore, patients infected with MRSA have more morbidity and mortality compared to patients infected with susceptible strains (Table 2).[37-44] Therefore, it is important to keep the prevalence of MRSA low.

Table 2. *Overview of studies that illustrate the increase in hospitalisation days, and the increase in mortalitity and costs of MRSA infections in comparison to susceptible S. aureus infections.*

Reference	Increase in admission days	Mortality (OR)#	Increase in costs (OR)
[39]	5	3.4	1.2
[40]	8	-	3.0
[41]	7	2.7	-
[42]	17	3.2	-
[43]	18	1.8	-
[77]	7	1.1	-
[78]	-	1.7	-
[38]	-	1.4	-
[44]	-	1.9	-

OR: odds ratio.
- not stated.

The MRSA prevalence in the Netherlands is low: less than 1% of the clinical isolates is methicllin resistant.[45] This can be achieved by maintaining a restrictive antibiotic prescription policy and by screening and isolation of patients at risk for MRSA carriage (e.g. repatriated patients) until screening cultures prove negative. MRSA carriers need to stay in isolation and need to receive decolonization treatment. MRSA positive hospital personnel are relieved from their duties, send home, and should be treated for MRSA carriage. They can return to work after eradication therapy.[46]

Decolonization strategies.

In populations in which *S. aureus* nasal carriage is identified as a risk factor for infection it is conceivable that elimination of carriage would reduce the infection rate. Three approaches for elimination of carriage are available: (1) local antibiotic therapy with nasal ointments, (2) systemic antibiotics, (3) bacterial interference, and combinations of these strategies (e.g. nasal ointment and systemic antibiotics). The available options are summarized in Table 3.

Table 3. *Strategies[#] for eliminating S. aureus from the nose (from reference 79, with permission).*

Decolonizing therapy	Frequency	Efficacy	Remark
Topical*:			
Mupirocin nasal ointment 2%	2-3 times daily	Very good	Beware of resistance.
Polysporin	2-3 times daily	Good	Use when therapy failure.
Bacitracin	3 times daily	Moderate	Anaphylaxis reported.
Chlorhexidine	4 times daily	Poor	Anaphylaxis reported.
Lysostaphin nasal cream	not registered	Potential	Trial expected soon.
Povidone-iodine cream	unclear	Potential	Needs more evaluation.
Tea tree oil 4%	unclear	Potential	Needs more evaluation.
Systemic:			
Rifampicin	600 mg/day	Good	Don't use as single therapy.
Clindamycin	1200 mg/day	Potential	Needs more evaluation.
Combinations:			
Fusidic acid 2% and oral cotrimoxazole	3 times daily	Very good	As effective as mupirocin
Rifampicin and other oral or topical drug.	depends on combination	Very good	
Interference:			
S. aureus 502A	not registered	Good	Prevents (re)colonization. Needs more evaluation.
Corynebacterium spp.	not registered	Potential	Eliminates *S. aureus*. Needs more evaluation.

most strategies are effective after 5 to 10 days. Always be aware of the possibility of resistant micro-organisms. Short-course therapies prevent resistance formation.

* for MRSA decolonization, most strategies are combined with antiseptic skin scrub, which is the most effective for *S. aureus* decolonization.

For the first option, mupirocin nasal ointment, has shown to be efficacious in eliminating *S. aureus* carriage. Mupirocin is active against a wide variety of gram-positive bacteria, including *S. aureus*. Mupirocin inhibits bacterial protein synthesis by reversibly and specifically binding to bacterial isoleucyl transfer-RNA synthetase.[47] Mupirocin is well tolerated and, when used appropriately (application to the nose twice daily for 5 days) development of resistance is minimal. However, mupirocin resistance does occur, by modification of isoleucyl transfer-RNA synthetase. Also plasmid mediated high level mupirocin resistance has been reported.[48] An extensive review of the literature on mupirocin has been published by Hudson and Laupland.[47,49] Doebbeling et al. has found that when mupirocin was applied to the nose twice daily for 5 consecutive days, this resulted in elimination of carriage in 91% of stable nasal carriers.[50] Four weeks post-treatment, 87% of the subjects remained free of nasal carriage, at six months 48%, and at 12 months 53%. In patients on hemodialysis mupirocin is less effective. Apparently, in this group of patients other body sites exist were *S. aureus* can maintain itself.[51] *S. aureus* is capable of internalization into host epithelial cells, which can be triggered by antibiotic use.[52,53] The role of *S. aureus* internalization in mupirocin failure has not been established.

Although development of resistance to mupirocin was not observed in clinical studies for

eradication of carriage it has been reported repeatedly in the literature.[54] Generally, mupirocin resistance emerged in cases of prolonged and extensive use, especially for staphylococcal skin diseases. The resistance mechanism is transmissible and this causes concern about the future spread of mupirocin resistance, when it is used on a large scale. Therefore, restricted usage of this antimicrobial agent is recommended. Restricted means only in selected patient groups and for short courses.

Polysporin ointment (containing bacitracin, polymixin B, and gramicidin) has been proven successful in 82 percent of 11 cases whom had previously failed a 1 week course of topical mupirocin.[55] This ointment should be reserved for resistant organisms and/or treatment failure. Topical bacitracin alone is half as effective as mupirocin for nasal decolonization and is therefore not considered an option for the purpose of decolonization.[56] A comparative study of topical mupirocin versus oral cotrimoxazole plus topical fusidic acid, both in conjunction with a chlorhexidine soap bath, yielded equal efficacy and safety for the eradication of MRSA from nasal and extra nasal sites.[57]

Novel agents that may be helpful in the future in *S. aureus* decolonization, are lysostaphin, tea tree oil, and povidone-iodine cream. Lysostaphin is a rapidly bactericidal anti-staphylococcal agent that hydrolyzes the cell wall. An old study showed an elimination rate of 90 percent.[58] Recently an intranasal lysostaphin cream has been developed and clinical trials are underway. Tea tree oil has a wide spectrum of antimicrobial activity and is relatively non-toxic when applied topically.[59] A controlled trial showed that tea tree oil is more effective than chlorhexidine at clearing superficial skin sites from MRSA, but is inferior to mupirocin in decolonizing the anterior nares.[60] In-vitro studies with povidone-iodine cream indicate that this ointment has potential and is suitable for clinical trials.[61]

The second approach to eliminate *S. aureus* nasal carriage, i.e. by administering systemic antibiotics, has been disappointing for most agents. Only rifampicin has proven to be an effective systemic agent.[62] When prescribing rifampin, one must be aware of its side effects and the prevalence of rifampin resistant *S. aureus* mutants. It is advised to combine rifampin with another oral drug or a topical drug, like bacitracin or mupirocin. A potentially effective drug is clindamycin, a bacteriostatic agent that achieves high tissue concentrations. In a small study of seven carriers, clindamycin was able to decolonize all these carriers.[62] This drug should be studied more extensively for the indication of nasal decolonization. Also quinolones achieve eradication rates of up to 70 percent and warrant further evaluation. [62]

The third strategy is bacterial interference. This is based upon the finding that when two

competing micro-organisms vie for the same ecological niche, the organism arriving first will usually prevail. Micro-organisms can accomplish this by blocking receptor sites and by quorum sensing mechanisms. However, the exact mechanism for bacterial interference has not been clarified. Colonization with a virulent strain of *S. aureus* can be prevented by active colonization with a non-virulent strain of *S. aureus* (e.g. with strain 502A) and other bacterial species. This strategy was used successfully in nurseries during outbreaks of *S. aureus* infections in the 1960's and to treat patients with recurrent furunculosis.[63-65] However, due to a published fatal infection with *S. aureus* 502A, this strategy has been abandoned. Apart from this incident, the benefits of the *S. aureus* 502A interference program far outweighed the hazards at that time.

Recently *S. aureus* 502A has been used in a trial with CAPD patients. *S. aureus* 502A was able to colonize the nares after eliminating the resident strain and was found to colonize the exit site after some time [17]. A Japanese study in healthy volunteers has shown successful eradication by application of corynebacteria in the nose.[66] More studies are needed to see if these strategies are practical for daily clinical practice and beneficial for patient's outcome.

For all strategies, recolonization or colonization with new *S. aureus* strains have been described. Therefore, follow-up of individual patients by nasal culturing is warranted and treated when these cultures are positive. Staying ahead of antibiotic resistance by developing alternative effective eradication strategies, stresses the point that the exact mechanisms of *S. aureus* nasal carriage need to be elucidated.

Does decolonization prevent infection in surgical patients?

To prevent *S. aureus* infection, elimination of *S. aureus* nasal carriage seems to be the most straightforward strategy. The introduction of mupirocin ointment for this indication, in the late 1980s, lead to several intervention studies. In this section we will discuss the different clinical trials with mupirocin nasal ointment, which are summarized in Table 4.

Table 4. *Summary of randomized controlled intervention studies (from reference 79, with permission).*

Ref	Intervention	Population	Outcome
[68]	Mupirocin	Surgical	Two-fold reduction in nosocomial *S. aureus* infections.
[67]	Mupirocin	Orthopedic	Non-significant 1.7 fold reduction in surgical site infection rate. Five-fold reduction in endogenous *S. aureus* infection.
[74]	Vaccine	Hemodialysis	Two-fold reduction for approximately 40 weeks in Development of *S. aureus* bacteremia.
[70]	Mupirocin	Hemodialysis	Four-fold reduction in *S. aureus* infection.
[73]	Mupirocin	CAPD[$]	Three-fold decrease in exit-site *S. aureus* infection. Not cost effective.

$: chronic ambulatory peritoneal dialysis

One study compared cardio-thoracic surgery patients who received mupirocin prophylaxis (n=868) with a historical control group (n=928).[8] The surgical wound infection rate in the control group was 7.3% and was 2.8% in the treated group ($p<0.001$).[8] Two randomized controlled trials have been published, studying the efficacy of mupirocin in a general surgical and an orthopedic patient population.[67,68] Perl and co-workers included 3,864 patients in her study, both carriers and non-carriers, who were randomized to either mupirocin or placebo. Overall, 2.3% of mupirocin recipients and 2.4% of placebo recipients had *S. aureus* infections at the surgical site. Nasal carriage of *S. aureus* was eliminated in 83.4% of patients who received mupirocin, versus 27.4% of those who received placebo. Among the *S. aureus* nasal carriers (n=891), 4.0% of those who received mupirocin had overall nosocomial *S. aureus* infections, as compared with 7.7% of those who received placebo (odds ratio for infection, 0.49 [0.25-0.92]).

Kalmeijer *et al.* also included carriers and non-carriers, before an orthopedic surgical intervention.[67] A total of 315 and 299 patients were randomized to receive mupirocin and placebo, respectively. The preoperative nasal carriage rate was approximately 30%. Eradication of nasal carriage was significantly more effective in the mupirocin group (eradication rate, 83.5% versus 27.8%). In this study, mupirocin nasal ointment did not reduce the *S. aureus* surgical site infections rate significantly (3.8% in the mupirocin group and 4.7% in the placebo group), nor the duration of hospital admission. In the mupirocin group, the rate of endogenous *S. aureus* infections (i.e. the strain that causes the infection has the same genotype as the strain previously cultured from the nose) was 5 times lower than in the placebo group (not significant).

Does decolonization prevent infection in dialysis patients?

Several oral and topical antibiotics have been studied for eradication of *S. aureus* nasal carriage in hemodialysis patients and are summarized by Chow and Yu.[62] Rifampicin in conjunction with nasal bacitracin can result in a significant reduction of the *S. aureus* infection rate in hemodialysis patients. However, emergence of rifampicin-resistant strains has been observed. Short course therapies and combination therapies may prevent the emergence of resistant isolates Mupirocin has also been evaluated extensively in hemodialysis patients, and has been reviewed by Boelaert. [69] In a randomized, double-blind placebo controlled trial, stable nasal carriers were treated with mupirocin for two weeks three times daily, and then thrice weekly for a total of 9 months.[70] A significant reduction in the *S. aureus* infection rate (1/104 patient-months among treated and 6/147 patient-months among non-treated) was observed. The administration of mupirocin to nasal carriers was later adjusted to an initial course of 5 days, 3 times per day, and thereafter once a week during the remaining period on hemodialysis. Using this schedule a highly effective elimination of carriage was achieved and this was accompanied by a four to six-fold reduction in the *S. aureus*-bacteremia rate.[70]

The effect of decolonizing the nares from *S. aureus* has also been studied in peritoneal dialysis patients. The effects of intermittent administration of rifampicin in patients on CAPD was studied in a randomized controlled trial.[71] No significant difference in the *S. aureus* peritonitis rates was found. Until now two reports have been published studying the effects of mupirocin on the infection rate in CAPD patients. A case-control study in a CAPD patient population found that the *S. aureus* peritonitis rate was significantly reduced in *S. aureus* nasal carriers who were given mupirocin.[72] There was a significant lower catheter loss due to exit-site infections in the treated group. The overall peritonitis rate was not reduced, mainly due to a significantly higher rate of peritonitis caused by gram-negative bacteria in the treated group compared to the not-treated group. Recolonization occurred frequently, especially after three months.

Also a randomized controlled study was performed in this patient population.[73] Nasal carriers were treated with mupirocin or placebo ointment twice daily for five days and was repeated every four weeks. In 1,144 patients screened, 267 carriers were identified (23.3%). No overall differences in the rates of catheter tunnel or exit site infections or peritonitis were found. The *S. aureus* exit-site infection rate was significantly lower in the treated group (1 in 99.3 patient months versus 1 in 28.1 patient months, $p=0.006$).[73] There was no significant increase in gram-negative infections and development of resistance to mupirocin was not observed. The possibility of development of resistance should be accounted for when using mupirocin for prolonged periods such as in CAPD patients. It can be concluded that elimination of *S. aureus* nasal carriage

in patients on CAPD decreases the exit site infection rate. The effect on the peritonitis rates remains unclear. Researchers and clinicians should be cautious for organism replacement. Prevention of *S. aureus* infections is meaningless if other, potentially more serious, infections come in place.

Vaccination

The past 100 years many attempts have been made to develop a vaccine to control Staphylococcal disease in humans and cattle. The fact that an infection with *S. aureus* does not protect against a new infection with *S. aureus*, illustrates that vaccine development is not easy. Some recent advances in vaccine development do show some protective action. A double-blind trial in patients receiving hemodialysis, has evaluated the use of a conjugate vaccine with *S. aureus* type 5 and 8 capsular polysaccharides.[74]

These two capsular types account for approximately 85 percent of all clinical isolates and can induce a type-specific opsonophagocytic killing by neutrophils in vitro and confer protection in animals. The study has shown that this vaccine can confer partial immunity against *S. aureus* bacteremia for approximately 40 weeks, after which protection wanes as antibody levels decrease. Nearly 90 percent of the patients had a response to the vaccine and the decrease in vaccine efficacy paralleled the decrease in levels of specific antibodies. It would be interesting to study the efficacy of this vaccine or an improved version of this vaccine in other patient populations at risk for *S. aureus* infection.

Is prophylaxis cost-effective?

Cost-effectiveness studies have been performed for mupirocin prophylaxis in hemodialysis patients, peritoneal dialysis patients, and thoracic surgery patients.[2,75,76] Bloom et al evaluated three management strategies: (1) all patients are screened by a nasal culture every three months and those carrying *S. aureus* are treated with mupirocin, twice daily for five days, (2) all patients are treated, irrespective of their carrier state, with mupirocin weekly for 3 days, twice daily, (3) no preventive measures are taken, only infections are treated. It was assumed that 75% of *S. aureus* infections are attributable to nasal carriage in hemodialysis patients and eliminating nasal carriage of *S. aureus* reduces the number of infections with 45 percent to 55 percent. The annual savings of the first strategy were $784,000 per thousand dialysis patients and of the second strategy the savings were $1,117,000 per thousand dialysis patients. Both strategies prevented death and improved the quality of life. Since the risk of development of resistance with widespread use of mupirocin is increased, the first strategy would be preferred.

Davey et al also performed a cost-effectiveness study in peritoneal dialysis patients, on basis of a randomized placebo controlled trial, described earlier.[73,76] Patients in the mupirocin group had lower antibiotic and hospitalization costs. However, overall antibiotic costs, including mupirocin, were significantly higher in the mupirocin group. Mupirocin prophylaxis would have been cost neutral if the exit site infection rate in the placebo group increases to 75 percent, or if the costs of screening was reduced from 15 English pounds to 3 pounds, or if the costs of mupirocin treatment was reduced from 93 pounds to 40 pounds per patient-year. This study did not include the patient's quality of life and the long-term effects of *S. aureus* infection into consideration. One may conclude that short-term savings of mupirocin prophylaxis in dialysis patients in health care costs are unlikely to be sufficiently great to offset the cost of mupirocin.

Vandenbergh et al assessed the cost-effectiveness of perioperative intranasal application of mupirocin calcium ointment in cardiothoracic surgery, based on results of an intervention study with historical controls.[2] Postoperative costs were increased significantly in patients with a surgical-site infection, in comparison with uninfected patients. The mean attributable costs of these surgical site infections were estimated at $16,878. The incidence of surgical site infections was 7.3% in the control group and 2.8% in the mupirocin group. A sensitivity analysis showed that of the four variables, which could influence the resulting cost-effectiveness, being the cost of mupirocin, the effectiveness of the intervention, the cost of a surgical site infection and the incidence of surgical site infection without using mupirocin, only the costs of a surgical site infection had a major influence on the model. Therefore, they conclude that, provided that perioperative mupirocin reduces the surgical site infection rate, mupirocin prophylaxis in patients undergoing cardiothoracic surgery is cost-effective.

CONCLUSION

This review has summarized the clinical impact of *S. aureus* nasal carriage and the effect of several prophylactic measures on these infections. *S. aureus* nasal carriers are at increased risk of acquiring invasive *S. aureus* infections. So far, there is only evidence that mupirocin prophylaxis is efficacious in hemo- and peritoneal dialysis patients, and patients undergoing surgery. In CAPD patients, mupirocin is only effective in preventing exit-site infections and not deeper infections like peritonitis and tunnel infections. For surgical patients, the profile of patients that are most at risk should be identified to make this strategy more effective. More studies should be performed to identify other patient categories that may benefit from prophylaxis. Since infections with multi-resistant *S. aureus* strains rising, more effort should be put in elucidating the

mechanisms leading to *S. aureus* carriage and infection, to be able to develop new and better effective prophylactic strategies.

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PART II

STAPHYLOCOCCUS AUREUS NASAL CARRIAGE AND NOSOCOMIAL INFECTION

CHAPTER 3

MUPIROCIN PROPHYLAXIS AGAINST NOSOCOMIAL *STAPHYLOCOCCUS AUREUS* INFECTIONS IN NONSURGICAL PATIENTS. A RANDOMIZED STUDY

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Annals of Internal Medicine 2004; 140: 419-425

NEUSZALF HELPT NIET TEGEN S. AUREUS

Profylaxe met de antibacteriële neuszalf mupirocine voorkomt infectie met *S. aureus* niet. Tenminste, niet bij patiënten die geen operatie ondergaan. Dat blijkt uit een studie van de universiteitsziekenhuizen in Rotterdam, Amsterdam en Nijmegen en van het Amphia Ziekenhuis uit Breda. Het onderzoek is gepubliceerd in Annals of Internal Medicine van 16 maart.

Mupirocine - aldus eerdere studies - kan infectie met *S. aureus* voorkómen bij patiënten die onder het mes moeten. De wetenschappers hebben onderzocht of dit ook geldt voor niet-chirurgische patiënten. Dit is niet het geval. In een placebo-gecontroleerd onderzoek onder 1602 patiënten blijkt dat tweemaal daags smeren met een 2 procent mupirocinezalf niet tot minder infecties leidt. Bij de patiënten in de placebogroep kreeg 2,8 procent van de patiënten een infectie, bij de met mupirocine behandelde patiënten 2,6 procent.

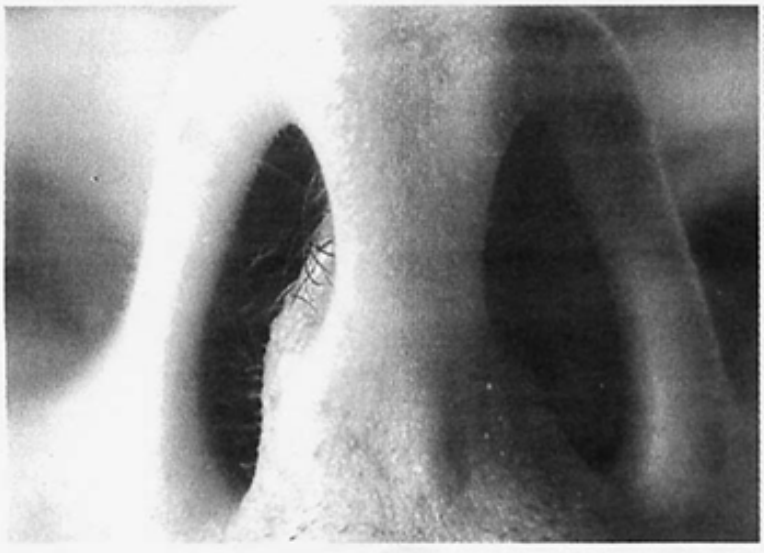


FOTO: SIZE-ISN'T-EVERYTHING.CO.UK

In een commentaar schrijven twee experts dat het afwachten is of andere doseringen van de neuszalf wel een preventief effect hebben. << *EJP*

Medisch Contact, 26 maart 2004

ABSTRACT

Background: *Staphylococcus aureus* nasal carriage is a major risk factor for nosocomial *S. aureus* infection. Studies show that intranasal mupirocin can prevent nosocomial surgical site infections. No data are available on the efficacy of mupirocin in non-surgical patients.

Objective: To assess the efficacy of mupirocin prophylaxis in preventing nosocomial *S. aureus* infections in nonsurgical patients.

Design: Randomized, double-blind, placebo-controlled trial.

Setting: 3 tertiary care academic hospitals and 1 nonacademic hospital.

Patients: 1602 culture-proven *S. aureus* carriers hospitalized in nonsurgical departments.

Intervention: Therapy with mupirocin 2% nasal ointment (n = 793) or placebo ointment (n = 809), twice daily for 5 days, started 1 to 3 days after admission.

Measurements: Nosocomial *S. aureus* infections according to defined criteria, in-hospital mortality, duration of hospitalization, and time to nosocomial *S. aureus* infection. *S. aureus* isolates were genotyped to assess whether infection was caused by endogenous strains.

Results: The mupirocin and placebo groups did not statistically differ in the rates of nosocomial *S. aureus* infections (mupirocin, 2.6%; placebo, 2.8%; risk difference, 0.2% [95% CI, –1.5% to 1.9%]), mortality (mupirocin, 3.0%; placebo, 2.8%; risk difference, –0.2% [CI, –1.9% to 1.5%]), or duration of hospitalization (median for both, 8 days). However, time to nosocomial *S. aureus* infection was decreased in the mupirocin group from 12 to 25 days ($P > 0.05$). A total of 77% of *S. aureus* nosocomial infections were endogenous.

Conclusion: Routine culture for *S. aureus* nasal carriage at admission and subsequent mupirocin application, does not provide an effective prophylaxis for nosocomial *S. aureus* infections in nonsurgical patients.

INTRODUCTION

Staphylococcus aureus is a frequent cause of nosocomial infections, including bacteremia and wound infections.[1,2] Approximately 25% of all nosocomial infections are caused by *S. aureus*, affecting both surgical and nonsurgical patients and leading to increased hospital stay, antibiotic use, costs, and mortality.[3-5] Nasal carriers of *S. aureus* have an increased risk for these infections.[6-9] Recent data show that 80% of nosocomial bacteremic *S. aureus* strains are endogenous and originate from the nose of *S. aureus* carriers.[7] Since 20% of the population carry this pathogen persistently and 60% carries it intermittently, a substantial number of these nosocomial infections may be prevented by eliminating *S. aureus* from the nose.[10]

Intranasal application of mupirocin twice daily for 5 days successfully eradicates *S. aureus* in 83% to 88% of carriers and reduces *S. aureus* hand carriage.[8,11-13] Several studies have shown that patients undergoing surgery or dialysis (peritoneal and hemodialysis) benefit from *S. aureus* eradication from the nose because of the reduction in nosocomial *S. aureus* infections.[10] Mupirocin prophylaxis has been proven to be effective in preventing nosocomial *S. aureus* infections in randomized, placebo-controlled trials among dialysis and surgical patients and patients with recurrent skin infections.[8,14-17] Although the efficacy of mupirocin prophylaxis use has been confirmed only in these patients, mupirocin has many extralabel indications. The resulting widespread use has lead to mupirocin resistance.[18] Since mupirocin is a major weapon to control methicillin-resistant *S. aureus* outbreaks, it should be used in a prudent and restrictive manner. Prudent use implies that it be used only for patients in whom it has proven efficacy.

The efficacy of mupirocin prophylaxis in a general nonsurgical patient population is not yet known. Therefore, we decided to study whether mupirocin prophylaxis in nasal *S. aureus* carriers hospitalized in nonsurgical wards decreases the incidence of nosocomial *S. aureus* infections. We assessed whether these nosocomial *S. aureus* infections were caused by endogenous strains, and we measured the effect of this intervention on mortality and duration of hospital stay.

METHODS

Design and Patients

This is a multicenter, randomized, double-blind, placebo-controlled trial. The 4 participating hospitals were Erasmus University Medical Center (Rotterdam, 1300 beds), University Medical Center St. Radboud (Nijmegen, 950 beds), VU University Medical Center

(Amsterdam, 730 beds), and Amphia Hospital, Langendijk (Breda, 500 beds). The first 3 hospitals are tertiary care hospitals, and all are teaching hospitals in the Netherlands. The institutional review board of each hospital approved the study.

Between 1 February 1999 and 1 February 2001, adult patients hospitalized in nonsurgical departments were screened for nasal *S. aureus* carriage at the time of admission. All patients whose screening cultures grew *S. aureus* within 72 hours after admission were eligible for the study. Additional inclusion criteria were age 18 years or older, not discharged or expected to be discharged within 1 day, not being transferred to a nonparticipating department, and provision of written informed consent. Exclusion criteria were known allergy to mupirocin or glycerin ester, presence of a nasal tube, recent or current mupirocin use (mostly patients undergoing hemodialysis or peritoneal dialysis), and any culture-proven *S. aureus* infection at the time of inclusion.

Trial participants were randomly assigned to receive mupirocin 2% nasal ointment or placebo ointment (both were obtained from GlaxoSmithKline, Harlow, United Kingdom) twice daily for 5 days. Mupirocin and placebo ointments were similar in appearance and odor and were supplied in identical tubes. Randomization was performed by a computer-generated allocation list and stratified for each hospital. The allocation list and study medication were stored by the departments of medical microbiology and infectious diseases at the participating centers. Study personnel and patients were blinded throughout the study. Study medication was dispensed by trained study personnel, who performed the first application according to the manufacturer's instructions. Subsequent applications were done by the patient or nursing personnel according to oral and written instructions. Patients and nurses were informed about possible adverse events (mainly local irritation, itching or burning, rhinorrhoea, and rarely hypersensitivity reactions). They were instructed to report any adverse event related to the treatment, and medication was withdrawn if necessary. Patients did not receive follow-up cultures to check for clearance of *S. aureus* nasal carriage.

Follow-up and Definitions

At randomization, the following patient data were collected: demographics, main diagnosis, underlying illnesses, immunosuppressive and antibiotic medication, and presence of indwelling devices or prosthetic material. The main diagnosis was coded according to the International Classification of Diseases, Ninth Revision (ICD-9).

Nosocomial *S. aureus* infections were followed up by checking the microbiological culture data from any site of all included patients on a weekly basis until 6 weeks after discharge. In

case of a positive culture result, hospital records were checked and, if necessary, the treating physician was interviewed. Nosocomial infections were defined according to criteria of the Centers for Disease Control and Prevention.[19] A nosocomial infection was caused by *S. aureus* when this pathogen was cultured from the site of infection. Patients with nosocomial *S. aureus* infection were considered to have sepsis if 2 or more of the following conditions were present: temperature greater than 38° C or less than 36° C; heart rate greater than 90 beats/min; respiratory rate greater than 20 breaths/min or $PaCO_2$ level less than 4.3 kPa; and leukocyte count greater than 12×10^9 cells/L or less than 4×10^9 cells/L; or greater than 10% immature (band) forms, according to standard criteria.[20] Infections that were not clearly nosocomial were classified by an expert panel of 2 infectious disease specialists not related to the trial.

Microbiology

Nasal swabs were collected by nursing personnel at admission. The swabs were streaked onto 5% sheep blood agar plates (Becton Dickinson, Le Pont de Claix, France), incubated for 48 hours at 35° C, and checked each day for bacterial growth. Suspected colonies were identified as *S. aureus* with the Staphaurex Plus agglutination test (Abbott Murex, Chatillon, France). Patients with positive culture test results were eligible for randomization. The identity of all positive isolates was later confirmed by an automated system (MicroScan Walk-a-Way, Dade-Behring Inc., West Sacramento, California). Strains yielding negative results on confirmation were retested with the AccuProbe hybridization test (Gen-Probe Inc., San Diego, California), according to the manufacturer's guidelines. Patients were incorrectly categorized as nasal carriers of *S. aureus* if the agglutination screening test result was positive but both the subsequent determination with the automated system and the hybridization test result were negative. Susceptibility to mupirocin was only tested in strains causing infections and was performed by disk diffusion.[21]

Infections were treated by the patients' physician, and treatment was not influenced by the trial team members. Cultures were processed according to standard microbiologic methods. All *S. aureus* strains were stored in glycerol medium at –80° C. Nasal and clinical *S. aureus* isolates from the same patient were genotyped by pulsed-field gel electrophoresis and considered to be clonally related if their genotype patterns did not differ by more than 3 bands, according to standard criteria.[22]

Sample Size and Statistical Analysis

On the basis of a literature review and pre-study data from the participating centers, we estimated a priori the incidence of nosocomial *S. aureus* infections among *S. aureus* nasal carriers to be 6%.[9,23] Thus, about 800 patients in each treatment group would demonstrate a statistically significant 50% reduction in nosocomial *S. aureus* infections in patients treated with mupirocin (with a power of 80% and an alpha level of 0.05).

The primary end point was the incidence of nosocomial *S. aureus* infections. Secondary outcome measures were time to nosocomial *S. aureus* infections, duration of hospitalization, and in-hospital mortality.

Data were analyzed by using SPSS 10.0 for Windows (SPSS Inc., Chicago, Illinois). The risks for nosocomial *S. aureus* infection and mortality in the 2 treatment groups were compared by estimating odds ratios, risk differences, and their 95% CIs per type of infection. Odds ratios with CIs not containing unity and risk differences with CIs not containing 0 were considered statistically significant. Differences per treatment group in duration of hospitalization and time to infection were tested for significance by the Mann-Whitney test. Other categorical variables were compared by Pearson chi-square or Fisher exact test where appropriate. Variables that differed between the 2 treatment groups by univariate analysis ($P < 0.1$) were included in a logistic regression model. A P value less than 0.05 was considered statistically significant.

Data were analyzed on an intention-to-treat and per-protocol basis. The intention-to-treat analysis contained all randomized patients fulfilling the inclusion criteria. The per-protocol analysis excluded the following patients: those with false-positive diagnoses of *S. aureus* carriership, those who did not complete the treatment course, and those who developed nosocomial *S. aureus* infection before the end of their prophylactic course.

Role of the Funding Source

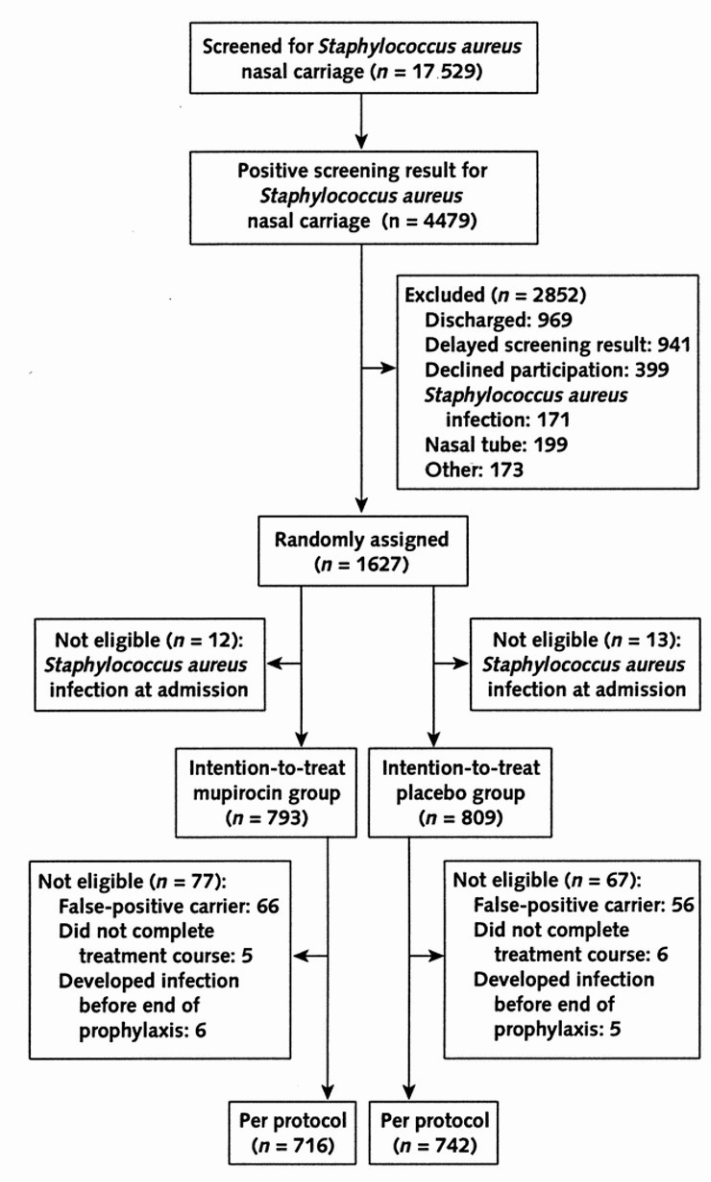
This study was financed by Zon-Mw, The Netherlands Organization for Health Research and Development. This organization had no involvement in the study design, data collection, data analysis, data interpretation, or writing of this report.

RESULTS

Enrollment

A total of 17 529 nonsurgical patients were screened for nasal carriage of *S. aureus*. Of these patients, 4479 (25.6%) patients were found to have *S. aureus* nasal carriage and 1627 were initially randomly assigned (Figure 1). There were 627 patients randomly assigned at Erasmus Medical Center, 462 patients randomly assigned at the University Medical Center St. Radboud, 126 patients randomly assigned at the VU University Medical Center, and 412 patients randomly assigned at the Amphia Hospital.

Figure 1. *Study profile*



The demographic characteristics of excluded patients did not differ from those of included patients (data not shown). In 25 patients hospitalized with a *S. aureus* infection, the culture results became known after randomization and these patients were excluded from analyses (Figure 1). Mupirocin was administered to 793 patients and placebo to 809 patients. Application commenced at a mean of 1.8 days (range, 1 to 3 days) after admission.

The demographic and clinical characteristics of the 2 treatment groups were similar (Table 1). In 24 patients (14 receiving placebo and 10 receiving mupirocin), obstacles to ointment application occurred. Eleven of these patients stopped the prophylaxis prematurely. Four of the 24 patients (2 of which used mupirocin ointment) reported side effects (itching or burning sensation of the nose). No serious adverse events were observed or reported.

Intention-to-Treat Analysis

The overall cumulative incidence of nosocomial *S. aureus* infections was 21 of 793 (2.6%) in the mupirocin group and 23 of 809 (2.8%) in the placebo group (risk difference, 0.2% [CI, –1.5 to 1.9]) (Table 2). In addition, in-hospital mortality (risk difference, –0.2% [CI, –1.9 to 1.5]) and duration of hospitalization did not differ between treatment groups. In each group, 1 death could directly be related to a nosocomial *S. aureus* infection. In patients developing a nosocomial *S. aureus* infection, the median time to infection was 25 days for the mupirocin group and 12 days for the placebo group (P = 0.28). The multiple logistic regression showed that the following variables were independent risk factors for nosocomial *S. aureus* infections: male sex, being immunocompromised, and the presence of an indwelling device (Table 3). Sepsis was diagnosed in 94% of the patients with nosocomial *S. aureus* bacteremia and in 83% of patients with *S. aureus* pneumonia.

All strains causing nosocomial *S. aureus* infections were mupirocin sensitive. Another 1039 *S. aureus* nasal strains from this study sample were tested, and none was found to be mupirocin resistant. Only 1 nasal strain was methicillin resistant (prevalence, 0.06%). Genotyping of nasal and subsequent infection strains revealed that 34 of 44 (77.3%) of these strains were clonally related to the nasal strain (Table 2).

Per-Protocol Analysis

In the per-protocol cohort, the overall cumulative incidence of nosocomial *S. aureus* infections was 14 of 716 (1.9%) in the mupirocin group and 18 of 742 (2.4%) in the placebo group (risk difference, 0.5% [CI, –1.1 to 2.1]). There were no statistically significant differences in mortality (risk difference, –0.2% [CI, –2.1 to 1.6]) or duration of hospitalization

(Table 2). In patients developing nosocomial *S. aureus* infections, the median time to infection was 32 days in the mupirocin group and 13 days in the placebo group (P = 0.02). The same variables in the intention-to-treat analysis were used for logistic regression analysis. In this analysis, an indwelling device was the only independent risk factor (Table 3).

Table 1. *Patient Characteristics**

Characteristic	**Mupirocin Group (*n* = 793)**	**Placebo Group (*n* = 809)**
Mean (±SD) age, *y*	57.6 ± 16.5	57.4 ± 17.3
Men, *n* (%)	456 (57.5)	453 (56.0)
Hospitalized in intensive care unit, *n* (%)	34 (4.3)	53 (6.6)
Underlying illness, *n* (%)		
Diabetes	126 (15.9)	137 (16.9)
Autoimmune disorder	46 (5.8)	56 (6.9)
Neoplasms	136 (17.2)	123 (15.2)
Obstructive pulmonary disease	85 (10.7)	99 (12.3)
Skin disease	99 (12.5)	117 (14.5)
HIV positive	10 (1.3)	8 (1.0)
Post-transplantation	28 (3.5)	14 (1.7)
Renal insufficiency	35 (4.4)	28 (3.5)
Liver function disorder	80 (10.1)	68 (8.4)
Medication, *n* (%)		
Chemotherapy	55 (7.0)	65 (8.0)
Corticosteroids	123 (15.6)	126 (15.6)
Immunosuppressive therapy	44 (5.6)	32 (4.0)
Antibiotics	107 (13.5)	107 (13.3)
Foreign bodies or indwelling devices, *n* (%)		
Central venous access	15 (1.9)	14 (1.7)
Implant	98 (12.4)	95 (11.8)
Urine catheter	29 (3.7)	29 (3.6)
Other indwelling device	24 (3.0)	26 (3.2)

* HIV = human immunodeficiency virus.

Table 2. *Study Outcomes and Corresponding Risk Differences.*

Outcome	**Intention to Treat**		
	Mupirocin (*n*=793)	**Placebo (*n*=809)**	**Risk Difference* (95% CI)**
Nosocomial *S. aureus* infections, *n* (%)			
All†	21 (2.6)	23 (2.8)	0.2 (-1.5 to 1.9)
Bacteremia	7 (0.9) ‡	10 (1.2)	0.3 (-0.7 to 1.5)
Pneumonia	5 (0.6)	1 (0.1)	-0.5 (-1.4 to 0.2)
Surgical site infection, *n* (%)	5 (0.6)	8 (1.0)	0.4 (-0.6 to 1.4)
Skin or soft tissue infection, *n* (%)	2 (0.3)	4 (0.5)	0.2 (-0.5 to 1.0)
Urinary tract infection, *n* (%)	2 (0.3)	0	-0.3 (-0.9 to 0.3)
In-hospital mortality, *n* (%)	24 (3.0)	23 (2.8)	-0.2 (-1.9 to 1.5)
Median Hospitalization (interquartile range), d§	8 (5.0 to 14.0)	8 (5.0 to 15.5)	

* CIs not containing unity were considered significant. For skin or soft-tissue and urinary tract infections, no estimates are given in case these infections did not occur in 1 of the treatment groups.
† Identical nasal and clinical isolates as determined by pulsed-field gel electrophoresis: overall, 34 of 44 (77.3%); bacteremia, 14 of 17 (82.4%); pneumonia, 6 of 6 (100%); surgical site infection, 9 of 13 (69.2%); skin or soft-tissue infection, 4 of 6 (66.7%); and urinary tract infection, 1of 2 (50.0%).
‡ 1 patient had endocarditis.
§ Mann-Whitney test: $P > 0.2$.

Table 2. (Continued)

Outcome	**Per Protocol**		
	Mupirocin (*n*=793)	**Placebo (*n*=809)**	**Risk Difference* (95% CI)**
Nosocomial *S. aureus* infections, *n* (%)			
All†	14 (1.9)	18 (2.4)	0.5 (-1.1 to 2.1)
Bacteremia	4 (0.6)	8 (1.1)	0.5 (-0.5 to 1.6)
Pneumonia	4 (0.6)	1 (0.1)	-0.5 (-1.3 to 0.3)
Surgical site infection, *n* (%)	4 (0.6)	5 (0.7)	0.1 (-0.8 to 1.1)
Skin or soft tissue infection, *n* (%)	0	4 (0.5)	0.5 (-0.1 to 1.4)
Urinary tract infection, *n* (%)	2 (0.3)	0	-0.3 (-1.0 to 0.3)
In-hospital mortality, *n* (%)	23 (3.2)	22 (3.0)	-0.2 (-2.1 to 1.6)
Median Hospitalization (interquartile range), d§	8 (4.0 to 14.0)	8 (5.0 to 16.0)	

§ $P = 0.19$

Table 3. *Independent Relationship of Possible Risk Factors for Nosocomial Staphylococcus aureus Infection**

Variable	Odds Ratio (95% CI)†	
	Intention to Treat	Per Protocol
Sex		
Men	2.25 (1.12--4.53)	1.9 (0.90--4.39)
Women	1	
Renal insufficiency		
Present	2.71 (0.97--7.57)	2.93 (0.92--9.37)
Absent	1	
Solid tumor		
Present	1.65 (0.79--3.39)	1.89 (0.82--4.39)
Absent	1	
Liver dysfunction		
Present	1.76 (0.77--3.99)	1.84 (0.72--4.68)
Absent	1	
Immunocompromised		
Present	2.15 (1.13--4.09)	1.61 (0.75--3.47)
Absent	1	
Indwelling device		
Present	3.41 (1.29--8.98)	3.35 (1.04--10.81)
Absent	1	
Study medication		
Mupirocin	0.92 (0.50--1.70)	0.77 (0.38--1.57)
Placebo	1	

* Obtained by multiple logistic regression. Along with mupirocin prophylaxis vs. placebo, we included variables in the regression model that were significant ($P < 0.1$) in the univariate analysis and included skin disease as a confounder.
† CIs not containing unity were considered statistically significant.

DISCUSSION

This study showed that screening for *S. aureus* nasal carriage on admission by routine culture and applying mupirocin in *S. aureus* carriers to prevent nosocomial *S. aureus* infections in nonsurgical patients is not an efficacious strategy. None of the odd ratios and risk differences for the different types of nosocomial infections and mortality indicated sufficient mupirocin effectiveness to merit treatment (risk difference for overall infection, 0.2% [CI, –1.5 to 1.9]; risk difference for mortality, –0.2% [CI, –1.9 to 1.5]; P > 0.05). We found that 82.4% of the bacteremic strains were clonally related to the nasal strain at admission, which confirms the results found by Von Eiff and colleagues.[7]

Although the rate of *S. aureus* nasal carriage found in this study (25.6%) is within the range described in the literature (19% to 55%), the incidence of nosocomial *S. aureus* infections was far lower than that estimated à priori.[10]

The observed low incidence can be explained by the relatively small proportion of patients in intensive care in our study sample. Also, the national trend for shorter hospitalizations reduces the period at risk for nosocomial infections and increases the chance of missing

nosocomial *S. aureus* infections.[24] Furthermore, the few risks described in the literature are mainly based on patients in the intensive care unit who are at a greater risk for infection.[9,23]

We detected nosocomial infections by checking the microbiology reports. This may not be optimal, although 1 study found this method to have a sensitivity of approximately 90%.[25] We believe that we detected most of these infections, since *S. aureus* infections usually lead to clinically evident disease. Since the study was blinded, missed infections would be evenly distributed between the treatment groups. A nonsurgical patient population in general probably has a relatively low risk for nosocomial *S. aureus* infections. This is illustrated by the 1.2% incidence of nosocomial *S. aureus* bacteremia in a similar patient sample, which was found by Von Eiff and colleagues.[7] We found a similar incidence in our placebo group and thus conclude that our study did not have exclusion bias.

Two other randomized, controlled trials that studied the efficacy of mupirocin in a general surgical and an orthopaedic patient sample have recently been published.[8,26] These studies also showed little to no efficacy of mupirocin prophylaxis. The general surgery study included both carriers and non-carriers who were randomly assigned to either mupirocin or placebo. Overall, 2.3% of mupirocin recipients and 2.4% of placebo recipients had *S. aureus* infections at surgical sites. Among the *S. aureus* nasal carriers, mupirocin-treated patients had significantly fewer nosocomial *S. aureus* infections at any site (4.0%) than placebo-treated patients (7.7%; odds ratio, 0.49 [CI, 0.25 – 0.92]). However, prophylactic mupirocin did not significantly reduce the rate of *S. aureus* infection at surgical sites.[8] The orthopaedic trial also included carriers and non-carriers receiving a surgical intervention.[26] In this study, mupirocin did not reduce the rate of *S. aureus* infection at surgical sites (mupirocin, 3.8%; placebo, 4.7%) or the duration of hospital stay. In the mupirocin group, the rate of endogenous *S. aureus* infections was 5 times lower than that in the placebo group ($P > 0.05$).

In our study, the time to infection shifted by almost 2 weeks in the subgroup of patients with nosocomial *S. aureus* infection. Patients in the mupirocin group, who had a prolonged hospital stay, seemed to catch up in infection probability after this delay. This may be due to recolonization with *S. aureus* from extra-nasal sites several weeks after mupirocin prophylaxis was stopped. Several studies show recolonization with *S. aureus* occurs in 38% to 43% of patients after 4 to 6 weeks after mupirocin application.[11,12,27] The role of *S. aureus* carriage at extra-nasal sites (for example, throat, skin, and perineum) in recolonization after mupirocin treatment and in developing infections needs further study. *S. aureus* present in a lesion (for example, exit site of an indwelling device) may not be eradicated by solely applying mupirocin to the nose. Topical mupirocin application to such sites may be needed to

reduce nosocomial *S. aureus* infections, such as line-related sepsis in patients with tunnelled, cuffed hemodialysis catheters.[28]

To prevent recolonization, repetitive mupirocin application to patients with prolonged hospital stay may have resulted in more efficacy of this prophylactic regimen, which is the case for dialysis patients.[10] However, this would affect a small proportion of all patients, since 90% of the patients in this study were already discharged within 25 days. Also, many nosocomial *S. aureus* infections occur early after admission. These infections may not be preventable by nasal application of mupirocin given a few days after admission. Future studies should consider screening high-risk patients and starting prophylaxis before admission or using a rapid molecular-based screening method and treating carriers the same day.

Although we did not find mupirocin-resistant strains in our study, large-scale use might induce more mupirocin-resistant organisms in the sample.[18] Therefore, future intervention trials should preferably focus on patients who are known *S. aureus* carriers and at high risk for *S. aureus* infections, including immunocompromised patients and patients requiring indwelling devices, as shown by the regression analysis in this study. This analysis also suggests that *S. aureus* carriers with chronic renal insufficiency, without dialysis indication, have an increased risk for *S. aureus* infection.

This study does not support the strategy of routine culture at admission and subsequent mupirocin application in *S. aureus* nasal carriers to prevent *S. aureus* nosocomial infection in a general nonsurgical population. Because more than 80% of nosocomial cases of *S. aureus* bacteremia are endogenous, strategies that can effectively and safely eliminate *S. aureus* carriage from relevant sites may still play an important role in preventing infections with this pathogen. We recommend continued effort in elucidating the mechanisms leading to *S. aureus* carriage and subsequent infection and ongoing development and testing of prophylactic strategies.

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CHAPTER 4

RISK AND OUTCOME OF NOSOCOMIAL *STAPHYLOCOCCUS AUREUS* BACTEREMIA IN NASAL CARRIERS VERSUS NON-CARRIERS

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Lancet 2004; 364: 703-705

De zware last van dragerschap

Staphylococcus aureus komt op de tweede plaats van veroorzakers van in het ziekenhuis opgedane bacteriëmie. Om die reden hebben Wertheim et al. 14.008 niet-chirurgische patiënten die bij opname geen van allen een bacteriëmie hadden, gescreend op dragerschap van S. aureus in de neus, waarna werd gekeken bij wie van hen tijdens het verblijf in het ziekenhuis een bacteriëmie ontstond. Een dergelijke nosocomiale bacteriëmie met S. aureus deed zich 3 maal zo vaak voor bij S. aureus-dragers als bij niet-dragers (relatief risico: 3,0; 95%-BI: 2,0-4,7). Bij genotypering bleek dat van de stammen die bij dragers een bacteriëmie veroorzaakten, 80% endogeen was. Opvallend is echter dat de totale sterfte en de sterfte die samenhing met de S. aureus-bacteriëmie, bij niet-dragers statistisch significant groter waren dan bij dragers (respectievelijk p = 0,005 en p = 0,006).

Ned Tijdschr Geneeskd, 8 januari 2005

ABSTRACT

Staphylococcus aureus is the second most frequent cause of nosocomial blood infections. We screened 14,008 non-bacteremic, non-surgical patients for *S. aureus* nasal carriage at admission, and monitored them for the development of bacteremia. Nosocomial *S. aureus* bacteremia was three times more frequent in *S. aureus* carriers (40/3420: 1.2%) than in non-carriers (41/10588: 0.4%; relative risk 3.0; 95% CI: 2.0 to 4.7). However, in bacteremic patients, all cause mortality was significantly higher in non-carriers (19/41: 46%) than in carriers (7/40: 18%; p=0.005). Additionally, *S. aureus* bacteremia-related death was significantly higher in non-carriers than in carriers (13/41 [32%] versus 3/40 [8%]; p=0.006). *S. aureus* nasal carriers and non-carriers differ significantly in risk and outcome of nosocomial *S. aureus* bacteremia. Genotyping revealed that 80% of the strains causing bacteremia in carriers were endogenous.

INTRODUCTION

Staphylococcus aureus accounts for about 13% of all nosocomial blood stream infections, and is the second most common cause of these infections after coagulase-negative staphylococci.[1] *S. aureus* bacteremia increases length of hospital stay, antibiotic use, costs, and mortality.[1] *S. aureus* nasal carriage is a risk factor for acquiring nosocomial infections.[2] Von Eiff and colleagues have shown that 80% of nosocomial *S. aureus* bacteremia episodes in *S. aureus* carriers are attributable to an endogenous source.[3] However, the risk is known only for selected patient groups and not for the general patient population in hospitals.[2] Risk estimates are mostly based on case-control studies or on studies in which carriership was assessed after patients had a positive blood culture. The latter studies do not rule out the possibility that *S. aureus* nasal colonisation developed secondary to infection. A valid risk estimate is important in cost-benefit assessment of regimens aimed at preventing *S. aureus* infections. We estimated the al risk of *S. aureus* carriers versus non-carriers of acquiring nosocomial *S. aureus* bacteremia in a general, non-surgical hospital population.

METHODS

This study was performed in four hospitals in The Netherlands: Erasmus MC in Rotterdam (1300 beds), UMC St. Radboud in Nijmegen (950 beds), VU University Medical Centre in Amsterdam (730 beds), and Amphia Hospital in Breda (500 beds). The first three are tertiary care hospitals and all four are teaching hospitals. Institutional Review Board approval was obtained.

Between February 1st, 1999 and February 1st, 2001, nurses took nasal swabs from the anterior nares of all adult patients at admission to participating non-surgical departments, were collected at admission by nursing personnel, after oral informed consent was obtained (some patients declined participation). One-fourth of *S. aureus* nasal carriers were included in a clinical randomised trial that evaluated the efficacy of mupirocin versus placebo nasal ointment in preventing nosocomial *S. aureus* infections. Mupirocin was not efficacious in this trial and mupirocin-treated patients were therefore not excluded from the present study.[4] Identification and susceptibility testing of *S. aureus* was performed with conventional methods. In case of uncertainty, identification was confirmed with the AccuProbe® hybridisation test (Gen-Probe Inc., San Diego, California, USA). Methicillin resistance was rare: 0.03% of isolates tested. Patients screened at more than one admission were included only once, and only the first bacteremic episode with *S. aureus* counted. Blood specimens

were routinely obtained for culture in case of fever (body temperatures of ≥38.5° C), or when bacteremia was suspected for other reasons. For patients with blood cultures positive with *S. aureus*, taken 2-120 days after a nasal swab specimen, hospital records were checked, blinded to carrier status, and if necessary, the attending physician was interviewed. Infections were classified as nosocomial using standard criteria, and those that could not be classified as such, were deemed to be community-acquired.[5] We judged in-hospital mortality to be linked to *S. aureus* bacteremia if there was clinical or microbiological evidence of infection at time of death.

Nasal and blood *S. aureus* isolates from the same patient were genotyped by pulsed-field gel electrophoresis (PFGE), and were interpreted according to criteria by Tenover and collaegues.[6] Statistical analysis included calculation of the relative risk with 95% confidence intervals, Chi-square test for dichotomous variables, and Mann-Whitney test for non-parametric comparisons, using statistical software (SPSS version 10.0, SPSS Inc., USA). Logistic regression was performed to identify risk factors for mortality in patients with *S. aureus* bacteremia. P-values below 0.05 were regarded as significant.

RESULTS

A total of 14,014 patients were initially screened. Of these, six seemed to have community-acquired *S. aureus* bacteremia and were excluded, leaving a total of 14,008 who were included in the study. Of these 3,420 (24.4%) carried *S. aureus* in the nose. During follow-up 81 developed *S. aureus* bacteremia. Carriers had a threefold higher risk than non-carriers of acquiring nosocomial *S. aureus* bacteremia (Table 1). All *S. aureus* isolates causing infection were meticillin sensitive. Genotyping by PFGE, revealed that 32/40 (80%) of invasive *S. aureus* strains of carriers were identical to the nasal strain detected at admission, and were thus considered to be of endogenous origin. In 44/81 (54%) of the cases, the probable source of nosocomial *S. aureus* bacteremia was an intravascular catheter (Table 2). Follow-up of patients with *S. aureus* bacteremia revealed that non-carriers had significantly higher in-hospital mortality compared to carriers (Table 2). Logistic regression including all possible risk factors (Table 2) as covariates and all-cause mortality as the outcome variable showed that only carrier status (OR 0.2, p=0.016) and having a central venous catheter (OR 4.7, p=0.016) were independent risk factors. With *S. aureus* bacteremia-related mortality as the outcome variable, only carrier status proved a significant (protective) covariate (0.1, p=0.013). Although bacteremic non-carriers were older than carriers, age was not identified as

a confounder (p=0.85), probably because the age distribution in of those who died was similar to that of those who survived.

Table 1. *Relative risk of nosocomial S. aureus bacteremia by S. aureus nasal carriership.*

	Nosocomial *S. aureus* bacteremia		
	Yes n (%)	No n (%)	Relative risk (95% confidence interval)
S. aureus carrier	40* (1.2)	3,380 (98.8)	3.0 (2.0 – 4.7)
Non-carrier	41 (0.4)	10,547 (99.6)	1.0

* Nasal and subsequent bacteremic *S. aureus* isolates were clonally related in 80% of the cases, as determined by pulsed field gel electrophoresis. Excluding carriers with exogenous bacteremia resulted in a relative risk of 2.4 (95% CI: 1.5 to 3.8).

Table 2. *Comparison of patients with nosocomial S. aureus bacteremia by nasal carriage status.*

	S. aureus carrier (n=40)	Non-carrier (n=41)	P-value Univariate
Age, mean (±SD)*	53.7 ± 18.6	64.6 ± 16.3	0.007
Male sex, n (%)[#]	23 (58.5)	28 (68.3)	0.32
Reason for admission, n (%):			
-Cardiac	9 (22.5)	21 (51.2)	0.001
-Malignancy	9 (22.5)	4 (9.8)	0.12
-Infection	5 (12.5)	3 (7.3)	0.43
-Other	15 (37.5)	8 (19.5)	0.07
Underlying disease or risk factor, n (%):			
-Diabetes	10 (25.0)	9 (22.0)	0.75
-Immunocompromised	14 (35.0)	5 (12.2)	0.02
-Central venous access	18 (45.0)	19 (46.3)	0.90
-Other indwelling device	13 (32.5)	22 (53.7)	0.05
Outcome			
Hospitalisation days, median (± SD)	25 ± 72	50 ± 64	0.01
Days to bacteremia, median (± SD)	11 ± 21	16 ± 25	0.22
In-hospital mortality (all causes)§	7[$] (17.5)	19 (46.3)	0.005
In-hospital mortality (*S. aureus* related)	3 (7.5)	13 (31.7)	0.006
Source of bacteremia, n(%):			
-Intravascular device-related	21 (52.5)	23 (56.1)	0.75
-Wound	9 (22.5)	3 (7.3)	0.05
-Other	6 (15.0)	8 (19.5)	0.59
-Unknown	4 (10.0)	7 (17.1)	0.36

* Mean age of all carriers was 57.0 ± 18.4 and of non-carriers 59.8 ± 17.4 years.
[#] 56% of all carriers and 53% of all non-carriers were male.
[$] All bacteremic strains of carriers who died were from an endogenous origin.

DISCUSSION AND CONCLUSION

The current prospective study demonstrates that *S. aureus* nasal carriers have a heightened risk of developing nosocomial *S. aureus* bacteremia. However, non-carriers with *S. aureus* bacteremia had higher mortality risk than did carriers, which could not be accounted for by differences in underlying disease and age. As a result, in-hospital mortality (all causes) after

S. aureus bacteremia occurred in both groups, was similar (0.2%). Only having a central venous catheter or being a non-carrier were correlated with increased mortality, possibly indicating more severe disease.

Duration of hospital stay, both until bacteremia and discharge, was longer for bacteremic non-carriers than for carriers. Extended hospital stay in non-carriers with *S. aureus* bacteremia could be the result of more severe underlying disease of bacteremic non-carriers compared to carriers. Longer hospital stays increases the time at risk of colonisation with an exogenous *S. aureus* strain, which may lead to subsequent infection. On the other hand, more severe bacteremia in non-carriers than in carriers might have resulted in prolonged length of stay. Since most strains causing nosocomial *S. aureus* bacteremia in carriers were of endogenous origin, carriers could be immunologically adapted to their *S. aureus* strain. They could, therefore have a more adequate immune response than non-carriers, or, alternatively, exogenous strains might be more virulent than endogenous strains.

We conclude that *S. aureus* nasal carriers and non-carriers differ significantly in risk and outcome of nosocomial *S. aureus* bacteremia. Carriers suffer a threefold higher risk of nosocomial *S. aureus* bacteremia, but if they get bacteremic they have a lower mortality risk compared to non-carriers. Possibly, differential strategies to prevent *S. aureus* bacteremia are needed in both carriers and non-carriers. In understanding *S. aureus* disease and related mortality, we now need to know whether nasal carriers of *S. aureus* really have a reduced risk of mortality from invasive *S. aureus* infection and what the underlying immunological mechanisms are.

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CHAPTER 5

THE EFFECT OF MUPIROCIN TREATMENT ON NASAL-, PHARYNGEAL-, AND PERINEAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS* IN HEALTHY ADULTS

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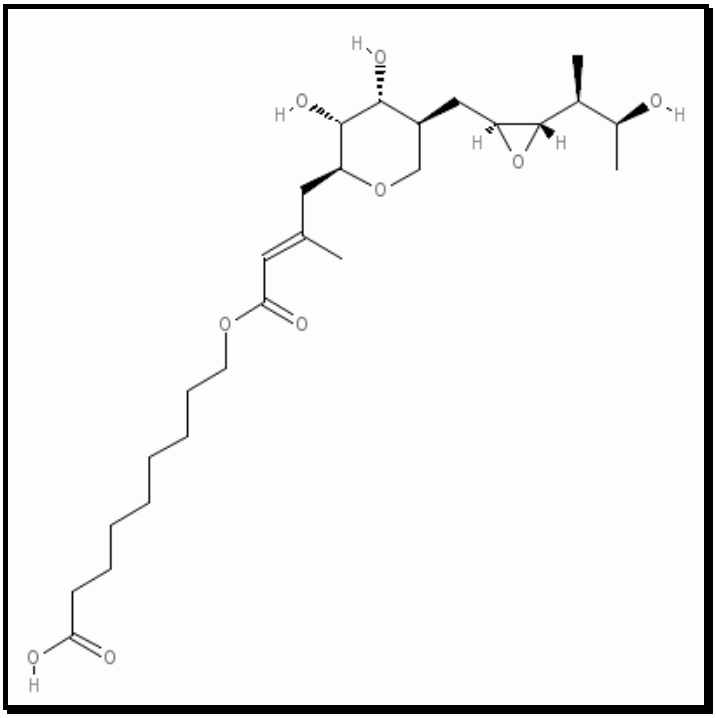
Antimicrobial Agents and Chemotherapy 2005; 49: 1465-1467



Mupirocin structure

ABSTRACT

Nasal carriage with *Staphylococcus aureus* is an important risk factor for *S. aureus* infections. Mupirocin nasal ointment is currently the treatment of choice for decolonizing the anterior nares. However, recent clinical trials show limited benefit from mupirocin prophylaxis in preventing nosocomial *S. aureus* infections, probably due to (re)colonization from extra-nasal carriage sites. Therefore, we studied the effectiveness of mupirocin nasal ointment treatment on the dynamics of *S. aureus* nasal and extra-nasal carriage. Twenty non-carriers, 26 intermittent carriers, and 16 persistent carriers had nasal, throat and perineum taken one day before and five weeks after mupirocin treatment (twice daily for five days) and assessed for growth of *S. aureus*. Identity of cultured strains was assessed by restriction fragment length polymorphisms of the coagulase and protein A genes. The overall carriage rate (either nasal-, pharyngeal-, perineal carrier, or a combination of those) was significantly reduced after mupirocin treatment from 30 to 17 carriers (P=0.003). Of those 17 carriers, 10 (60%) were still colonized with their old strain, 6 (35%) with an exogenous strain, and one with both (5%). Two non-carriers became carriers after treatment. The acquisition of exogenous strains after mupirocin treatment is a common phenomenon. The finding warrants that mupirocin should only be used in proven carriers for decolonization purposes. Mupirocin is overall effective in decolonizing nasal carriers, but less effective in decolonizing extra-nasal sites.

INTRODUCTION

In humans, the nose is the primary reservoir of *S. aureus* [1,2]. Approximately 30% of the healthy population carry *S. aureus* in the nose, which is an important risk factor for *S. aureus* infections [1]. *S. aureus* nasal carriers have a threefold increased risk for nosocomial *S. aureus* bacteremia compared to non-carriers[3]. Approximately 80% of invasive nosocomial *S. aureus* infections is of endogenous origin in nasal carriers [3,4].

Mupirocin nasal ointment is effective in temporarily eradicating *S. aureus* from the nose. When mupirocin is applied to the nose twice daily for five consecutive days, it has been reported that this results in elimination rates of 91% directly after therapy, 87% after 4 weeks, and 48% after six months [5]. However, despite these high elimination rates, three recent clinical studies found little to no efficacy of mupirocin in preventing nosocomial *S. aureus* infections [6-8].

In order to determine the effect of mupirocin treatment on *S. aureus* carriage at extra-nasal sites re, we studied the effect of mupirocin treatment on different carrier types: persistent-, intermittent-, and non-nasal carriers of *S. aureus.* Pharyngeal carriage of *S. aureus* was assessed as well, since nasal application of mupirocin results in low concentrations of this drug in the pharynx [9,10]. Furthermore, perineal carriage was assessed, since perineal carriers are known to disperse more *S. aureus* into the environment [11]. Non-carriers were included as well, to be able to identify whether mupirocin application in non-carriers may lead to carriage due to loss of colonization resistance [12]. To assess the role of extra nasal carriage sites in recolonization of the nose after mupirocin treatment, all strains were genotyped.

MATERIALS AND METHODS

Study-population and general study design

Healthy, adult volunteers (n=165) were screened for nasal carriage of *S. aureus* on at least five separate occasions, one week apart. A participant was labeled as a persistent carrier if at least 80% of the cultures were *S. aureus* positive, as a non-carrier if all nasal cultures were *S. aureus* negative, and as an intermittent carrier in all other cases. Only participants who attended all culture occasions were included in the study.

Treatment and follow-up

Participants, who gave informed consent, self-administered mupirocin 2% nasal ointment (SmithKline Beecham, Rijswijk, The Netherlands) twice daily, for five days, according to

manufacturer's guidelines. Nasal, pharyngeal and perineal samples, were taken just before mupirocin treatment, and once five weeks after treatment. Therapy failure was defined as having a positive nasal culture with *S. aureus* five weeks after the end of treatment. A nasal culture was taken by rotating a sterile swab four times in the anterior nares (Transwab; Medical wire & equipment Co. Ltd., Corsham, Wilts, England). All swabs were processed on the same day. The swab was plated on a Columbia blood agar plate-medium (Becton-Dickinson, Etten-Leur, the Netherlands) and submerged in phenol red mannitol broth (PHMB). Plates were read after one and two days of incubation and broths after three days of incubation 35 °C. Broths with color change from red to orange-yellow were subcultured on blood-agar plates. Identification of *S. aureus* was based on colony morphology, gram stain, catalase test and latex-agglutination test (Staphaurex Plus, Murex, Dartford, UK).

Genotyping

Genotyping was performed on the last cultured *S. aureus* strain before mupirocin treatment and on those strains cultured after mupirocin treatment. *S. aureus* DNA was obtained according to Boom method [13]. Restriction fragment length polymorphisms (RFLP) of the coagulase and protein A genes were determined for typing of all cultured *S. aureus* strains, as described previously [14,15]. Pulsed-field gel electrophoresis (PFGE) was performed to confirm the results obtained by RFLP, when appropriate, according to described methods [16]. Strains were considered to be unrelated in case the RFLP pattern of either the coagulase gene or protein A gene differed from the other strain. PFGE patterns were compared using the criteria by Tenover [17].

Statistical analysis

Volunteers were classified, as described above, as persistent-, intermittent- and non-nasal carriers using the results of at least five screening cultures. Per carriage type, the efficacy of mupirocin was assessed by comparing the culture results of the samples taken just before mupirocin treatment, with the culture results of the samples taken five weeks after mupirocin treatment. Mupirocin therapy was considered to have failed if an individual carried *S. aureus* in the nose five weeks after treatment, irrespective of extra-nasal carriage. Non-parametric paired tests were used where appropriate. P-values, two-sided, below 0.05 were considered significant.

RESULTS

At least five serial cultures were obtained from 62 individuals from the initial cohort of 165 volunteers. Twenty volunteers were non-carrier (NC; 32%), 26 were intermittent carrier (IC; 42%) and 16 were persistent carrier (PC; 26%; Table 1) and all participated in the mupirocin intervention. No serious side effects were observed and all volunteers completed the treatment. The overall carriage rate (either nasal-, pharyngeal-, perineal carrier, or a combination of those) was significantly reduced after treatment from 30 to 16 carriers (Table 1; P=0.003).

Table 1. *Change in carriage sites, just before and 5 weeks after mupirocin treatment.*

Carriage site	Persistent Carrier (n=16)		Intermittent carrier (n=26)		Non Carrier (n=20)	
	Before treatment *	After treatment	Before treatment ^	After treatment	Before treatment	After treatment
nose alone	6	3	5	1	0	1
nose-throat	6	1	5	3	0	1
nose-throat-perineum	0	1	1	1	0	0
nose-perineum	3	0	0	0	0	0
throat alone	0	2	2	0	1	1
throat-perineum	0	0	1	0	0	0
perineum alone	0	0	0	1	0	0
All	15	7	14	6	1	3

* one persistent carrier had a negative nasal culture just before mupirocin treatment
^ 15 intermittent carriers had negative culture results just before mupirocin treatment.

Mupirocin significantly reduces nasal carriage in persistent carriers (n=16)

Of the sixteen persistent carriers, one carrier had a negative nasal culture just before mupirocin treatment. Five (31%) carriers had therapy failure five weeks after mupirocin treatment (Table 1). Four remained colonized with the same strain of which all had at least one extra nasal carriage site (3 throat, 1 perineum). One volunteer acquired a new strain and never carried *S. aureus* on extra-nasal sites. In persistent carriers, mupirocin was effective in decolonizing *S. aureus* from the nose five weeks after treatment (P=0.002), but not effective in decolonizing throat and perineal carriage (P=0.69 and P=0.5 respectively).

No significant reduction of nasal carriage in intermittent nasal carriers after mupirocin treatment (n=26)

From the 26 intermittent carriers, 11 (42%) carried *S. aureus* just before treatment. Three of these (27%) had therapy failure. Two remained colonized with the same strain of which one

was a perineal carrier before treatment. From those who did not carry *S. aureus* just before treatment, two became colonized after treatment, of which one was a combined pharyngeal and perineal carrier just before treatment. Overall, mupirocin treatment did not significantly reduce nasal (P=0.11), throat (P=0.29), and perineal (P=1.0) carriage, in this subgroup of intermittent carriers.

Rare acquisition of exogenous *S. aureus* after mupirocin treatment in non nasal carriers (n=20)

Within the non-carrier group there was one apparent throat carrier before mupirocin treatment. After treatment two non-carriers became colonized with *S. aureus* (10%), of which none carried *S. aureus* on extra nasal sites before treatment. The pharyngeal carrier remained pharyngeal carrier with the same strain.

Special emphasis on pharyngeal carriage (n=16) and perineal carriage (n=5).

In general there were 16 pharyngeal carriers before treatment, irrespective of carriage at other sites (12 were also nasal carrier). In 5/12 (42%) cases the throat strain was different from the nasal strain. After treatment six (38%) remained throat carriers, of which one acquired a new strain, a significant reduction in throat carriage after mupirocin treatment (P=0.002). Interestingly, of those who were non-throat carrier before treatment (n=46), 5 (11%) became colonized in the throat with *S. aureus*. Four of these new throat carriers were nasal carriers before treatment and one was a non-carrier.

There were five perineal carriers (four were also nasal carrier) before treatment and three after treatment (non significant reduction). Only one perineal carrier remained carrier after mupirocin treatment with an identical strain. Two non perineal carriers became perineal carrier after treatment, of which one with an endogenous strain.

DISCUSSION

Our results showed similar mupirocin effectivity on nasal decolonization at five weeks post treatment as reported in previous studies [5]. We found a *S. aureus* nasal carriage elimination rate of 69% in persistent carriers and 58% in intermittent carriers. Therapy failure is not likely to be due to mupirocin resistance, since the prevalence of mupirocin resistant strains is very low in the Netherlands: none found in more than 1000 strains [6]. Only one strain was found to be mupirocin resistant after therapy in our study (data not shown). Though the MRSA

prevalence is very low in the Netherlands, the findings of this study may be extrapolated to an endemic MRSA setting, as long as these strains are mupirocin sensitive [18].

Mupirocin nasal ointment also had a significant effect on pharyngeal *S. aureus* carriage decolonization, but not on perineal carriage. The effectiveness of mupirocin in reducing the occurrence of perineum carriage in this cohort was low, due to new acquisition of *S. aureus* at this site. Unlike in nasal carriage, where the effectiveness is much higher, mupirocin does not seem to have a preventive effect in *S. aureus* perineum carriage. Though the nose is the primary reservoir for *S. aureus,* the perineum itself is not directly affected by local application of mupirocin to the nose, as we saw in our study. Application of a local antibiotic or disinfectant on the perineum could be an option for optimal decolonization.

Interestingly, ten volunteers became colonized at new sites, five weeks after mupirocin treatment, of which five were exogenous strains (two were non-carriers). Furthermore, two carriers became colonized with exogenous strains at their old sites after treatment. Overall we can state that of those 17 carriers at any site after treatment, ten (60%) were colonized with their old strain, six (35%) with an exogenous strain, and one (5%) with both old and exogenous strain. Therefore the acquisition of exogenous strains after mupirocin treatment is a common phenomenon. The finding that two non-carriers became carriers after treatment (17% of all therapy failures) warrants that mupirocin should only be used in proven carriers for decolonization purposes. Mupirocin also eradicates coagulase negative staphylococci and corynebacteria, which may be present in non-carriers, and this change in nasal flora may facilitate colonization with *S. aureus*, by eliminating the bacterial intereference [12].

We conclude that mupirocin is overall effective in decolonizing nasal carriers, but less effective in decolonizing extra-nasal sites. These extra-nasal sites may be a source for *S. aureus* infections. The majority of the *S. aureus* strains of those who remain colonized five weeks after treatment are endogenous. But acquisition of exogenous *S. aureus* strains occurs and warrants that decolonization should only be performed in proven carriers. Furthermore, patients treated with mupirocin should receive follow-up cultures to determine treatment failure, which is already introduced for dialysis patients.

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CHAPTER 6

ASSOCIATION BETWEEN *STAPHYLOCOCCUS AUREUS* GENOTYPE, INFECTION AND IN-HOSPITAL MORTALITY. A NESTED CASE-CONTROL STUDY

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Submitted

Dear Dr. Wertheim,

Your manuscript has been reviewed by our editors and other experts in your field. The reviewers felt that it contains interesting information and is potentially acceptable for publication in The Journal of Infectious Diseases.

David C. Hooper, editor.
April 16, 2005

ABSTRACT

We screened 14,008 non-surgical patients for *S. aureus* nasal carriage at admission and assessed them for invasive *S. aureus* disease and in-hospital mortality. Multi locus sequence typing was performed for endogenous invasive strains and nasal strains of matched asymptomatic carriers to investigate whether virulent clones could be identified in nasal carriers. Clonal complex (CC45) was significantly underrepresented (OR: 0.16; 95% CI: 0.04-0.59), and CC30 was overrepresented (not significant) among invasive strains (OR 1.91; 95% CI: 0.91-4.0). There was no clonality among invasive *S. aureus* strains in non-carriers. Patients infected with *S. aureus* strains belonging to a clonal complex had a higher mortality rate (OR 3.03; 95%CI: 1.09-8.43), indicating co-evolution of *S. aureus* virulence and spread among humans.

INTRODUCTION

Staphylococcus aureus is one of the leading causes of nosocomial infections, varying from superficial wound infections to more invasive infections, like deep abcesses, osteomyelitis and bacteremia.[1] These infections lead to prolonged hospital stay, increased antibiotic use, and increased costs.[2] *S. aureus* nasal carriers have a threefold increased risk of acquiring nosocomial *S. aureus* bacteremia compared to non-carriers, but have a lower mortality rate when infection occurs.[3] This higher survival rate of carriers may be due to partial immunity. Alternatively, *S. aureus* strains of asymptomatic nasal carriers of *S. aureus*, may belong to a less virulent genotype compared to strains from nasal carriers with proven invasive disease.
We, therefore, wanted to investigate whether certain *S. aureus* clones found in *S. aureus* nasal carriers are more or less likely to cause invasive disease, and whether invasive nosocomial *S. aureus* disease in non-carriers is due to a special clone. We analyzed a collection of *S. aureus* strains isolated from a previously described cohort of patients admitted to the hospital using a microarray based method for multilocus sequence typing (MLST).[3-5]

METHODS

Study design

We performed a nested case control study in a cohort of 14,008 adult non-surgical patients, who were screened for *S. aureus* nasal carriage at hospital admission.[5,6] All patients were monitored for the development of invasive *S. aureus* disease by checking microbiology data on a weekly basis, as described earlier.[3,5] The study was performed in four teaching hospitals from separate regions of the Netherlands. Medical ethical approval was obtained from all participating centers.
Nasal and invasive strains were genotyped by PFGE, of which the resulting data were interpreted according to standard criteria.[7]. MLST was performed for invasive strains, that were genetically similar as determined by PFGE, and for nasal strains of matched carriers, who did not develop invasive *S. aureus* infection (asymptomatic carriers). For each case of invasive nosocomial infection, two matched controls were included, who were matched for: *S. aureus* nasal carriage, the hospital of admission, date of admission (range one month), sex, age class (allowed difference: up to 5 years), and absence of *S. aureus* infections during follow-up. If more matched controls were possible, those with the age closest to the index were selected. Moreover, invasive *S. aureus* strains from non-carriers, cultured from blood or deep foci of infection, were analysed by MLST.

Multi Locus Sequence Typing.

For this study we used an oligonucleotide array for MLST of *S. aureus,* as described earlier [4]. Briefly, DNA was extracted using lysostaphine and the QIAamp DNA Minikit (Qiagen, Westburg, Leusden, The Netherlands). DNA was used in a multiplex PCR with specific primers targeting the seven housekeeping genes as defined by Enright *et al.* [8] PCR products were fragmented and labeled with a new DNA-amplicon labeling technique (bioMérieux, Marcy l'Etoile, France), purified with the QIAquick Nucleotide Removal kit (Qiagen) and hybridized with the oligoprobe arrays in the GeneChip Fluidics Station (Affymetrix, St.Clara, Calif.).[4] Each probe array was stained with streptavidine-RPE (phycoerythrin; Dako, France) and signal was measured with the GeneArray scanner (Agilent, Palo Alto, Calif.). Probe array cell intensities, base call, sequence determination, and reports were generated by functions available in the GeneChip software (Affymetrix). A candidate allele selection index was determined by the percentage homology between the experimentally derived sequence and the distinct reference sequence tiled on the array.

For some house keeping genes the oligo-mediated MLST procedure can generate ambiguous results, because polymorphisms can be present in the 5' and 3' proximal ten nucleotides of the amplicons.[8] These are not recognized by the oligoprobes. In such cases the entire housekeeping gene was reamplified and both strands of the amplicons were sequenced to identify possible polymorphisms.[4]

Statistical analysis.

Comparison of MLST results was based upon related sequence types (BURST) software, as described before.[9] Data were analysed with the help of SPSS software. Frequencies were compared by Chi-square test and continuous variables by T-test. P-values below 0.05 were considered significant. Odds ratio's with 95% confidence intervals were calculated for the case-control study.

RESULTS

General

Demographic data of patients (60 cases, 118 controls) are summarized in Table 1. Most invasive strains originated from blood cultures (92%). Two controls were excluded since these were found to have an *S. aureus* infection at a later stage, and were not replaced by new controls. Additionally, 34 invasive *S. aureus* strains originating from blood cultures of hospitalised non-nasal carriers of *S. aureus*, as described earlier, were selected for MLST analysis [3]. Five strains of the original cohort were lost and were therefore not analysed.

Table 1. *Patient characteristics.*
Cases are carriers who acquired invasive S. aureus disease during hospitalization with their own strain, and controls are carriers who did not acquire S. aureus invasive disease. Non-carriers are those who were not colonized by S. aureus in the nose at admission but did develop invasive S. aureus disease during hospitalization.

	Cases (n=60)	**Controls** (n=118)*	**Non-carriers** (n=34) .
Sex (n, % male)	33 (55.0%)	65 (55.1%)	24 (66.7)
Mean age (years ± SD)	53 (±17)	53 (±17)	64 (±17)#
Hospital (n, %) located in			
Nijmegen	17 (28.3%)	33 (28.0%)	12 (35.3%)
Amsterdam	8 (13.4%)	16 (13.5%)	5 (14.7%)
Breda	5 (8.3%)	10 (8.5%)	ND**
Rotterdam	30 (50.0%)	59 (50.0%)	17 (50.0%)
Strains obtained by:			
Blood culture (n, %)	55 (91.7%)	0 (0.0%)	34 (100%)
Other sterile site (n, %)	5 (8.3%)	0 (0.0%)	0 (0.0%)

* 2 controls were found to have a *S. aureus* infection at a later stage and were excluded from further analysis.
# Non-carriers were significantly older than cases (P=0.005)
** ND: not done. Breda did not store invasive *S. aureus* strains from non-carriers.

Sequence types, invasive disease and mortality

Overall, 32 different sequence types (STs) were identified (Figure 1). Nine STs accounted for 80% of all tested strains, of which three STs (30, 15 and 45), were the most prevalent (20%, 15%, and 12%, respectively). There were no significant differences in the distribution of STs per hospital (data not shown). STs were grouped by BURST analysis in clonal clusters (CC) as shown in Table 2. Singletons also included STs 5, 9, 12, and 22, which were identified as a clonal complex in earlier studies.[9] Only CC45 was significantly more prevalent among non-invasive strains (OR: 0.16; 95% CI: 0.04 - 0.59). Most invasive strains belonged to CC30 and many were different singletons. Invasive strains of non-carriers did not differ markedly in their distribution of STs as compared to invasive strains of carriers (Table 2).

Figure 1. *Dendrogram showing the relatedness of the found S. aureus sequence types.*

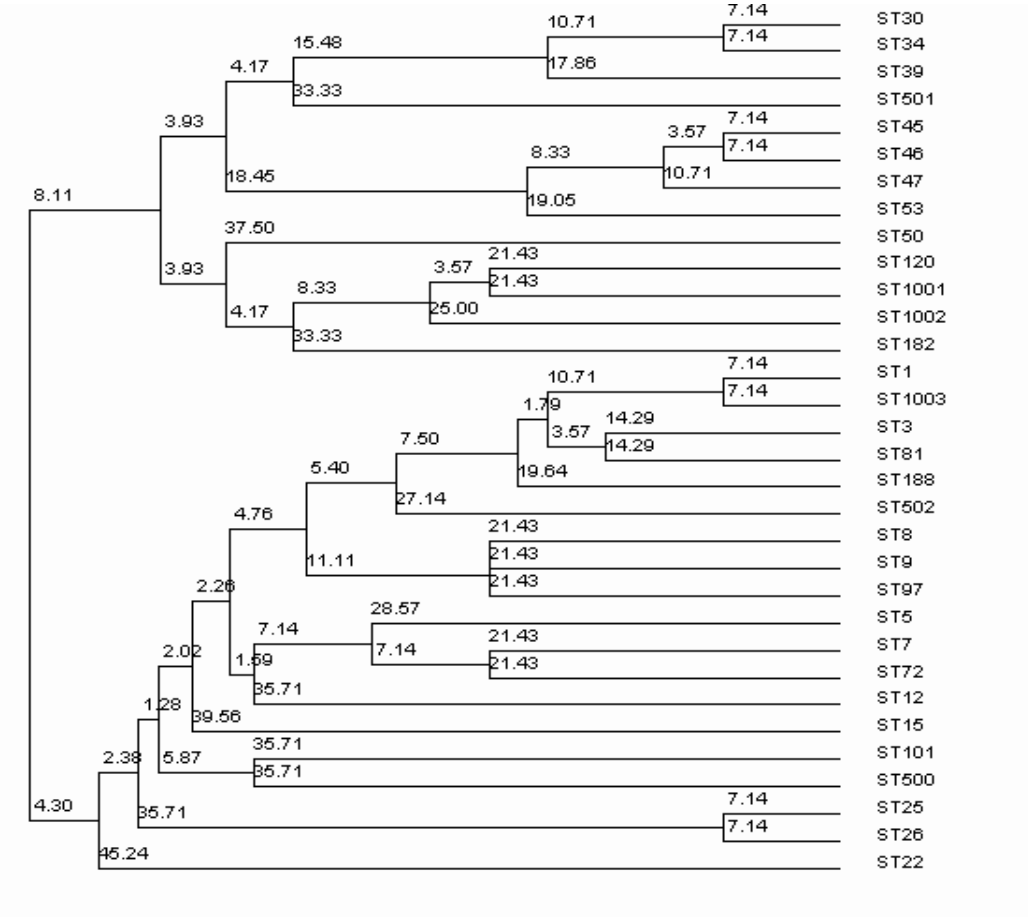

Table 2. *Distribution of identified clonal complexes in cases (S. aureus nasal carriers with invasive S. aureus infection) and their controls (asymptomatic S. aureus carriers). Corresponding odds ratios are given. The distribution of clonal complexes belonging to invasive S. aureus isolates from non-carriers is given.*

Clonal Complex (CC)	Cases	Controls	OR (95% CI)	Non-carriers
1	1 (1.7%)	4 (3.4%)	0.48 (0.02-4.75)	3 (8.8%)
25	1 (1.7%)	6 (5.1%)	0.32 (0.01-2.75)	3 (8.8%)
30	21 (35.0%)	26 (22.0%)	1.91 (0.91-4.00)	6 (17.6%)
45	3 (5.0%)	29 (24.6%)	0.16 (0.04-0.59)	5 (14.7%)
Singletons	34 (56.7%)	53 (44.9%)	1.60 (0.82-3.15)	15 (44.1%)
New ST	0	0	-	2 (5.9%)
Total	60	118	-	34

CC 1 includes sequence types: 1, 3, 81, and 188
CC 25 includes sequence types: 25 and 26.
CC 30 includes sequence types: 30, 34, and 39.
CC 45 includes sequence types: 45, 46, 47, and 53
Singletons include sequence types: 5, 7, 8, 9, 12, 15, 22, 50, 97, 120, 182, 500, 501, and 502.

Table 3. *Mortality data of patients with invasive S. aureus infection, by carriage status.*

	In-hospital mortality n/total (%)		
Clonal cluster	Carrier*	Non-carrier	Total
1	1/1 (100%)	2/3 (67%)	3/4 (75%)
25	1/1 (100%)	1/3 (33%)	2/4 (50%)
30	2/21 (10%)	4/6 (67%)	6/27 (22%)
45	0/3 (0%)	3/5 (60%)	3/8 (38%)
singleton	2/34 (6%)	5/15 (33%)	7/49 (14%)**
new	0/0 (-)	0/2 (0%)	0/2 (0%)**
total	6/60 (10%)	15/34 (44%)	21/94 (22%)

* Significant higher mortality in non-carriers as compared to carriers (Chi square: P=0.00015). No specific clonal cluster could be identified that was associated with the higher mortality rate in non-carriers.
** Significant higher mortality in those infected with *S. aureus* strains belonging to a clonal cluster versus those infected with singletons or new sequence types (P=0.029).

The overall mortality rate in non-carriers with invasive S. aureus infection was higher than in carriers, as described earlier (Table 3; P=0.00015) [3]. By the present MLST analysis we could not identify a specific clone in non-carriers with invasive S. aureus infection that could explain the higher in-hospital mortality in this group. However, there was a significant higher mortality rate in those infected with S. aureus strains belonging to a clonal cluster (14/43 [33%]) compared to those infected with strains classified as singletons or new sequence types (7/51 [14%]; P=0.029).

DISCUSSION

In a previous study, patients were screened for *S. aureus* nasal carriage at admission in 4 distinct teaching hospitals and followed for the development of nosocomial invasive *S. aureus* disease. In the present study, invasive *S. aureus* strains from *S. aureus* carriers were compared by MLST with carriage strains isolated from matched controls, who did not develop invasive *S. aureus* infections. The STs of invasive *S. aureus* strains from non-carriers were defined as well. The distribution of the STs was comparable with that found in other studies.[9]
Overall, no major clonal cluster could be identified that was responsible for invasive *S. aureus* disease. However, invasive strains belonged in 35% of the cases to ST 30 (not significant). Interestingly, we did identify a clonal complex (CC45) that was significantly more prevalent among non-invasive strains. We could not identify a clonal cluster that was significantly more prevalent in invasive strains of non-carriers compared to the other groups.

Earlier we reported a considerable higher mortality rate in *S. aureus* non-carriers with invasive *S. aureus* as compared to carriers with invasive *S. aureus* disease in the same cohort.[3] No single clonal cluster could be identified that could explain the higher mortality rate in this patient category. But this analysis is not definite since some clonal clusters had small numbers.

However, mortality rates were significantly higher among patients infected with a *S. aureus* strain belonging to a MLST clonal complex, compared to patients infected with a strain not belonging to a clonal complex. This finding indicates that staphylococcal clones that have successfully spread among humans, i.e. evolved into prevalent clonal complexes or lineages, are those that have more virulence factors associated with lethality of *S. aureus* disease. Further screening of the staphylococcal genome for virulence factors could aid in identifying the putative factor(s) responsible for the higher mortality rate of strains belonging to clonal complexes.

Feil et al also identified CC30 as a major clone in invasive nosocomial disease.[9] They attributed this observation to widespread presence of EMRSA-16 within this CC.[8,9] However, in our study no MRSA was identified, so this does not explain this finding. The prevalence of MRSA is very low in The Netherlands.[10] Clearly CC30 is a successful *S. aureus* lineage, irrespective of methicillin resistance. Due to the abundance of strains belonging to CC30, as found in our study and by Melles et al, the chances of this lineage to acquire a SCC*mec* are likely higher.[11] Once SCC*mec* is acquired, these ST 30 MRSA strains can replace ST 30 MSSA strains easily in settings with high antibiotic use, including hospitals.

Peacock and co-workers compared 155 *S. aureus* isolates from invasive disease with carriage isolates from healthy individuals, in the same cohort as Feil did.[12] They proposed that allelic variants of a polymorphic locus can make different contributions to the disease process. It remains unclear how. They also found evidence for considerable horizontal transfer of genes against a clonal background. It is now well established that within and between *S. aureus* clones there is a significant high level of exchange of mobile DNA coding for virulence and resistance.[13-15] Melles et al showed that all clones can cause life-threatening infections, but certain clones are more virulent than others.[11] It would be interesting to investigate whether there is a difference in competence for the uptake of (mobile) DNA between clones.

CONCLUSION

By multi locus sequence typing we could not identify a *S. aureus* clonal cluster that was more likely to cause invasive *S. aureus* infections. We did find that clonal cluster 45 was more prevalent in asymptomatic carriers. Clonal cluster 30 is in general a prevalent clone, independent of methicillin resistance. There were no prevalent clones of invasive *S. aureus* strains in non-carriers and no specific clone that could explain the higher in-hospital mortality rate. However, overall mortality, irrespective of carriage status, was significantly higher for those patients infected with strains belonging to a clonal complex, indicating co-evolution of *S. aureus* virulence and spread among humans.

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PART III

DETERMINANTS OF *STAPHYLOCOCCUS AUREUS* NASAL CARRIAGE

CHAPTER 7

NOSE PICKING AND NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS*.

Heiman F.L. Wertheim, Menno van Kleef, Margreet C. Vos, Alewijn Ott, Henri A. Verbrugh, Wytske Fokkens

Infection Control and Hospital Epidemiology. Accepted.

Dear Dr. Wertheim,

I am pleased to inform you that your revised manuscript, "Nose picking and nasal carriage of Staphylococcus aureus", has been accepted for publication in Infection Control and Hospital Epidemiology.

William R. Jarvis, Editor
Infection Control Hosp Epidemiol
March 21, 2005

ABSTRACT

Nasal carriage of *Staphylococcus aureus* is an important risk factor for subsequent *S. aureus* infections. We studied whether nose picking was associated with *S. aureus* nasal carriage in 238 Ear, Nose- and Throat disease (ENT) patients and 109 healthy employees by nasal culture, questionnaire (5 point scale answers) and nasal examination by an ENT-doctor (ENT patients only). Nose pickers are significantly more likely to carry *S. aureus* than non-pickers: 37/69 (53.6%) versus 60/169 (35.5%) respectively (RR: 1.51; CI: 1.03 - 2.19). There is a significant positive correlation between self-perceived frequency of nose picking and frequency of positive cultures (R: 0.31; P=0.004), and load of *S. aureus* in the nose (R: 0.32; P=0.003), suggestive for a causal relationship.

INTRODUCTION

Staphylococcus aureus, irrespective of resistance to methicillin, is a frequent cause of both community and hospital acquired infections, with substantial morbidity and mortality as a result.[1,2] About one-third of all persons is found to carry *S. aureus* in the nose.[3] Nasal carriage of *S. aureus* is a well-known risk factor for acquiring *S. aureus* infections and eradication of this micro-organism from the nose can be an effective preventive measure, mostly in surgical and dialysis patients.[3-6] The same prophylactic strategy is used for eradicating carriage of methicillin resistant *S. aureus* (MRSA), as an infection control policy.

Although numerous studies have been performed, a valid explanation for the *S. aureus* carriership has yet to be given. Since hand and nasal carriage of *S. aureus* are associated and *S. aureus* resides in the anterior part of the nose, we considered the habit of nose picking as a potential determinant of *S. aureus* nasal carriage.[7] In a pilot study, we demonstrated a positive trend between nose picking and *S. aureus* nasal carriage (Wertheim et al. Presentation at 9th International Symposium on Staphylococci and Staphylococcal Infections. Denmark 2000). We, therefore, studied this determinant in a larger cohort with predefined criteria for nose picking.

METHODS

Participants

Participants were patients who visited the Ear, Nose, and Throat (ENT) outpatient clinic and healthy volunteers, including personnel and medical students, of the Erasmus University Medical Centre Rotterdam, the Netherlands. Ethical review board approval was obtained. All participants gave written informed consent.

ENT-patients

Patients (≥ 18 years) who visited the ENT outpatient clinic between June 2001 and July 2002, and did not come primarily for nose complaints, were screened for nasal carriage of *S. aureus* and assessed for nose picking behaviour. The following exclusion criteria were used: signs of rhinitis, use of antibiotics at the time of inclusion, and inability to understand the Dutch language. The following data were obtained: demographics, medical history, and medication. Patients were given a standardized questionnaire on behaviour and symptoms related to the nose on which they could give answers on a five-point scale (Table 1). Patients were not informed that the primary determinant of this investigation was their nose picking behaviour.

Table 1. *Topics addressed in the questionnaire.*

Complaints	Behaviour
-Epistaxis	-Smoking
-Nasal dryness	-Exhaling smoke through the nose
-Nasal itchiness	-Blowing the nose
-Nasal crusts	-Turning up the nose
-Nasal wounds	-Picking the nose
-Runny nose	-Rubbing the nose externally
-Rhinitis	

A nasal examination was performed by an ENT specialist, who was blinded for *S. aureus* carriage status of the patient and the patient's answers to the questionnaire. The following symptoms and signs were scored: vestibulitis, recurrent epistaxis, septal hyperkeratosis, scratch effects in the vestibulum nasi, wounds and erosions in the vestibulum nasi, septum perforation and any nasal injury that was considered by the ENT doctor to be potentially due to nose picking. Only if these signs could not be explained otherwise they were scored as a sign of nose picking. In conclusion, the ENT specialist had to state whether the examined patient was considered a nose picker according to his/her clinical expertise. The anterior nares were cultured once, just prior to the ENT examination.

Patients were identified as nose pickers if they answered to pick at least sometimes and had at least one nose picking sign found by nasal examination.

Healthy volunteers

Between January 2002 and May 2003, nasal swabs were obtained from the healthy volunteers (≥18 years). At least 5 nasal swabs were obtained with one-week intervals to differentiate between the different carrier types of *S. aureus*. Volunteers were excluded if they used antibiotics.

Frequent carriers were defined as having at least two-third of their cultures positive for *S. aureus*, moderate carriers had one- to two-third of the cultures positive, occasional carriers had fewer than one-third of the cultures positive, and non-carriers had none of the cultures positive for *S. aureus.* These persons filled in the same questionnaire as described above, but were not examined by an ENT specialist.

Microbiology

Nasal specimens were obtained using sterile cotton-wool swabs and transport medium (Transwab, Medical Wire & Equipment Co. Ltd., Corsham, United Kingdom). Both the left and right anterior nares were swabbed by rubbing the swab four times in each nostril. The swabs were immediately placed in Stuart's medium and were cultured within 24 hours.

Nasal swabs from ENT patients were cultured quantitatively on selective media: phenol-red mannitol salt agar (PHMA) and phenol red mannitol salt broth (PHMB), as described earlier.[8] Colonies morphological suspect of *S. aureus* were subcultured overnight on Columbia blood agar (BA) plates (Becton-Dickinson B.V., Etten-Leur, The Netherlands) and a catalase- and latex-agglutination test (Staphaurex Plus^{R}, Murex, Dartford, UK) were performed.

Nasal swabs from healthy volunteers were cultured on BA plates and suspended in PHMB. The media were incubated for 48 hrs at 35 °C and checked each day for bacterial growth. Suspected *S. aureus* colonies were identified with catalase- and latex-agglutination test. The degree of growth was ascertained in a semi-quantitative manner.

Statistics

For data analysis we used SPSS version 10.0 statistical software. The five point-scale answers to the questionnaire (never, rarely, sometimes, regular, frequent) and the different degrees of carriage were coded from 0 to 4. The number of *S. aureus* CFU's were recorded quantitatively and then 10Log-transformed (10Log[CFU+1]) to obtain a normal distribution. Correlations were measured with the Spearman method. For 2 by 2 tables, the Fisher's exact test was used. Means were compared by unpaired t-tests and one-way ANOVA, where appropriate. P-values < 0.05 were considered significant.

RESULTS

ENT-patients

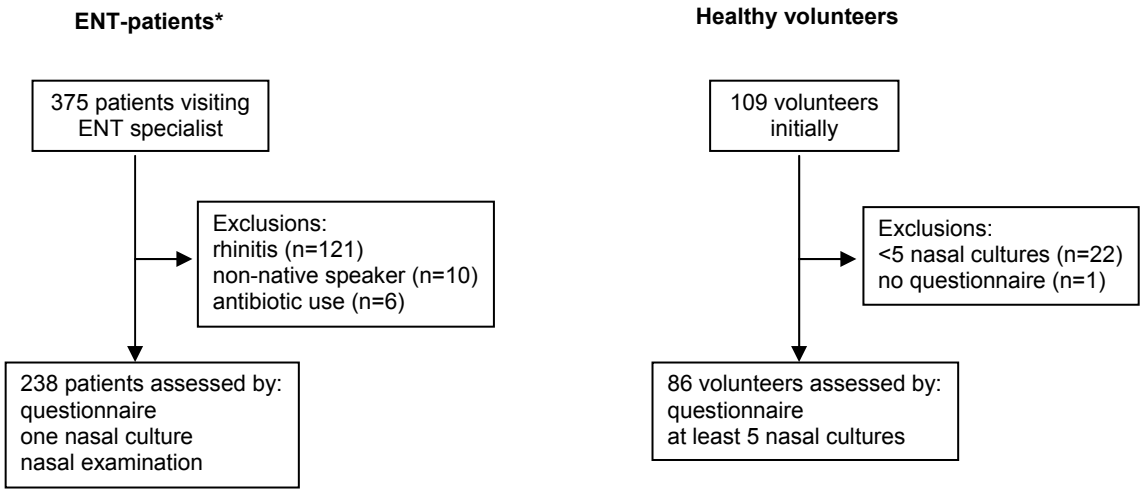
A total of 375 patients were asked to participate in this study of which 137 patients were excluded (Figure 1). The 238 included patients (58% male; mean age 47 years) had as most common primary complaint ear and hearing problems (71%). In this study population 97/238 (41%) patients were *S. aureus* nasal carriers and we found 69/238 (29%) to meet the criteria for nose picking. Nose pickers, as identified by questionnaire and nasal examination, were more often *S. aureus* carrier than non-pickers, 37/69 (54%) versus 60/169 (36%) respectively, resulting in a relative risk of 1.51 (95% CI: 1.03-2.19; Figure 2). This result was confirmed when only using the ENT-specialist classification of nose picking by physical examination:

59% of the patients classified as nose picker were *S. aureus* nasal carrier versus 35% of those considered non-picker (P=0.019).

In this patient population there was no correlation between the answers given to the questionnaire and number of *S. aureus* CFU's (R: 0.10; P = 0.18). However, significantly more CFU's were detected in patients who mentioned to pick at least sometimes (geometric mean CFU's: 1.9), versus those who mentioned not to pick their nose at all (geometric mean CFU's: 0.9; P=0.02).

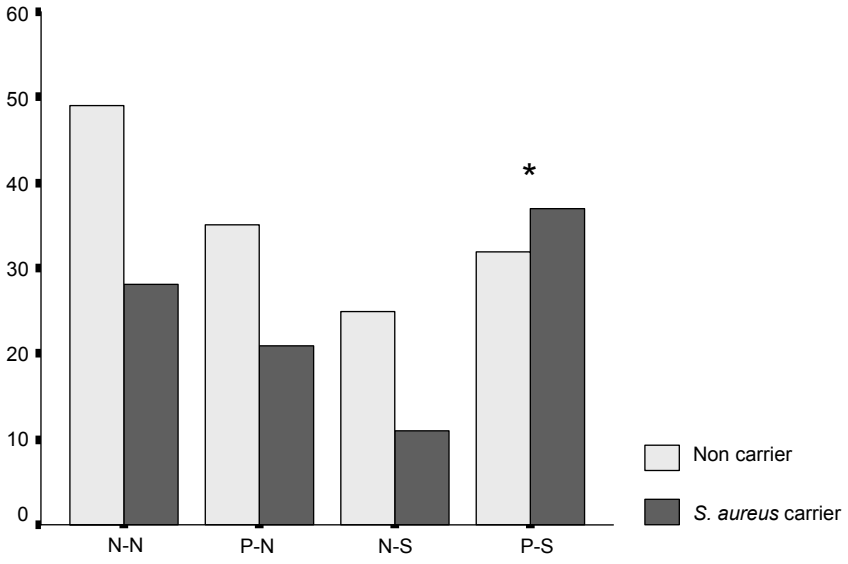
Self-reported nose picking was significantly correlated with self-reported nasal itchiness (R: 0.25; P<0.001), nasal crusts (R: 0.423; P<0.001), nasal dryness (R: 0.21; P=0.001), nasal wounds (R: 0.20; P=0.001), turning up once nose (R: 0.19; P=0.004) and nose rubbing (R: 0.31; P<0.001). Self-reported nose picking frequency was only significantly correlated with nasal crusts found during nasal examination (R: 0.16; P=0.013). There were no significant associations between the separate signs of nose picking and *S. aureus* carriage. If we take the number of *S. aureus* CFU's into account, there was a significant correlation with *S. aureus* load and nasal wounds (R: 0.14; P=0.032), nasal crusts (R: 0.13; P=0.048), and vestibulitis (R: 0.14; P=0.035).

Figure 1. *Study profile*



*ENT: Ear-, Nose-, Throat disease.

Figure 2. *Frequency of S. aureus nasal carriers in ENT-patients per nose-picking category.*



Categories: N-N: rarely to never picks and no signs of nose picking; P-N: picks at least sometimes, but no signs of nose picking; N-S: rarely to never picks, but signs of nose picking present; P-S: picks at least sometimes and signs of nose picking present.
* Category P-S included significantly more *S. aureus* carriers compared to all other categories (P=0.013), and compared to category N-S (P=0.024) and N-N (P=0.036), but not category P-N (P=0.072).

Healthy volunteers

Eighty-six volunteers (33.3% male; mean age: 23 years; Figure 1), who filled in the questionnaire and had at least 5 nasal cultures taken, were included in the study. On average 7 cultures were obtained per volunteer (range: 5-10 cultures). Carrier types detected were: 33 non-carriers (38.5%), 22 occasional carriers (25.5%), 9 moderate carriers (10.5%), and 22 frequent carriers (25.5%). There was a significant positive correlation between self perceived frequency of nose picking and frequency of positive cultures (R: 0.31; P=0.004; figure 3), and the semi-quantitative count of *S. aureus* CFU's (R: 0.33; P=0.002; figure 4). The reported frequency of nose picking was significantly correlated with the self-reporting on having nasal crusts (R: 0.45; P<0.001), nasal rubbing (R: 0.23; P=0.033), and turning up once nose (R: 0.29; P=0.007).

Figure 3. *Correlation between self reported frequency of nose picking and the proportion of positive nasal cultures in healthy volunteers.*

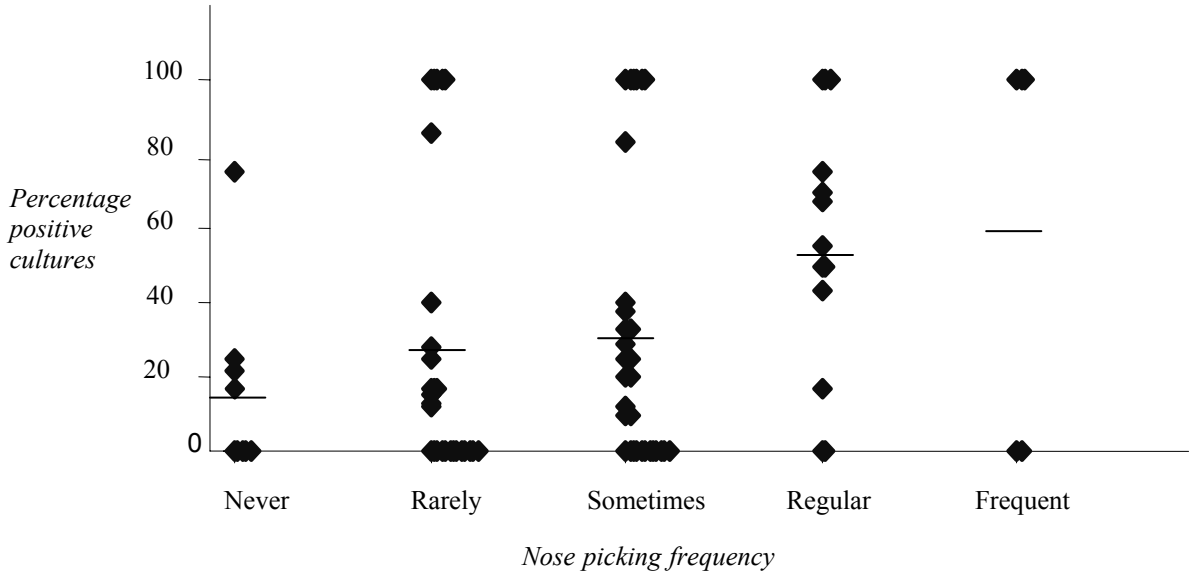
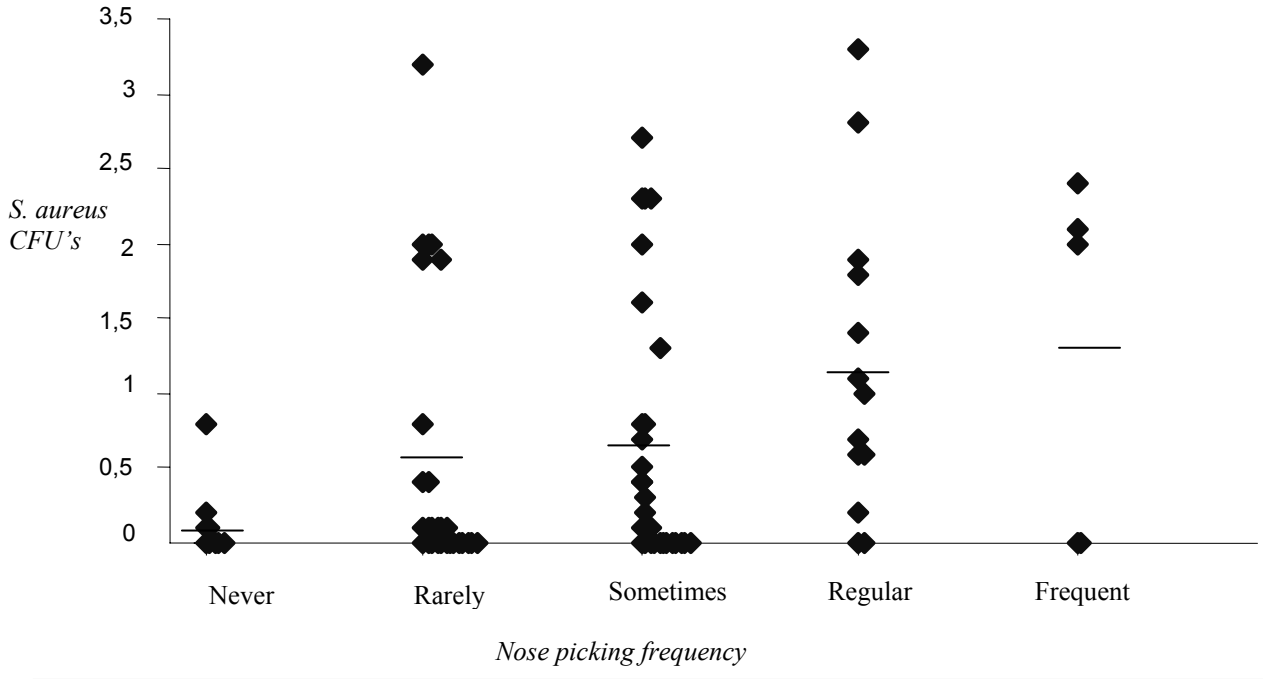


Figure 4. *Density of nasal S. aureus and nose picking frequency in healthy volunteers*



DISCUSSION

The findings of this study support the hypothesis that nose picking is associated with nasal carriage of *S. aureus*. The habit of nose picking is probably initiated by having nasal crusts. In the first study sample of ENT-patients, significantly more carriers were found in those classified as nose picker by predefined criteria. Also those patients classified as nose picker by the ENT-specialist, based on his clinical expertise, had a significant higher *S. aureus* carriage rate. Furthermore, there exists a significant correlation of proportion of positive cultures and the load of *S. aureus* present in the nose with self-graded frequency of nose picking. This positive dose-response suggests a causal relation between nose picking and nasal carriage of *S. aureus*.

We realize that the used questionnaire is a subjective measurement. It was unfeasible for us to observe participants secretly for their nose picking behaviour, which led us to the used study design. This disadvantage is partly compensated by blinding the participant for his/her carriage status and not telling the objective of the study. The questionnaire was also anonymous, which probably improved the sincerity of the given answers. We also included the nasal examination by an ENT specialist to score objective signs of nose picking.

Jefferson *et al*. studied the habit of nose picking and found that 90% of his study population picked their nose with various frequencies and degrees of severity, leading even to a perforated septum in two cases.[9] To be classified as a nose picker in our study, one needed at least to have one objective clinical sign assumed to be due to nose picking. Therefore patients classified as nose pickers all had some form of traumatic lesions, probably due to nose picking. Interestingly, nose picking signs with a negative answer to the questionnaire, was not predictive for *S. aureus* nasal carriage. Patients with rhinitis were excluded, since the associated inflammation impeded determination of nose picking signs.

We classified patients as nose pickers in case of observed damages to the nasal mucosa and dermis. This surface acts as a first line defence to microbial colonization and invasion. Lesions therein will expose extra-cellular matrix molecules, including fibronectin and collagen, to which *S. aureus* can adhere.[2,10,11] However, recent in-vitro studies found that *S. aureus* cell wall teichoic acid, clumping factor B and other cell-wall associated adhesins may be involved in adhering to nasal epithelial cells, suggesting that exposure of extra-cellular matrix molecules may not be essential for colonization.[12-15] However, *S. aureus* is well known to heavily colonize skin lesions, including eczematous lesions, indicating that, in-vivo, *S. aureus* exhibits high affinity to extra-cellular matrix molecules.[11]

Alternatively, carriage of *S. aureus* in the nose may elicit an immune response with irritation and itchiness as a result, that may elicit more frequently or rigorously nose picking. A recent study suggests that *S. aureus* colonization induces a local inflammatory response.[16] Eradicating *S. aureus* from the nose may result in reduced inflammation and itchiness. It still needs to be resolved whether nose picking is a cause or consequence of *S. aureus* nasal carriage.

Hand carriage is known to be associated with nasal carriage of *S. aureus*. The number of staphylococci on the fingers rises with increasing nasal counts.[17] Furthermore, nasal *S. aureus* carriers are more likely to be hand carriers of *S. aureus* and nasal eradication of *S. aureus* often leads to disappearance of the micro-organism from the hands as well.[18] A study by Hare et al., elegantly demonstrated that nine students, observed during a one hour lecture, touched their mouth or nose on 6 to 23 separate occasions.[19] Another study showed that nasal *S. aureus* carriers carry different loads of *S. aureus* on their left and right fingers.[17] Clearly, hands are the major vector for transmitting *S. aureus* from the environment into the nose, and vice versa. It is likely that staphylococci are introduced into the nose by the hand and that persistence of carriage may in part be determined by the frequency, duration and intensity of nose picking. The data as presented in figure 2 suggest that nose picking or nasal trauma alone do not lead to a higher carriage rate. It is probably a combination of both the introduction of *S. aureus* by the finger and having nasal trauma (either by nose picking or other causes) that facilitates *S. aureus* nasal carriage. Future *S. aureus* eradication studies could incorporate an advice to shed the habit of picking one's nose, reducing the probability of recolonization. Understanding the pathogenesis of *S. aureus* nasal carriage helps optimising prophylactic strategies to prevent *S. aureus* disease and spread of MRSA.

We conclude that nose picking is associated with nasal carriage of *S. aureus*, and may well be causal.

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PART IV

METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

CHAPTER 8

IMPROVED DETECTION OF METICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) USING A PHENYL MANNITOL BROTH CONTAINING AZTREONAM AND CEFTIZOXIM

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Journal of Clinical Microbiology 2001; 39: 2660-2662

... Vloeibare media verhogen de detectiekans en maken het afnemen van meerdere sets kweken overbodig. Derhalve wordt het gebruik hiervan aangeraden ... Een benadering is het toevoegen van antibiotica. In een studie werd hiermee een significant betere detectie gevonden in vergelijking met vaste media bij een aanmerkelijke vereenvoudiging van de laboratorium werkzaamheden. Dit medium bestaat uit een phenyl-mannitol bouillon met aztreonam en ceftizoxim. Ceftizoxim is gekozen omdat hierdoor de expressie van meticillineresistentie wordt verbeterd. Men kan de bouillon, ongeacht de kleur, na 48 uur afenten op een bloedagar, waarne de bloedagar verder wordt bewerkt. Echter men kan ook afenten nadat een kleuromslag van rood naar oranje-geel is opgetreden. De kleuromslag wordt beoordeeld na 48 en na 72 uur. Indien de laatste werkwijze wordt gekozen, wordt een gelijktijdig ingezette bloedagar beoordeeld op groei van niet-fermentatieve gram-negatieve staven. Bij aanwezigheid hiervan wordt de bouillon altijd afgeënt, ongeacht de kleur. Non-fermenters maken het milieu alkalisch waardoor de kleuromslag mogelijk niet meer optreedt. Uit de eerder genoemde studie blijkt dat bij deze werkwijze bij ongeveer 75% van alle bouillons geen afenting nodig is ...

NVMM richtlijn detectie van MRSA in Nederland 2002

ABSTRACT

We tested a phenyl mannitol broth containing ceftizoxim and aztreonam ($PHMB^+$) for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) with reference MRSA strains and, subsequently, with clinical samples (n=1,098). All reference MRSA strains induced color change in $PHMB^+$ after 24–72 hours incubation. In a clinical setting, 40 MRSA strains were detected with $PHMB^+$ versus only 23 with a routine method. Thus, this selective broth significantly ($p<0.001$) improved the rate of MRSA detection.

INTRODUCTION

Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical samples continues to be important, since infections due to MRSA have a high morbidity and mortality. Moreover, some MRSA strains have the potential to spread rapidly and colonize other patients. In The Netherlands, therefore, patients who are suspected for MRSA carriage are isolated until screening cultures are repetitively negative for MRSA. Methods to detect MRSA in clinical samples should ideally have a high sensitivity and a short time to reporting. To increase the sensitivity one can simply take more screening samples on the same day or on consecutive days, but this is more cumbersome and increases the time to reporting. Another way to increase the sensitivity is to use a broth in addition to agar plates as was demonstrated previously. [1-5] To increase the sensitivity of the detection of MRSA from a single sample and to improve laboratory efficiency, we developed a new selective broth containing phenol red, mannitol, aztreonam and ceftizoxim. First, we tested the broth with laboratory reference strains. Subsequently, we compared our routine method of direct plating of specimens onto blood agar plates and mannitol salt agars with the new selective broth combined with a blood agar plate.

METHODS

The selective broth ($PHMB^+$) was made by adding 5 µg/ml ceftizoxim (Yamanouchi) and 75 µg/ml aztreonam (Bristol-Myers Squibb) to phenyl mannitol broth with 0.05% salt (Becton Dickinson, Le Pont de Claix, France). See table 1 for a recipe of the broth. We tested $PHMB^+$ with 5 different MRSA and 5 different methicillin sensitive *S. aureus* (MSSA) strains isolated from patients. Methicillin resistance was confirmed by *MecA* PCR, according to the method described by Murakami.[6] At first, all 10 strains were subcultured onto Brucella blood agar and incubated for 18 hours at 37° C. From each strain a suspension was made in 0.9% NaCl with a density of 0.5 McFarland (10^8 cfu/ml) and dilution series of 10^8 cfu/ml to 10^0 cfu/ml were made. Five-hundred microliters of each dilution were pipetted in 5 $PHMB^+$ broths (4.5 ml) of different production dates, each 1 week apart. Every batch of $PHMB^+$ was prepared by the same person and stored at 4° C until use. One hundred microliters of the original solution of 0.5 McFarland was streaked on a Brucella blood agar plate as a control for the density of cfu's. The broths were incubated for 14 days at 37° C, and were inspected on a daily basis for color change from red to orange/yellow.

From June 1997 to December 1997 the Department of Medical Microbiology and Infectious

Diseases received 1,098 consecutive specimens for the detection of MRSA. These specimens originated from patients and employees and were either screening samples or samples taken during a putative MRSA outbreak. From employees, only the anterior nares were cultured. From patients, specimens were taken from rectum, nose, throat, wounds, insertion sites of venous and arterial lines, and urine if a urine catheter was present. Samples were collected and transported with commercial swabs (Transwab®, Medical Wire & Equipment Co. Ltd., Wiltshire, United Kingdom) to the laboratory and then stored for a maximum of 16 hours at 4° C until inoculation. Only one swab was available per collection site.

For the routine culture of MRSA the swabs were streaked on 5% sheep blood agar plates (BA, Becton Dickinson, Le Pont de Claix, France) and phenyl mannitol salt (7%) agar plates (PHMA, Becton Dickinson, France). Subsequently, the swabs were submerged in $PHMB^{+}$. All media were incubated for 3 days at 37° C and checked for growth of Staphylococci each day. The broth was examined daily for color change from red to orange/yellow for three days. When the color of the broth had changed to orange/yellow, a loop of broth was subcultured on BA. If growth of a non-fermenter was observed on the primary BA, the broth was subcultured on BA irrespective of the color of the broth. This subculture was examined for suspect colonies after incubating 18- 24 hrs at 37° C. Colonies suspected for *S. aureus* were identified with a Staphaurex Plus® agglutination test (Abbott Murex, Chatillon, France) and tested with methicillin disk diffusion performed according to the NCCLS guidelines.[7] All morphologically different strains were tested. Staphaurex Plus® positive strains were confirmed with the AccuProbe® hybridization test (Gen-Probe Inc., San Diego, USA), according to guidelines of the manufacturer. Methicillin resistance was confirmed with *MecA* PCR.[6] MecA positive strains were send to the laboratory of the National Institute of Public Health and the Environment (RIVM, Bilthoven, The Netherlands) for MRSA phage typing (unpublished method). The difference in proportion of detected MRSA strains between the two methods was statistically tested with the Sign test for paired samples using SPSS software, $p<0.05$ was considered significant.

RESULTS

In the experimental setting the MSSA strains did not produce any color change in the $PHMB^{+}$ broth, irrespective of the concentration of cfu's, of the incubation time, or the storage time of the broth. All MRSA strains gave a distinct color change at the dilution step corresponding to approximately 10^{0} cfu/ml after incubating for 72 hours. At densities of 10^{5} cfu/ml and higher, the color change was observed within 24 hours. The storage life of the broth was at least 4 weeks at

4° C in the dark (data not shown).

In the clinical setting a total of 1,098 cultures were performed. The cultures were taken from nares (n=466), perineum (n=220), throat (n=215), wounds (n=101), exit sites catheters (n=43), urine (n=22), and other sites (n=31). One-hundred-thirty-six (12 %) of these cultures were positive for *S. aureus* of which 40 (29 %) were methicillin resistant (*MecA* PCR positive). The MRSA strains were cultured from eight different patients. Phage typing of the MRSA strains showed 5 distinct phage types and one was untypable. Twenty-three (57%) of the MRSA strains grew on both BA, PHMA and in PHMB$^+$. Seventeen additional strains only grew in PHMB$^+$, and not on BA or PHMA (Sign test: $p<0.001$). The PHMB$^+$ showed 263/1,098 (24%) color changes (Table 2). The most prevalent organisms in positive PHMB$^+$, apart from MRSA, were coagulase-negative *Staphylococci* (n=107) and *Enterococcus spp*. (n=33).

DISCUSSION AND CONCLUSION

The results show that by using the selective broth we detected almost twice as many MRSA strains compared with the routine technique. Furthermore, only a small fraction of the PHMB$^+$ broths need to be subcultured due to the presence of selective antibiotics. At the time of this study our laboratory used methicillin agar diffusion instead of oxacillin to test for methicillin resistance. Since this test was used for both culture techniques we do not believe this will have a great effect on our results. This is the only study that presents a selective broth with antibiotics inhibiting growth of both gram-positive and gram-negative bacteria for the selection of MRSA strains. Previous studies have used high concentrations of salt for selectivity, with or without aztreonam or oxacillin.[1-5,8-12] By using only salt one selects MRSA as well as MSSA strains and salt has the disadvantage that some MRSA strains will not grow when concentrations exceed 2.5%.[10] The rationale for using ceftizoxim and aztreonam in the selective broth instead of oxacillin and colistin was that earlier studies had shown that both oxacillin and colistin resulted in inhibited or slower growth of MRSA strains (data not shown). Furthermore, ceftizoxim is known to increase the phenotypic level of resistance to methicillin.[13-15]

This study was designed to improve the efficiency and sensitivity of detecting MRSA, and in this respect "the need for speed" remains important. The use of the BA plate is still necessary to detect non-fermenters that produce an alkaline environment in the broth, thereby prohibiting the phenol red to turn yellow. Therefore, broths should always be subcultured when a non-fermenter grows on BA. When there is an outbreak with a new MRSA strain we suggest to immediately determine it's growth characteristics in the PHMB$^+$. Do this by making a dilution series of the

cultured strain, incubate and check the time required until color change. From the results one can choose the optimal incubation time for specimens from contact patients and health care workers. The present study clearly shows that MRSA screening with a selective phenyl mannitol broth including aztreonam and ceftizoxim is efficient and sensitive. This method is now implemented in the routine MRSA screening of our and other Dutch hospitals.

Table 1. *Recipe of the selective broth PHMB$^+$.*

Step 1
Mix 21 mg of dehydrated Phenol Red Mannitol Broth (PHMB, Becton Dickinson) with 1000 ml destilled water. Sterilize for 15 minutes at 121°C. Let it cool down to room temperature.

Step 2
Mix 5 mg Ceftizoxim (Yamanouchi) with 5 ml destilled water. Add to PHMB and mix.

Step 3
Mix 75 mg Aztreonam (Bristol-Myers Squibb) with 5 ml destilled water. Filter through FP 030/2 filter (Schleicher & Schuell). Add to PHMB and mix.

Step 4
Fill sterile tubes with 8 ml PHMB$^+$. Store at 4° C in the dark. Shelf-life is at least 4 weeks.

Table 2. *MRSA detected with the routine method versus PHMB$^+$.*

Species	**Routine**	**PHMB$^+$**
Coagulase-negative Staphylococci[a]	NR	111
Methicillin resistant *S. aureus* (MRSA)[b]	**23**	**40**
Enterococcus spp.[c]	NR	37
Coagulase-negative Staphylococci + *Enterococcus spp.*	NR	35
Other[d]	NR	40

Note: most frequent microorganisms causing color change in the PHMB$^+$ are mentioned (n=263).
[a] In combination with other species (n=4).
[b] Sign test: $p < 0.001$
[c] In combination with other species (n=4).
[d] Gram positive rods, yeasts and methicillin sensitive *S. aureus* (n=3).
NR: not registered.

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CHAPTER 9

LOW PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AT HOSPITAL ADMISSION IN THE NETHERLANDS

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Journal of Hospital Infection 2004; 56: 321-325

Ned Tijdschr Geneeskd 2004; 148: 1044-1048

MRSA-infecties in de VS nu ook onder de gewone bevolking

In de Verenigde Staten komt ongeveer een op de vijf infecties met meticilline-resistente *Staphylococcus aureus* (MRSA), de 'superbug', voor onder de gewone bevolking. Dat is verrassend, want er is altijd gedacht dat die bacterie zich buiten het ziekenhuis niet zou kunnen verbreiden, omdat hij dan wel resistent was tegen antibiotica, maar overigens te 'zwak' om zich in de strijd met andere huid- en keelholte- en anusstreekbewonende huidbacteriën te handhaven. Van de met MRSA geïnfecteerde patiënten heeft 8 tot 20 procent geen duidelijke risicofactoren voor deze besmetting, blijkt nu. Deze mensen waren bijvoorbeeld niet kort daarvoor in een ziekenhuis geweest.

Vroeger kon een stafylokokkeninfectie dodelijk verlopen. De ontdekking van penicilline rond de Tweede Wereldoorlog was dan ook een waar wonder. Maar al snel doken de eerste voor dit antibioticum ongevoelige bacteriestammen op. Dat gat is in 1960 gedicht met meticilline. Daarna ging het lange tijd goed tot in 1980 de eerste meticilline-resistente stafylokokken (MRSA) opdoken, meest in ziekenhuizen, waar de bacterie zich kan handhaven omdat er veel mensen verblijven met een verzwakt afweersysteem. Dan rest eigenlijk alleen nog het antibioticum vancomycine. Er zijn tegenwoordig wel alternatieve medicijnen maar die zijn peperduur. Als MRSA zich gaat verbreiden onder de gewone bevolking, is de kans groot dat steeds meer stafylokokken ook voor vancomycine resistent worden. Dan kunnen stafylokokkeninfecties weer vooroorlogse proporties aannemen.

Om te bepalen hoe vaak MRSA onder de gewone bevolking voorkomt, hebben medewerkers van het Amerikaanse National Center for Disease Control and Prevention alle MRSA-gevallen in Atlanta, Minnesota en Baltimore in de periode 2001-2002 geanalyseerd (*The New England Journal of Medicine*, 7 april). In het totaal ging het om ruim 12.500 patiënten. Bij 2500 van hen was er geen duidelijke oorzaak voor de besmetting. Bij de meeste van deze 'verrassingspatiënten' had de resistente bacterie

de huid of het onderhuidse weefsel geïnfecteerd. Bij 23 procent was de infectie zo ernstig dat die in het ziekenhuis moest worden behandeld. Eén patiënt overleed. De onderzoekers hebben zoveel mogelijk niet te verklaren MRSA-patiënten telefonisch gevraagd naar mogelijke risicofactoren. Ze hebben 41 procent van die mensen bereikt en op basis daarvan concluderen ze dat ruim 2000 patiënten (variërend van 8 procent in Baltimore tot 20 procent in Atlanta) de MRSA-infectie buiten het ziekenhuis moet hebben opgelopen; dat zijn dus 'community acquired'-MRSA-infecties.

In Nederland (en in de Scandinavische landen) is nu nog minder dan 1 procent van de stafylokokken resistent tegen meticilline. Dat is heel weinig vergeleken met bijvoorbeeld België (28 procent), Frankrijk (33 procent) en de Verenigde Staten (meer dan 50 procent). Dat is vooral te danken aan het 'zoek-en-vernietig'-beleid ('search and destroy'), in 1994 opgesteld door de Werkgroep Infectie Preventie. Patiënten met MRSA worden volledig geïsoleerd, afdelingen worden gesloten en medewerkers gescreend. Dat is ingrijpend en kostbaar maar blijkbaar werkt het.

In 2000 is ook hier geïnventariseerd hoe vaak MRSA voorkomt bij patiënten zonder duidelijke risicofactoren (*Nederlands Tijdschrift voor Geneeskunde* 2004;148:1044-8). De belangrijkste kans op besmetting hebben Nederlanders in een buitenlands ziekenhuis. De onverklaarbare besmetting bleek heel zeldzaam te zijn, zo'n 0,03 procent. In de VS zijn er al plannen om alle patiënten voorafgaand aan een ziekenhuisopname te testen op MRSA, maar dat is hier dus voorlopig niet noodzakelijk.

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NRC Handelsblad, 9 april 2005

ABSTRACT

In the Netherlands, less than 1% of clinical isolates of *Staphylococcus aureus* is methicillin-resistant (MRSA). A national Search and Destroy policy prevents MRSA from becoming endemic. Some MRSA outbreaks cannot be related to patients at risk for MRSA carriage. This study was designed to measure the prevalence of MRSA among patients without risk factors for MRSA carriage at the time of admission to the hospital.

In 4 Dutch hospitals, patients admitted to non-surgical departments in the period 1999-2000 were screened for MRSA nasal carriage. Nasal swabs were streaked on 5% sheep blood agar (BA), submerged in a selective broth, and incubated for 2-3 days at 35°C. Colonies suspected for *S. aureus* were identified with an agglutination test. Susceptibility testing was performed by an automated system and additional oxacillin disk diffusion. Methicillin resistance was confirmed by a DNA hybridisation test and *MecA* PCR. MRSA strains were genotyped by pulsed field gel electrophoresis (PFGE).

Twenty-four percent (2,332/9,859) of the patients were *S. aureus* nasal carriers. Only 3 (0.03%) patients were MRSA carriers. These patients were not repatriated, nor known to be MRSA carrier prior to screening. Genotyping revealed that the strains were not clonally related and were not related to MRSA outbreaks in the hospital where the patients were admitted.

We conclude that at routine admission to a Dutch hospital (excluding high-risk foreign admissions) the MRSA prevalence is low (0.03%), due to the Dutch Search and Destroy policy and restrictive antibiotic prescribing.

INTRODUCTION

The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the Netherlands is among the lowest in the world. The European Antimicrobial Resistance Surveillance System (EARSS), a European resistance surveillance network, shows that the prevalence of MRSA among clinical *S. aureus* isolates is below 1% in the Netherlands.[1] Prevalences in other countries are much higher: Belgium 28%, France 33%, Germany 19%, and the United States 50%.[1,2] A great threat is the emergence of vancomycin-resistant *S. aureus* isolates, of which the first has been isolated in the United States in 2002.[3] A low MRSA prevalence may prevent the emergence of such highly resistant isolates.

The low prevalence in The Netherlands can largely be explained by our national Search and Destroy policy, in combination with restrictive antibiotic use.[4] The Search and Destroy policy implies that patients that are repatriated from countries outside the Netherlands and contacts of MRSA patients are strictly isolated at hospital admission until screening cultures for MRSA prove negative ('search'; Table 1). In case of MRSA carriage, individuals are kept in isolation and treated to eradicate MRSA ('destroy'). This policy is according to a guideline, established by the Dutch Working Group Infection Prevention (WIP guideline 35a; available at http://www.wip.nl). This guideline has recently been updated after this study was performed. Also, the use of antibiotics in the Netherlands is very low due to a restrictive prescribing policy: the defined daily doses of antibiotics used per 1000 people per day (DDD) in primary health care is 8.9, compared to 36.5 DDD in France.[5] This low antibiotic pressure in the Netherlands, probably limits the selection of resistant micro-organisms, including *S. aureus*.[5,6]

Since 1995, the Netherlands were confronted with a few MRSA outbreaks that could not be related to patients with known risk factors for MRSA carriage as mentioned in Table I.[7] If MRSA strains are circulating in the community outside the hospitals, the risk factors as described in Table 1 would not be sufficient for a successful Search and Destroy policy, and further outbreaks could be the consequence. This study measured the prevalence of MRSA nasal carriage in non-risk patients at admission.

Table 1. *Risk factors for MRSA carriage in the Netherlands, according to the national guidelines of the Working Group Infection Prevention (WIP-guideline 35a; available at: http://www.wip.nl). We do not refer to the new guideline because it was developed after this study.*

1.	All patients transferred from a foreign hospital or nursing home, who
	- have been admitted there for at least 24 hours
	or
	- have been operated there
	or
	- have a drain or catheter in place at the time of transfer
	or
	- are intubated
	or
	- have open wounds or infections like abscesses or furuncles
2.	All patients that are known positive for MRSA.
3.	Contacts of a MRSA carrier.

METHODS

Between April 1999 and April 2000, 9,859 patients of non-surgical departments were screened for MRSA nasal carriage at admission. The participating hospitals were: Erasmus MC in Rotterdam (1300 beds), UMC St. Radboud in Nijmegen (950 beds), VU Medical Center in Amsterdam (730 beds), and Amphia hospital in Breda (500 beds). Medical Review Board approval was obtained.

Nose swabs were obtained by nursing personnel at admission. Swabs were inoculated on blood agar plates (Becton Dickinson, France) and in a selective phenyl mannitol broth containing aztreonam and ceftizoxime (PHMB+), as described previously.[8] The selective broth was examined daily for color change from red to orange/yellow for 3 days. When the color of the broth had changed to orange/yellow, a loop of broth was subcultured onto a blood agar plate. Growth suspect for *S. aureus* was tested with an agglutination test (StaphaurexPlus, Abbott Murex, France). All StaphaurexPlus positive strains were send to Erasmus MC, where the identification of *S. aureus* was confirmed and susceptibility testing performed by an automated system (Microscan-Walk-Away, Gram positive panel, Dade-Behring, USA). Susceptibility for oxacillin was performed by disk diffusion according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS).[9] The minimal inhibiting concentration (MIC) for oxacillin was measured by E test® (AB biodisk, Solna, Sweden). Breakpoints of all MIC results were according to NCCLS criteria.[9] StaphaurexPlus positive strains with an antibiotic susceptibility profile suspect for methicillin resistance were confirmed by a *S. aureus* specific DNA hybridisation test (AccuProbe, Gen-Probe Inc., USA) and a PCR to identify the *MecA* gene. MRSA strains were genotyped by pulsed field gel

electrophoresis (PFGE) and compared with other circulating MRSA strains of the hospitals involved. MRSA isolates were considered to be identical if their PFGE patterns did not differ by more than three bands, according to standard PFGE interpretation criteria.[10] Detection of a MRSA strain from a patient in this study would not lead to the standard practice of isolation measures, because the susceptibility testing was performed at a later stage (after discharge).

RESULTS

During the study period 9,859 patients were screened for MRSA nasal carriage. Patients were screened on average 1.8 days after admission (range: 0-3 days). Twenty-four percent (2,332/9,859) of the patients were *S. aureus* nasal carrier. Thirty-three strains were lost for susceptibility testing (random error). Only 3 (0.03%) patients were MRSA carriers and all 3 patients originated from the same hospital (Hospital D). These patients had no known risk factors for MRSA carriage. Two of the three patients were hospitalised previously in hospital D, the other patient (patient 1) was hospitalised previously elsewhere (Table 2).

To investigate whether these MRSA strains were isolated earlier in hospital D, PFGE patterns were compared to patterns of other MRSA strains isolated in hospital D from 1993 to 2002. Genotyping showed that all three MRSA strains were unique, indicating that they were not related to any MRSA outbreak in hospital D. This indicates that these strains were neither acquired nor disseminated in that hospital.

The three "new" strains were send to the National Institute of Public Health and Environment (RIVM, Bilthoven, The Netherlands) for genotyping (PFGE) and compared with the PFGE genotypes of national MRSA strains, isolated since 2002. Only one of the three MRSA strains belonged to a known PFGE cluster (cluster 153), the other two MRSA strains were unknown.

Table 2. *Characteristics of the colonised patients and their MRSA strains.*

Patient characteristics							MRSA characteristics					
Patient	Age Years	Disease	Isolated	MRSA risk	Admitted Before	Days admitted	RIVM [a]	Oxa [b]	Cli	Cip	Gen	Rif
1, male	52	ischemic heart disease	No[c]	No	Yes	14	153	4	S	S	S	S
2, male	26	ulcerative colitis	No	No	Yes	3	250	256	S	R	R	S
3, male	79	arrhythmia	No	No	Yes	1[d]	251	32	S	S	S	S

Oxa: oxacillin; Cli: clindamycin; Cip: ciprofloxacin; Gen: gentamicine; Rif: rifampicin; S: susceptible; R: resistant.
[a] PFGE genotyping result RIVM: PFGE cluster 153 is a known cluster. Clusters 250 and 251 are new.
[b] Minimal inhibiting concentration for oxacillin (mg/L).
[c] This patient was admitted to a single room.
[d] This patient was readmitted 3 weeks later, again for a single day.

DISCUSSION

This study illustrates that the MRSA prevalence at hospital admission in the Netherlands, among patients without risk factors for MRSA carriage, is very low (0,03%). This prevalence is much lower than the 10% prevalence of MRSA carriage among patients repatriated from foreign countries and admitted to Hospital D in the year 2000. Extending the screening procedure to patients without risk factors as mentioned in Table I seems therefore not indicated.

This low prevalence level illustrates that the Dutch Search and Destroy policy in combination with restrictive antibiotic prescription policy, is still effective. A less stringent MRSA policy would probably lead to an increase of MRSA carriage in the community, as observed in France where the prevalence of MRSA at admission to the hospital is 1,3%.[11] Once MRSA is endemic in the hospitals, the prevalence in the community will be higher than in the Netherlands, as shown in an American meta-analysis: 1.3%.[2] Furthermore, it is known that patients who have ever been hospitalised, have more risk to be MRSA carrier (RR: 2,35), than persons without a history of hospitalization.[2]

Any MRSA outbreak in a Dutch hospital, results in unpopular hygienic measures, not always fully appreciated by clinicians and hospital administrators. The study clearly indicates that Search and Destroy in the present Dutch situation is still effective. Dropping this policy would certainly lead to endemic MRSA in due time, with all consequences for our current antibiotic prescription policy and for patients with MRSA infections. Being forced to replace our current first choice antibiotic (flucloxacillin) by glycopeptides or oxazolidinones will increase health-care costs. A recent Dutch study showed the Search and Destroy policy to be cheaper than the presence of endemic MRSA.[12] A recent meta-analysis illustrated that the mortality of patients with an invasive MRSA infection is double the mortality of patients with an invasive methicillin-susceptible *S. aureus* infection[13]. Furthermore, the replacement of penicillins by glycopeptides increases the risk that vancomycin resistant microorganisms, including *S. aureus,* may appear in the Netherlands.

Since 2002 more MRSA outbreaks are observed in the Netherlands. In addition to the Search and Destroy policy and restrictive antibiotic use, we need a national registration system of MRSA patients and of hospitals experiencing a MRSA outbreak. This could ensure that patients, colonised with MRSA and those who are transferred from a hospital with a MRSA outbreak, can be tagged and traced and control measures can be initiated. It is expected that in the near future, molecular techniques will allow to significantly reduce the time of the MRSA

screening to a few hours. Rapid MRSA testing will be of great benefit for both the patient and nursing personnel.[14]

It is striking that none of the three MRSA isolates in this study caused an outbreak, since none of these three patients was isolated. For patient 1 this can be explained by the fact that he received mupirocin nasal ointment soon after admission, as a consequence of enrolment in a clinical trial. Follow-up nasal cultures of this patient were negative. This patient was also admitted to a single room, which would further contribute to the prevention of MRSA transmission to other patients. The duration of hospitalisation of the other two patients was short (1 and 3 days), possibly too short for transmission to other patients or hospital personnel.

We conclude that the MRSA prevalence at admission to the hospital in the Netherlands in the years 1999 and 2000 was very low (0.03%). This low prevalence is due to our national Search and Destroy policy and due to restrictive antibiotic use. We need to make sure that we can maintain this low MRSA prevalence, since this is beneficial for patients (less morbidity and mortality) as well as for the healthcare system (less costs). Therefore, we believe that a national registry of MRSA positive patients and hospitals with MRSA outbreaks is necessary.

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PART V

DISCUSSION

CHAPTER 10

SUMMARY

SUMMARY

General

At its introduction in the 80's, mupirocin was reported to be highly effective in eliminating *S. aureus* nasal carriage, thus raising hopes that *S. aureus* nosocomial infections could be better prevented than in the past. Unfortunately, these hopes are not fulfilled. *S. aureus* remains high upon the list of causative organisms of nosocomial infections. Furthermore, *S. aureus* has become more resistant than ever.[1] Prevalence rates of MRSA strains in blood cultures have skyrocketed in most countries (the Netherlands are among the few countries with low MRSA prevalence rates), to prevalence rates of more than 40 percent.[2-4] Three vancomycin- and methicillin-resistant *S. aureus* strains have been cultured from three different patients in the United States since 2002.[5-7] These observations would predict that *S. aureus* infections will become more difficult to treat. Preventing *S. aureus* infections is, therefore, now more important than ever.

Preventing nosocomial *S. aureus* infection

Earlier studies, have shown that eradication of *S. aureus* from the nose of patients with mupirocin nasal ointment may prevent subsequent *S. aureus* infection.[8] We studied the efficacy of mupirocin nasal ointment in preventing nosocomial *S. aureus* infections in a non-surgical patient population with a randomised controlled trial (**chapter 3**). The findings of our study do not support the strategy of routine culture at admission and subsequent mupirocin application in *S. aureus* nasal carriers to prevent *S. aureus* nosocomial infection in a general non-surgical population. However, we did find that more than 80% of nosocomial cases of *S. aureus* bacteremia are of endogenous origin, which confirms the data of Von Eiff *et al*.[9] This illustrates that strategies that can effectively and safely eliminate *S. aureus* carriage from relevant sites may still play an important role in preventing infections with this pathogen.

Several explanations can be given for the observed lack of efficacy of mupirocin prophylaxis in this study.

1. First, in our study, there was an overall low rate of acquiring a nosocomial *S. aureus* infection, rates being lower than the a priori risk estimate. The sample size, therefore, was too small to detect potentially significant (albeit small) differences in rate of nosocomial infections.
2. Secondly, mupirocin prophylaxis was started 2-3 days after admission. Within this period, the risk of nosocomial infections is already present, which was observed in our

population. However, excluding these patients with such early-onset nosocomial *S. aureus* infections from our analysis did not lead to a significant risk reduction. Therefore, it is recommended to investigate the efficacy of screening and starting mupirocin treatment of patients earlier, preferably on the day of admission. Molecular and other novel diagnostic techniques that allow rapid and accurate detection of *S. aureus* carriage, have recently become available and make this feasible.[10]

3. Thirdly, most nosocomial *S. aureus* infections developed in patients with a relatively long hospital stay. We observed that in the placebo group infections occurred on day 12 (median) after admission, while the median number of admission days of all included patients was eight, in both the placebo and mupirocin group. Most nosocomial *S. aureus* bloodstream infections occur late during hospitalization, which is confirmed by a U.S. study.[1] Therefore, future interventions should be focused on patients at risk for a prolonged hospital stay.
4. Fourth and last, mupirocin treatment alone may not be sufficient for *S. aureus* decolonization. Several studies show recolonization with *S. aureus* occurs in 38% to 43% of patients after 4 to 6 weeks after mupirocin application.[11-13] Extra-nasal body reservoirs of *S. aureus* probably play an important role in nasal recolonization.

The fourth point described above made us study the role of *S. aureus* carriage at extra-nasal sites (throat and perineum) in recolonization after mupirocin treatment in a group of healthy volunteers (**chapter 5**). We found that mupirocin was overall effective in decolonizing the anterior nares, but less effective in decolonizing extra-nasal sites. The majority (60%) of the *S. aureus* strains of those volunteers who remained colonized five weeks after treatment were of endogenous origin (i.e. the same strain as before treatment present in the nose). However, acquisition of exogenous *S. aureus* strains is also common (40%), suggesting that decolonization should only be performed in proven carriers. This is even more stressed by the fact that we found one mupirocin resistant strain after treatment in this study and that two non-carriers became carriers after treatment. Decolonization may be improved by adding washing with disinfectant soap to the regimen. Also new promising compounds for nasal decolonization are being developed, including lysostaphin and fatty acid (lauric esthers) compounds, that may aid in improving decolonization strategies.[14,15]

The risk of *S. aureus* nasal carriage

Chapter 4 provides solid evidence that nasal carriers of *S. aureus* are indeed at increased risk of *S. aureus* bloodstream infections once they become admitted to the hospital. Nasal carriers have a threefold increased risk of acquiring nosocomial bloodstream infections as compared to non-carriers. In contrast, there was a fourfold decreased risk of in-hospital mortality in nasal carriers who acquired nosocomial *S. aureus* bloodstream infections, as compared to patients who were non-carrier at admission, but developed a similar infection anyway. Although, the two patient groups differed in age and clinical background, correcting for underlying disease and demographic characteristics did not significantly alter these findings. Previous studies identifying risk factors for fatal outcome of *S. aureus* bacteremia never included *S. aureus* nasal carriage. These studies did identify older age, infection with a methicillin-resistant strain, central venous access, disease severity, and underlying illness as risk factors for fatal outcome.[3,16-19] The higher mortality rate observed among non-carriers with infection needs confirmation. Novel strategies need to be developed to prevent *S. aureus* infection in non-carriers, who presumably acquire this micro-organism through cross-transmission.[20]

Several explanations can be given for the found higher mortality in non-carriers versus carriers with a *S. aureus* infection:

1. Non-carriers maybe infected with a more virulent hospital *S. aureus* clone, compared to carriers, acquired during hospital admission. We, therefore, compared the genotypes (sequence types) of invasive *S. aureus* strains of both carriers and non-carriers, as determined by multilocus sequence typing (MLST).[21] The sequence types of *S. aureus* strains causing invasive disease in non-carriers did not differ from carriers (see Chapter 6). This suggests that there probably is not a prevalent virulent 'hospital' *S. aureus* clone causing disease in non-carriers through cross-transmission. However, one study showed that staphylokinase production, a virulence factor, was lower in patients with lethal outcome of *S. aureus* bacteremia.[22] Although, a bacterial explanation for the higher mortality in this patient category is less likely, a detailed screen for potential virulence genes associated with fatal outcome still needs to be performed.
2. Host-related immunological mechanisms may provide an alternative explanation for the lower mortality rate observed in *S. aureus* nasal carriers with invasive disease. One study shows that S. aureus nasal carriers have neutralizing antibodies against *S. aureus*

superantigens, whereas non-carriers have no antibodies or very low titers.[23] A mouse model illustrates that intranasal application of *S. aureus* superantigens protects against subsequent death due to S. aureus infection.[24] Furthermore, certain viral and bacterial vaccines can effectively be applied intranasally and result in protective immunity in both humans and animals.[25-27] Clearly, *S. aureus* cells in the nares of carriers may likewise lead to some sort of immune response. Research in this field is lacking. One study showed that *S. aureus* nasal colonization induces a neutrophil mediated inflammatory response, but this response fails to clear the colonizing bacteria.[28] Since in more than eighty percent of the cases, *S. aureus* cells causing invasive disease in carriers are identical to the same strain found á priori in the nose, some level of cellular and/or humoral immunity to this endogenous strain may already be present, which may help reduce the risk of fatal outcome.

In **Chapter 6**, we compared the genotypes (sequence types) of invasive *S. aureus* strains of both carriers and non-carriers, as determined by MLST (see above).[21] The sequence types of *S. aureus* strains causing invasive disease in non-carriers did not differ significantly from carriers. We did identify a clonal complex (CC45) that was significantly more prevalent among non-invasive strains. No major clonal cluster could be identified that was responsible for invasive *S. aureus* disease in *S. aureus* carriers. Though, fifty percent of the invasive strains belonged to CC30, this was not statistically significant. Patients infected with a *S. aureus* strain belonging to a clonal cluster had a significant higher risk of dying, than those infected with a singleton or new sequence type. At this moment we cannot explain the higher mortality rate in those infected with *S. aureus* strains belonging to a clonal cluster. Strains belonging to a clonal cluster are in general more prevalent and, therefore, seem to be better adapted to the human host. It could be that specific virulence factors are needed for this adaptation, which can result in a higher mortality rate in those infected. These findings warrant further analysis of the *S. aureus* genomic structure and expression of virulence genes in relation to disease.

Nose picking behaviour and *S. aureus* nasal carriage

Since *S. aureus* nasal carriers are at increased risk for invasive disease, it is important to elucidate the mechanisms leading to *S. aureus* nasal carriage, to be able to develop new eradication strategies. We decided to investigate nose picking as a possible determinant, since hand carriage is known to be associated with nasal carriage of *S. aureus* (**Chapter 7**).

Previous studies show that the number of staphylococci on the fingers rises with increasing nasal counts.[29] Furthermore, nasal *S. aureus* carriers are more likely to be hand carriers of *S. aureus* and nasal eradication of *S. aureus* often leads to disappearance of the micro-organism from the hands as well.[12] A study by Hare et al., demonstrated that nine students, observed during a one hour lecture, touched their mouth or nose on 6 to 23 separate occasions.[30]

In our nose picking study, significantly more *S. aureus* carriers were found among those classified as nose picker by predefined criteria. Also those patients classified as nose picker by the Ear-, Nose-, Throat specialist, had a significant higher *S. aureus* carriage rate. Furthermore, we found a significant correlation between the number of positive cultures, the load of *S. aureus* present in the nose, and the self-graded frequency of nose picking. This 'dose-response' relationship suggests a causal relation between frequency of nose picking behaviour and *S. aureus* nasal carriage. Nose picking or nasal traumas alone do not lead to a higher carriage rate. Possibly a combination of both the introduction of *S. aureus* by the finger and having nasal trauma may suffice in establishing *S. aureus* nasal carriage.

The nasal mucosa and dermis is a first line defence to microbial colonization and invasion. Lesions therein will expose extra-cellular matrix molecules, including fibronectin and collagen, to which *S. aureus* can adhere.[2,31,32] However, recent in-vitro studies found that *S. aureus* cell wall teichoic acid, clumping factor B and other cell-wall associated adhesins may be involved in adhering to nasal epithelial cells, suggesting that exposure of extra-cellular matrix molecules may not be essential for colonization.[33-36] However, *S. aureus* is well known to heavily colonize skin lesions, including eczematous lesions, indicating that, in-vivo, *S. aureus* exhibits high affinity to extra-cellular matrix molecules.[32] It still needs to be resolved whether nose picking is a cause or consequence of *S. aureus* nasal carriage.

Methicillin-resistant *S. aureus* (MRSA)

In preventing *S. aureus* infections, it is essential to keep the prevalence of methicillin-resistant *S. aureus* (MRSA) strains low. Infections with MRSA can only be treated with usually less effective and generally more expensive antibiotics. Furthermore, MRSA infections have a worse prognosis than infections with susceptible strains. In the Netherlands, patients at risk for MRSA carriage are, according to national guidelines, screened and isolated until MRSA screening cultures are proven negative.[37] Identified MRSA carriers will remain in isolation and are offered an eradication treatment. This strategy is also known as a 'search and destroy'

policy. Due to this policy, the MRSA prevalence within clinical *S. aureus* isolates is still below one percent.[4] Since the prevalence is low it is essential to have a very sensitive and specific test to screen for MRSA.

In **Chapter 8** we describe the development of a novel sensitive diagnostic method to screen for MRSA. At the time of the study (1997), screening a patient for MRSA was usually performed by direct plating patient samples solely on solid culture media, e.g. plating on Columbia blood agar. The results of our study show that by using a selective broth with antibiotics inhibiting growth of both gram-positive (ceftizoxim) and gram-negative bacteria (aztreonam), twice as many MRSA strains were detected as compared with the routine technique. We used ceftizoxim, since this agent is known to increase the phenotypic level of resistance to methicillin.[38] This method is now implemented in the routine MRSA screening of our and many other Dutch hospitals.

Since 1995, MRSA outbreaks were reported in the Netherlands that could not be related to risk factors as defined by our national guideline.[39,40] We, therefore, decided to screen patients not at risk for MRSA carriage, to assess whether our guideline was still sufficient (**Chapter 9**). We found that the MRSA prevalence at hospital admission in the Netherlands, among patients without risk factors for MRSA carriage, is still very low (0.03%). Changing the risk factors for MRSA carriage in our national guideline is, therefore, not indicated. The low prevalence level illustrates that the Dutch 'search and destroy' policy in combination with restrictive antibiotic prescription policy, remains effective. Adherence to this policy may be improved by implementing rapid molecular techniques for the detection of MRSA, which will be of great benefit for both the patient (shorter isolation) and hospital (lower costs).[41]

Conclusions and recommendations for the clinician

- *S. aureus* screening of patients at admission by routine nasal culture at and subsequent application of mupirocin ointment to the anterior nares for five days in *S. aureus* nasal carriers is not effective in preventing *S. aureus* nosocomial infections in a general non-surgical population, and, therefore, can not be recommended.
- When an attempt is made to eradicate *S. aureus* nasal carriage in an individual, follow-up nasal cultures are needed to monitor recolonization with *S. aureus* is a common phenomenon.

- As compared to patients that do not carry *S. aureus* in the nose at admission, nasal carriers of *S. aureus* have a threefold increased risk of acquiring nosocomial *S. aureus* bloodstream infections.
- The outcome of nosocomial *S. aureus* bacteremia is, in part, dependent on the premorbid *S. aureus* nasal carriage status: when non-nasal carriers of *S. aureus* acquire a nosocomial *S. aureus* bloodstream infection, they have a fourfold increased risk of dying, as compared to *S. aureus* nasal carriers with nosocomial *S. aureus* bloodstream infections. Premorbid nasal carriage with *S. aureus* may, thus, confer paradoxical effects in patients admitted to the hospital: it increases the risk of invasive disease, but simultaneously provides them with partial protection against a fatal outcome in case such infections develop.
- Nose picking and nasal carriage of *S. aureus* are associated. This association may well be causal since there exists a positive correlation between frequency of nose picking, and both the number of positive cultures and *S. aureus* load.
- Clinicians should continue to support and follow the Dutch national policy of 'search and destroy', with regards to MRSA, as outlined by the Working Party on Infection Prevention (WIP). The Netherlands has, so far, been spared from the burden of endemic MRSA, and that should stay so.

Conclusions and recommendations for the investigator

- In future studies aimed at preventing nosocomial *S. aureus* infections, it is recommended to test for *S. aureus* nasal carriage and treat carriers as soon as possible, i.e. on the day of admission. The recent development of accurate and rapid diagnostic techniques to screen for *S. aureus* carriage makes this approach possible.
- Future studies aimed at preventing nosocomial *S. aureus* bloodstream infections should be focused on patient categories likely to have a prolonged hospital stay (e.g. 5 days).
- Since nasal application of mupirocin ointment alone was found to be insufficient to prevent *S. aureus* infection during hospital admission, other body sites should be considered as potential sources of *S. aureus* infection. Total body washing with a disinfecting soap may, therefore, be a necessary augmentation of the intervention strategy.
- The role of *S. aureus* carriage at extra-nasal sites in the development of *S. aureus* infections needs further study.
- The higher mortality rate in non-carriers versus carriers of *S. aureus* associated with *S. aureus* bloodstream infections should be confirmed and the underlying mechanism(s) unravelled.

- The type of host immune response to *S. aureus* nasal colonization needs to be re-investigated. So far, the immunological consequences of *S. aureus* nasal carriage have not been studied in much detail.
- Future *S. aureus* eradication studies may incorporate an intervention aimed at shedding the habit of picking one's nose, since nose picking is associated with a higher *S. aureus* nasal carriage rate.
- Further analysis of the *S. aureus* genomic structure and expression of specific genes in relation to clinically well-defined types of *S. aureus* diseases should be performed. A cost-effective, high throughput screening method for a complete *S. aureus* virulence profile, is a prerequisite for this kind of research.
- A screening-test for MRSA should continue to include an enrichment broth culture, until another technique is proven to be more sensitive and specific than this current gold standard.
- A rapid sensitive and specific test should be developed to screen patients for both methicillin-sensitive and methicillin-resistant *S. aureus* carriage (i.e. with a turn-around-time of two hours).

Recommendations for the policymaker

- Spend more money on studies aimed at preventing *S. aureus* infections, since this is likely to be highly cost-effective. In the Netherlands there are 1.6 million hospital admissions per year. Approximately 0.1 percent of these patients (n=1600) will acquire a nosocomial *S. aureus* bloodstream infection during their hospital stay.[1] A *S. aureus* bloodstream infection costs 10.000 euros on average, and carries an associated mortality of 25% (400 deaths/year).[1,42] In the Netherlands, therefore, nosocomial *S. aureus* bloodstream infections alone, will cost society 16 million euros every year. To this sum should also be added the costs of other types of nosocomial *S. aureus* infections, including surgical wound infections, urinary tract infections, osteomyelitis, and pulmonary infections.
- More continuing effort should be given to promoting, on both a national and international level, the Dutch national 'search and destroy' policy for controlling MRSA. This policy has been very effective for almost two decades, whereas less stringent policies adopted by other countries have miserably failed to contain the spread of MRSA within their borders.

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CHAPTER 11

SAMENVATTING

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Algemeen

Sinds medio vorige eeuw is men op de hoogte dat dragers van *S. aureus* in de neus een verhoogd risico hebben op *S. aureus* infecties. Met de introductie van mupirocine neuszalf (antibioticum) in de jaren tachtig, ter eradicatie van *S. aureus* uit de neus, leidde tot de hypothese dat *S. aureus* infecties voor een groot deel konden worden voorkomen. Helaas is deze hypothese niet uitgekomen. *S. aureus* staat nog steeds hoog op de ranglijst van micro-organismen die ziekenhuisinfecties veroorzaken. Verder wordt *S. aureus* meer resistent tegen antibiotica.[1] De frequentie van meticilline-resistente *S. aureus* (MRSA) stammen die uit bloedkweken worden geïsoleerd, is fors gestegen in veel landen. Van de gekweekte *S. aureus* stammen zijn in sommige landen zelfs meer dan 40 procent meticilline-resistent.[2-4] Tevens zijn sinds 2002 drie vancomycine- en meticilline-resistente *S. aureus* stammen gekweekt uit verschillende patiënten in de Verenigde Staten.[5-7] Dit betekent dat de behandeling van infecties met resistente *S. aureus* stammen nog moeilijker zal gaan worden met minder effectieve antibiotica. Daarom is het voorkomen van *S. aureus* infecties nu belangrijker dan ooit.

Preventie van nosocomiale *S. aureus* infecties

Eerder onderzoek, in voornamelijk chirurgische patiënten, laat zien dat eradicatie van *S. aureus* uit de neus met mupirocine neuszalf, *S. aureus* infecties kan voorkomen.[8] Wij hebben de effectiviteit van mupirocine neuszalf in het voorkomen van *S. aureus* infecties onderzocht in een niet-chirurgische ziekenhuispopulatie middels een gerandomiseerde placebo gecontroleerde studie (hoofdstuk 3). De resultaten van dit onderzoek ondersteunen niet het routinematig aantonen van *S. aureus* neusdragerschap middels kweek en het vervolgens behandelen van dragers met mupirocine neuszalf om *S. aureus* infecties te voorkomen in niet-chirurgische patiënten. Dit onderzoek laat zien dat meer dan 80% van nosocomiale *S. aureus* bacteriëmieën van endogene oorsprong zijn. Deze bevinding is een aanmoediging om strategieën te blijven ontwikkelen die effectief en veilig *S. aureus* kunnen decolonizeren van het lichaam, om infecties met dit pathogeen te voorkomen.

Er zijn meerdere verklaringen mogelijk voor de gevonden gebrek aan effectiviteit van mupirocine profylaxe in deze studie.

1. Allereerst was de incidentie van *S. aureus* ziekenhuisinfecties lager dan tevoren ingeschat. De onderzoeksgrootte is daarom achteraf gezien te klein om potentiële (echter kleine) statistisch significante verschillen in het voorkomen van ziekenhuisinfecties te kunnen aantonen.
2. Een andere mogelijke verklaring is dat mupirocine pas werd toegediend twee tot drie dagen na opname, omdat dan pas de kweekresultaten bekend waren. In deze periode kan een patiënt al een ziekenhuisinfectie oplopen, zoals waargenomen in deze studie. Echter, uitsluiten van de analyse van patiënten met deze vroeg ontstane infecties, veranderde niet onze eerdere conclusies. Het is aanbevolen om het screenen op *S. aureus* dragerschap en vervolgens *S. aureus* eradicatie in dragers op dezelfde dag van opname te doen. Snelle en betrouwbare moleculaire methoden zijn nu beschikbaar om dit mogelijk te maken.[9]
3. De meeste *S. aureus* ziekenhuis infecties ontstaan in patiënten die relatief lang in het ziekenhuis liggen. In de placebo groep ontstonden de infecties pas na een mediane opnameduur van 12 dagen, terwijl de mediane opnameduur van alle geïncludeerde patiënten acht dagen was. Het gevonden tijdstip van ontstaan van *S. aureus* bacteriëmieën wordt bevestigd door ander onderzoek.[1] Hieruit kan worden geconcludeerd dat een potentiële interventie strategie zich voortaan moet richten op patiënten met een verlengde opnameduur om zo effectief mogelijk zijn.
4. De gevonden vertraging van 13 dagen in de tijd tot ontstaan van *S. aureus* ziekenhuisinfecties in de mupirocine groep suggereert dat na verloop van tijd met mupirocine behandelde patiënten weer worden gerekoloniseerd met *S. aureus*. Na rekolonisatie met *S. aureus* hebben deze dragers weer een verhoogd risico op het verkrijgen van infecties met dit micro-organisme. Dit betekent dat vervolgen op rekolonisatie is aanbevolen en dat, indien nodig, dit moet leiden tot een nieuwe eradicatiekuur.

De vraag resteert of mupirocine behandeling op zich zelf niet voldoende is voor effectieve *S. aureus* dekolonisatie. Verschillende studies laten rekolonisatie met *S. aureus* zien in de neus na 4 tot 6 weken in 38% tot 43% van de gevallen na mupirocine applicatie.[10-12] Deze bevindingen impliceren dat mupirocine alleen niet afdoende is. Andere reservoirs van het lichaam dan de neus spelen waarschijnlijk een rol in rekolonisatie van de neus. Daarom

hebben wij de rol van *S. aureus* dragerschap van de keel en perineum onderzocht in rekolonisatie in zowel dragers als niet dragers van *S. aureus*, die zijn behandeld met mupirocine (hoofdstuk 5). In dit onderzoek was mupirocine effectief in het dekolonizeren van het vestibulum nasi, maar minder effectief in het dekolonizeren van keel en perineum.
Bij falen van eradicatie met mupirocine, is onderzocht of de *S. aureus* stammen na mupirocine behandeling gelijk waren aan de stam voor behandeling middels genotypering. In 60 procent van de gevallen was dit het geval. Echter, de acquisitie van exogene (nieuwe) *S. aureus* stammen werd tevens gezien in 35 procent van de gevallen. In de resterende 5 procent was sprake van rekolonisatie met zowel endogene als exogene stammen. Twee niet-dragers zijn na mupirocine behandeling drager geworden. Mogelijk door het verstoren van de aanwezige nasale flora en expositie aan *S. aureus* heeft *S. aureus* dragerschap kunnen ontstaan. Mupirocine neuszalf dient te worden voorbehouden aan bewezen *S. aureus* dragers om te voorkomen dat niet dragers alsnog drager worden.
Eradicatie van *S. aureus* uit de neus kan mogelijk worden verbeterd door naast mupirocine applicatie in de neus, ook te wassen met desinfecterende zeep. Hierdoor wordt *S. aureus* dragerschap buiten de neus nog harder aangepakt. Verder worden nieuwe veelbelovende producten ontwikkeld voor neus dekolonizatie, zoals lysostaphine en vetzuren, die mogelijk een verbetering kunnen bewerkstelligen in het elimineren van *S. aureus*.[13,14] Toekomstige studies dienen uit te wijzen of dit inderdaad het geval is.

Risico van *S. aureus* neusdragerschap

Het onderzoek beschreven in hoofdstuk 4, laat zien dat *S. aureus* neusdragers een drievoudig verhoogd risico hebben ten op zichte van niet-dragers op het krijgen van een nosocomiale bloedbaan infectie met *S. aureus*. Wanneer men echter de mortaliteit berekent in de twee groepen, ziet men dat niet-dragers met een *S. aureus* bloedbaaninfectie een significant verhoogd risico hebben op overlijden dan dragers. Hoewel de twee onderzochte groepen zeer verschillend zijn in leeftijd en onderliggend lijden, veranderen de bevindingen niet na statistische correcties. Eerdere studies die determinanten van overlijden door invasieve *S. aureus* infecties hebben onderzocht laten zien dat leeftijd, meticilline-resistentie, en ernst van ziek zijn, belangrijke risico factoren zijn voor overlijden.[3,15-18] In deze studies is nooit *S. aureus* neusdragerschap meegenomen als variabele. De in onze studie gevonden hogere mortaliteit onder niet-dragers met een *S. aureus* bloedbaaninfectie dient wel te worden bevestigd in een volgende studie. Nieuwe methoden dienen te worden ontwikkeld om *S.*

aureus infecties te voorkomen in niet-dragers, die deze bacterie waarschijnlijk via kruisbesmetting oplopen.[19]

Meerdere verklaringen zijn mogelijk voor de hogere mortaliteit onder niet dragers met een *S. aureus* bloedbaaninfectie, te weten:

1. Niet-dragers raken geïnfecteerd met een meer virulente 'ziekenhuis' kloon. Om dit aan te tonen dan wel uit sluiten, hebben wij de genotypen (multi locus sequence typing, MLST) van invasieve *S. aureus* stammen van zowel dragers als niet dragers vergeleken met behulp van een DNA micro-array techniek (hoofdstuk 6).[20] De frequentie van de verschillende MLST typen van invasieve *S. aureus* stammen van zowel dragers als niet dragers zijn vergelijkbaar. Dat suggereert dat er geen sprake is geweest van een virulente *S. aureus* kloon die door kruisbesmetting infecties heeft veroorzaakt in niet-dragers.
2. *S. aureus* neusdragers met *S. aureus* infecties zijn beschermd tegen overlijden door gedeeltelijke beschermende immuniteit. Eén studie laat zien dat asymptomatische dragers antistoffen hebben tegen *S. aureus* superantigenen, welke afwezig of verminderd aanwezig zijn in niet-dragers.[21] Onderzoek in een muizenmodel laat zien dat intranasale applicatie van *S. aureus* superantigenen inderdaad beschermt tegen overlijden ten gevolge van een *S. aureus* infectie.[22] Ook is het inmiddels bekend dat mensen effectief tegen bepaalde virussen en bacteriën kunnen worden gevaccineerd, middels het toedienen van het vaccin in de neus.[23-25] De aanwezigheid van *S. aureus* in de neus zou op een dergelijke wijze kunnen leiden tot een beschermende immunologische respons. Dit dient nader te worden onderzocht. Aangezien meer dan 80 procent van de *S. aureus* infecties bij dragers van endogene oorspong is, lijkt het zeer waarschijnlijk dat er een mate van beschermende immuniteit van dragerschap uit gaat, met een lagere mortaliteit tot gevolg.

In hoofdstuk 6 wordt een onderzoek beschreven waarin invasieve en niet-invasieve *S. aureus* stammen met elkaar worden vergeleken middels genotypering (MLST). Op een dergelijke wijze kan men nagaan of bepaalde genotypes vaker of minder vaak worden aangetroffen bij invasieve infecties. In dit onderzoek is een prevalente *S. aureus* kloon (CC45) geïdentificeerd onder niet invasieve stammen. Er waren geen *S. aureus* kloons die significant meer voor kwamen onder invasieve stammen. Hoewel 50 procent van de invasieve stammen tot CC30 behoorde, was dit niet significant. Verder is bestudeerd of welke genotypes aan elkaar verwant zijn en een zogeheten klonaal complex vormen. Patiënten die waren geïnfecteerd met

een genotype behorend tot een klonaal complex hadden een hoger overlijdensrisico, dan patiënten die waren geïnfecteerd met een stam die niet tot een klonaal complex behoorde. Stammen die tot een klonaal complex horen, zijn in het algemeen prevalent en daarom waarschijnlijk beter aangepast aan de mens.

Neuspeuteren en *S. aureus* neusdragerschap

Aangezien *S. aureus* neusdragerschap een belangrijke risicofactor is voor het krijgen van een *S. aureus* infectie, is het belangrijk het mechanisme dat leidt tot dragerschap op te helderen om zodoende effectieve preventieve maatregelen te kunnen ontwikkelen. Het is reeds bekend dat dragerschap van *S. aureus* in de neus en op de handen en vingers sterk met elkaar zijn gecorreleerd.[26] Daarom hebben wij een onderzoek gedaan naar een mogelijke associatie tussen neuspeuter gedrag en dragerschap van *S. aureus* (hoofdstuk 7).

Wij vonden een significante associatie tussen *S. aureus* dragerschap en deelnemers die waren geclassificeerd als neuspeuteraar volgens standaard criteria. Ook onder deelnemers waarvan de KNO-arts van mening was dit een neuspeuteraar betrof, vond men significant vaker *S. aureus* in de neus. Tevens was er een significante positieve correlatie tussen de frequentie van neuspeuteren en het relatief aantal positieve neuskweken met *S. aureus*. De frequentie van neuspeuteren en de hoeveelheid *S. aureus* in de neus waren ook positief met elkaar gecorreleerd. Deze zogenaamde ‘dosis-respons’ relatie duidt op een causaal verband tussen mate van neuspeutergedrag en *S. aureus* neusdragerschap. Neuspeuteren of het hebben van lesies in de neus alleen is echter niet voldoende om vaker *S. aureus* drager te zijn. Het is mogelijk de combinatie van zowel het introduceren van *S. aureus* in de neus met de vinger door neuspeutergedrag en het hebben van lesies dat tot *S. aureus* dragerschap van de neus leidt.

Het neusslijmvlies is de eerste barrière voor microbiële invasie. Lesies in dit slijmvlies zorgen ervoor dat extracellulaire matrix moleculen geëxposeerd worden waaraan *S. aureus* zich kan hechten, waaronder fibronectine en collageen.[2,27,28] Echter, recente in-vitro studies laten zien dat clumping factor B en teichoinezuur, beide *S. aureus* producten, in staat zijn zich aan epitheliale cellen te hechten. Dit suggereert dat de expositie van extracellulaire eiwitten niet echt nodig is voor *S. aureus* om zich in de neus vestigen.[29-32] Echter, het is bekend dat *S. aureus* in grote hoeveelheden kan worden gekweekt uit huidlesies, zoals bij eczeem patiënten. Dit laat zien dat in-vivo, *S. aureus* een hoge affiniteit heeft voor extra-cellulaire matrix moleculen.[28] Of neuspeuteren nu een gevolg is van *S. aureus* dragerschap of een oorzaak daarvan dient nader te worden onderzocht.

Meticilline-resistente *S. aureus* (MRSA)

Om infecties met MRSA te voorkomen, is het essentieel om de prevalentie van MRSA dragerschap laag te houden. Infecties door MRSA, in vergelijking met meticilline-gevoelige *S. aureus* (MSSA), kunnen meestal alleen worden behandeld met minder effectieve en duurdere antibiotica. Daarenboven, hebben patiënten met een MRSA infectie een slechtere prognose dan patiënten met een MSSA infectie.[3] In Nederland worden patiënten met risicofactoren voor MRSA dragerschap volgens een nationale richtlijn gescreend op MRSA en geïsoleerd verpleegd totdat de MRSA screeningskweken negatief zijn.[33] Bij vastgesteld MRSA dragerschap blijft de patiënt in isolatie en wordt een MRSA eradicatiekuur ingesteld. Deze strategie staat ook bekend als het 'search and destroy' beleid. Onder meer door dit beleid is de prevalentie van meticilline resistentie onder klinische *S. aureus* isolaten vooralsnog onder de één procent.[4] Gezien deze lage prevalentie is het van belang een sensitieve en specifieke test te hebben voor het screenen op MRSA.

In hoofdstuk 8 wordt een nieuwe gevoelige methode beschreven voor het kweken van MRSA. In de tijd dat deze studie plaatsvond was het voor de detectie van MRSA meestal gebruikelijk om patiëntenmonsters op vaste media te enten, zoals bloedplaten. De resultaten van deze studie laten zien dat het gebruiken van een selectief ophopingsmedium (phenyl mannitol bouillon) met antibiotica (aztreonam en ceftizoxime), twee keer zoveel MRSA wordt gedetecteerd dan met vaste media alleen. Ceftizoxime is gebruikt omdat dit middel phenotypisch het expressie niveau van meticilline resistentie kan verhogen. Aztreonam is toegevoegd ter onderdrukking van gram-negatieve staven. Deze kweekmethode wordt inmiddels in meerdere Nederlandse ziekenhuizen naar tevredenheid gebruikt.

Sinds 1995 zijn enkele meldingen geweest van MRSA uitbraken in Nederlandse zorginstellingen die niet konden worden gerelateerd aan een patiënt met risicofactoren, zoals vastgesteld in de richtlijn van de Werkgroep Infectiepreventie (WIP).[34,35] Dit kan betekenen dat ongemerkt MRSA dragers worden opgenomen in Nederlandse ziekenhuizen. Om dit te onderzoeken hebben wij 10 duizend patiënten zonder risicofactoren voor MRSA dragerschap bij opname gescreend op MRSA neusdragerschap in vier verschillende Nederlandse ziekenhuizen (Hoofdstuk 9). Deze screening laat zien dat onder patiënten zonder risicofactoren voor MRSA dragerschap de prevalentie van MRSA neusdragerschap bij opname zeer laag is (0,03%). De risicofactoren zoals vastgelegd in de WIP richtlijn hoeven vooralsnog niet te worden aangepast. Deze lage prevalentie ondersteunt het huidige 'search en destroy' beleid in combinatie met het restrictieve antibioticagebruik. Dit beleid kan worden

verbeterd door het implementeren van snelle MRSA detectie technieken wat grote voordelen heeft voor de patiënt (kortere isolatieduur) en ziekenhuis (kosten).[36]

Conclusies en aanbevelingen voor de clinicus

- Het screenen van niet-chirurgische patiënten op *S. aureus* neusdragerschap bij opname middels kweek en het vervolgens toedienen van mupirocine neuszalf bij gevonden dragers, is niet effectief in het voorkomen van ziekenhuisinfecties met *S. aureus*, en wordt daarom niet aanbevolen.
- De behandeling van *S. aureus* neusdragerschap dient te worden vervolgd met neuskweken omdat rekolonisatie frequent kan voorkomen.
- Neusdragers van *S. aureus* hebben een drievoudig verhoogd risico op het krijgen van een ziekenhuis bloedbaaninfectie met *S. aureus*, in vergelijking met niet-dragers.
- Niet dragers van *S. aureus* in de neus, die wel een ziekenhuis bloedbaaninfectie oplopen, hebben een viervoudig verhoogd risico op overlijden, in vergelijking met *S. aureus* neusdragers die eenzelfde infectie doormaken.
- Neuspeuteren en *S. aureus* neusdragerschap zijn geassocieerd. Deze associatie is waarschijnlijk causaal omdat er een positieve correlatie bestaat tussen de frequentie van neuspeuteren en het aantal positieve kweken of de hoeveelheid van *S. aureus* in de neus.
- Clinici dienen het 'search en destroy' MRSA beleid te ondersteunen. Nederland is de last van endemisch aanwezige MRSA tot op heden bespaard gebleven, en dat moet zo blijven.

Conclusies en aanbevelingen voor de onderzoeker

- Voor nieuwe studies gericht op het voorkomen van *S. aureus* infecties, is het aan te bevelen om van de deelnemers snel een testresultaat van *S. aureus* dragerschap te hebben en snel te starten met behandeling, bij voorkeur op de dag van opname.
- Dergelijke studies dienen zich ook te richten op patiënten met een verhoogd risico op *S. aureus* bloedbaaninfecties, waaronder patiënten met een verwachte lange opnameduur.
- De rol van *S. aureus* dragerschap op plekken buiten de neus op het ontstaan van *S. aureus* infecties behoeft nader onderzoek.
- De hogere mortaliteit onder niet dragers versus dragers van *S. aureus* met een *S. aureus* bloedbaaninfectie behoeft confirmatie en het onderliggende mechanisme dient te worden onderzocht.

- De immunologische respons op *S. aureus* neusdragerschap behoeft grondig onderzoek. Het menselijke model die wordt gebruikt op onze afdeling leent zich hier uitstekend voor (niet beschreven in dit proefschrift).
- Indien men beoogt *S. aureus* neusdragerschap te behandelen, is mogelijk het advies om niet meer in de neus te peuteren een waardevolle aanvulling op het eradicatieschema.
- Voor het screenen van MRSA moet altijd een ophopingsmedium te worden gebruikt.
- Een snelle sensitieve en specifieke test moet worden ontwikkeld voor het screenen van patiënten op zowel meticilline-resistente als gevoelige *S. aureus* (uitslag binnen enkele uren).

Aanbevelingen voor de beleidsmaker

- Meer geld moet worden besteed aan onderzoek die zich richt op het voorkomen van *S. aureus* infecties want dit is naar alle waarschijnlijkheid kosten-effectief. In Nederland zijn er 1.6 miljoen ziekenhuisopnames per jaar (Prismant). Ongeveer 0.1 procent van deze patiënten (n=1600) zullen ziekenhuis bloedbaaninfectie met *S. aureus* oplopen tijdens de ziekenhuisopname. Zo'n *S. aureus* bloedbaaninfectie kost gemiddeld 10.000 euro, en heeft een mortaliteit van 25% (400 doden/jaar).[1,37] In Nederland kosten deze *S. aureus* bloedbaaninfecties de samenleving 16 miljoen euro per jaar. Bij dit bedrag dient ook nog de kosten van andere *S. aureus* te worden opgeteld, waaronder chirurgische wondinfecties, urineweginfecties, osteomyelitis, en luchtweginfecties.
- Meer energie moet worden gestoken in het promoten van het Nederlandse 'search and destroy' MRSA beleid, zowel op nationaal als op internationaal niveau. Dit beleid is al 20 jaar effectief, terwijl minder streng beleid in andere landen ten aanzien van MRSA hebben gefaald.

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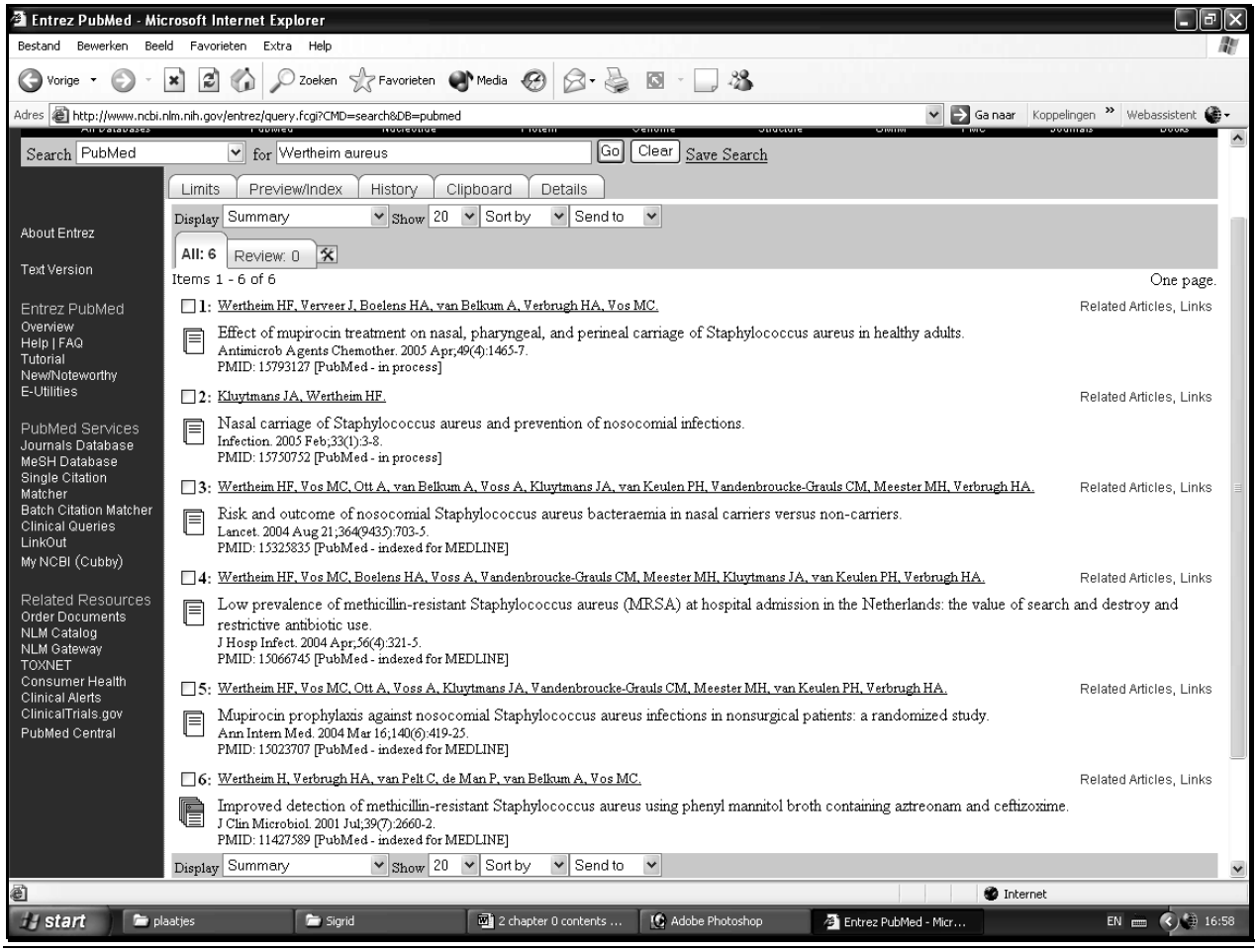

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DANKWOORD

Bij de neus genomen

- 4 ziekenhuizen
- 16 auteurs
- 5.000 *S. aureus* stammen
- 14.500 neuzen
- 18.000 wattenstokken
- 25.000 formulieren
- 370.000 euro

Dit groots opgezette onderzoek had ik uiteraard niet kunnen uitvoeren en voltooien zonder de medewerking, inzet en steun van een hele reeks personen. Graag wil ik alle mensen die op een of andere wijze een bijdrage hebben geleverd aan mijn onderzoek bedanken en niet in de laatste plaats alle patiënten en vrijwilligers die hun neuzen beschikbaar hebben gesteld voor de wetenschap.

Een persoonlijk woord van dank wil ik richten tot:

Henri Verbrugh: Beste Henri, zeer veel dank voor jouw vertrouwen in mij. Je hebt mij de vrijheid gegund om de dingen ook op mijn manier te doen, maar was tevens altijd bereikbaar als klankbord en graag bereid constructief mee te denken. Ik vind het inspirerend om met jou van gedachten te wisselen over nieuwe onderzoeksavonturen (Brazilië?).

Greet Vos: Ha Greet, je bent een heerlijke collega en de meest enthousiaste copromotor die ik me had kunnen wensen. Jouw aanstekelijke enthousiasme voor het vak gaf mij zeven jaar geleden een extra impuls om arts-microbioloog te worden. Fantastisch hoe je ruim de tijd nam om mij met raad en daad bij te staan.

Alewijn Ott: Beste Alewijn, het merendeel van de eerste analyses en interpretaties heb ik samen met jou gedaan. Jouw diepgaande kennis en ervaring in de epidemiologie en statistiek zijn voor mij, en eigenlijk voor de hele afdeling, goud waard. Voor mij was je als het ware een tweede ‘copromotor’.

Alex van Belkum: Beste Alex, jou ‘no nonsense’ instelling werkt verfrissend. Het is altijd snel en goed ‘zaken’ doen met jou. Neusdragerschap studies nemen een hele andere wending nu wij COGEM toestemming hebben verkregen om recombinant Stafylokokken in neuzen te gaan smeren. Het zal me benieuwen.

Jan Kluytmans: Beste Jan, stafylokokken-in-de-neus goeroe, jouw voorwerk heeft dit proefschrift mede mogelijk gemaakt. Ik vind het bewonderenswaardig hoe jij jouw ‘onderzoeksterrein’ en kennis deelt en uitbouwt met anderen vanuit een niet-academische setting.

Christina Vandenbroucke-Grauls: Beste Christina, jouw kritische commentaren op het onderzoeksprotocol en uiteindelijke manuscripten hebben mij zeer geholpen scherp te blijven en helder te formuleren. Ik hoop dat de ‘koek en zopie’ tijdens de MUP meetings jouw treinreizen naar Rotterdam enigszins hebben verzacht.

Andreas Voss: Beste Andreas, door jouw expertise en andere kijk op wetenschappelijk onderzoek was het leerzaam en door je gevoel voor humor ook ontzettend leuk om samen met jou onderzoek te doen. Gelukkig heeft jouw overstap naar het Canisius ziekenhuis verdere samenwerking niet belemmerd.

Peter van Keulen: Beste Peter, dank je wel voor jouw deelname aan en inzet voor de MUP-studie en de spin-off studies. Als enig deelnemend perifeer ziekenhuis hebben jullie een zeer groot deel van de patiënten data verzameld. Daarvoor ben ik ook mijn dank verschuldigd aan Annie Antonissen, Geert van de Sanden, Melanie Srodzinsky, en Laura Verputten.

Marlene Meester: Beste Marlene, jouw jarenlange ervaring in de infectiepreventie is zeer waardevol gebleken. Zo had jij ook ‘antieke’ proefschriften over stafylokokken in jouw bezit, welke mij een historisch perspectief ten aanzien van dit onderzoek hebben gegeven. Ook Joke van Wegen wil ik hier bedanken voor haar inzet voor de MUP-studie.

Wilma Kraak: Beste Wilma, jij was de stille kracht uit Nijmegen. Hoofdzakelijk in je eentje heb jij honderden patiënten ‘geronseld’ voor de MUP-studie en duizenden formulieren ingevuld. Grandioos!

Roel Verkooyen: Beste Roel, dankjewel voor jouw onmisbare hulp bij het ontwikkelen van de ‘case record forms’ die door een computer konden worden ingelezen. Ik zou niet weten hoe ik zonder jouw hulp 25.000 formulieren in een database zou hebben gekregen. Je know-how wordt nu weer gebruikt voor de vervolg studie.

Marius Vogel en Arjen van Vliet: Beste Marius en Arjen, de computer experts, wat moet onze afdeling zonder jullie? Verloren bestanden of ‘verzoekjes’ worden door jullie ergens uit een server tevoorschijn getoverd. Geweldig! Dank dank dank!

Wytske Fokkens: Beste Wytske, ik mis je hier wel in Rotterdam. Waren we eindelijk op dreef met het onderzoek, ging je naar Amsterdam! Met plezier denk ik terug aan onze neuspeuter brainstorm sessies op jouw oude kamer in de ‘ivoren toren’.

Willem van Leeuwen: Beste Willem, jouw MLST-chip voorwerk kwam goed van pas. Hele stammencollecties zijn door deze chip geanalyseerd, waaronder de MUP collectie, met leuke resultaten. Jammer dat het maken van een eigen virulentie-chip met Susan is mislukt om de karakterisering van invasieve versus niet invasieve stammen te completeren.

Susan Snijders: Beste Susan, jouw handen zijn onmisbaar gebleken, onder andere voor het ‘chippen’. Samen hebben we nog kunnen ploeteren met een virulentie-chip, echter zonder resultaat. Volgende keer beter met een gekochte chip.

Hélène Boelens: Beste Hélène, zonder jouw hulp zou ik heel wat minder artikelen hebben geproduceerd en pas jaren later zijn gepromoveerd. Honderden stammen heb jij met verschillende apparaten geanalyseerd. Veel dank.

Jan Nouwen, beste Jan, dank je wel voor het helpen schrijven van het protocol voor de MUP-studie (7 jaar geleden alweer). Uniek dat wij, na zeven jaar werken op dezelfde afdeling en aan hetzelfde micro-organisme, nul publicaties samen hebben. Wellicht komt daar binnenkort verandering in.

Marian Humphrey and Paula Jansen: lieve dames, dank voor al jullie steun en het mij wegwijs maken in de administratieve wirwar van het Erasmus MC.

Verder wil ik graag drie medische studenten bedanken voor hun hulp: Menno van Kleef, Jeroen Verveer en Jilling Bergman. Beste Menno, jij nam het werk wel heel serieus door neuswattenstokken als corsages op een studentengala uit te delen. Beste Jilling, bedankt voor de eerste typeringen en opzet database die hebben geleid tot het 'relatief risico' artikel. Beste Jeroen, het kweken van bijna tweehonderd studenten was een hele klus die je toch mooi hebt geklaard.

Unit Infectiepreventie: Myra, Cindy, Carol, Marja, en Gerard. Dank voor het includeren van patiënten op dagen dat ik met vakantie of cursus was (niet te vaak?). Margreet Filius, als enige niet-hygiënist, deed ook mee aan deze pool. Succes met het afronden van je boekje! Myra Behrendt wil ik nog in het bijzonder bedanken voor het mij wegwijs maken in de wereld van infectiepreventie en sepsis registratie. Cindy van Pelt en Peter de Man wil ik extra bedanken voor al het werk rondom het ontwikkelen en valideren van de MRSA bouillon.

Verder heeft iedereen van de afdeling Medische Microbiologie en Infectieziekten wel een keer op persoonlijke wijze een bijdrage geleverd aan mijn onderzoek: stafleden, analisten, en arts-assistenten. Bedankt! Nogmaals wil ik alle analisten bedanken voor het verwerken van al die duizenden neuswattenstokken en het bewaren van de 'kokken uit de gokken'.

Wim Ang: Beste Wim, paranimf, dank je wel voor het kritisch lezen van bijna al mijn manuscripten. Heerlijk hoe jij genadeloos strepen zet door hele alinea's.
Bernard Jan Verkoren, de andere paranimf, en dat zegt genoeg. L'Chaim!

Lieve ouders, dank voor een heerlijke jeugd en al jullie onvoorwaardelijke steun daarna. De gevleugelde woorden van vroeger: 'heb je snot, doe het vlug in een pot', neem ik nog steeds letterlijk.

Lieve Sigrid, met jou is het leven *vurrukkulluk*.
Lieve Lara en Peer, nu hebben jullie ook een boekje van papa voor het 'slapen gaan'.

Heiman

CURRICULUM VITAE

DEN HAAG

Zeeheldenkwartier wil einde last hondenpoep

door
Maarten Brakema

DEN HAAG | Een groep burgers uit het Haagse Zeeheldenkwartier wil dat de gemeente de overlast door hondenpoep veel harder gaat aanpakken. De werkgroep 'Stront aan de Knikker' heeft het afgelopen jaar duizend handtekeningen verzameld en hoopt via een burgerinitiatief de gemeente tot actie te dwingen.

De handtekeningen zijn inmiddels overhandigd aan de griffie van de gemeenteraad. Het presidium – het bestuur van de raad – moet beoordelen of het voorstel van het comité in aanmerking komt om te worden besproken door de politiek. De bewoners van het Zeeheldenkwartier willen dat het 'bestaande Haagse hondenpoepbeleid daadkrachtig en consistent' wordt gehandhaafd in hun wijk. 'Met als doel hondenpoepoverlast – ergernis nummer 1 – uit te bannen'. Het plan om de overlast van hondenpoep te beperken past binnen een reeks plannen in korte tijd. Zo werden ook burgerinitiatieven ontplooid om de sloop van de Zwarte Madonna tegen te houden, om een popmuseum in Den Haag te realiseren en om het openbaar vervoer gratis te maken. 'Stront aan de Knikker' vindt dat bij succes in het Zeeheldenkwartier de maatregelen ook in andere buurten moeten worden ingevoerd.

Bewoners wijzen erop dat de gemeente al regels heeft opgesteld die ervoor moeten zorgen dat de overlast door poep wordt beperkt. Zo zijn hondenbezitters nu al verplicht om om hun dier aan de lijn te houden, behalve op daarvoor aangewezen plekken. Daarnaast moeten de eigenaars de stront van hun dier opruimen, ook weer met uitzondering van aangewezen plekken. Maar, constateert het comité, de regels hebben geen effect. "Bezitters van loslopende honden hebben de gewoonte hun hond uit te laten zonder acht te slaan op waar hun hond zijn behoefte doet." Daarom wil 'Stront aan de Knikker' dat de gemeente nu 'gedegen' voorlichting gaat geven over de regels. Daarnaast moeten deze 'strikt' worden gehandhaafd. Overtreders dienen te worden geconfronteerd met lik-op-stukbeleid. "Politie en handhavingsteams moeten worden geïnstrueerd om mensen die hun hond niet aanlijnen of de hondenpoep niet opruimen een boete te geven. Gedogen of waarschuwen is er niet meer bij", aldus de bewoners van het Zeeheldenkwartier. Ook dienen er voldoende vuilnisbakken te komen voor hondenpoepzakjes en moet er een onderzoek komen naar de uitbreiding van het aantal uitlaatplekken. 'Stront aan de Knikker' schat dat de totale kosten van het voorstel 21.000 euro bedragen. Daar staat tegenover dat er meer boetes worden uitgedeeld en de gemeente ook inkomsten heeft uit de hondenbelasting.

Den Haag telt naar schatting 15.000 hondenbezitters. Volgens de initiatiefnemers moet het hondenpoepprobleem bij de wortel worden aangepakt: bij de hondenbezitter dus. De boodschap moet volgens hen zijn: 'uw hond, uw stront.

Haagsche Courant, 17 februari 2005

CURRICULUM VITAE

Personalia:

Naam	Wertheim, Heiman Frank Louis
Geboortedatum	7 december 1970 te ‘s-Gravenhage
Nationaliteit	Nederlandse
Burgerlijke staat	Gehuwd met Sigrid Heck
Kinderen	Lara (30-11-2001) en Peer (21-11-2004)

Opleiding:

1998-2005	AGIKO medische microbiologie, Erasmus MC, Rotterdam.
1998-2002	Klinische epidemiologie, Netherlands Institute for Health Sciences, Rotterdam.
1989-1997	Geneeskunde, Rijksuniversiteit Leiden.
1985-1989	International school, Hilversum, Nederland.
1983-1985	Curundu Junior High School, Panama.
1982-1983	Collegio Episcopal, Panama.

Werk ervaring

Vanaf juli 2005	Arts-microbioloog, Erasmus MC, Rotterdam.
2004	Waarnemend arts-microbioloog als senior arts-assistent, RDGG, Delft.
1998-2005	AGIKO medische microbiologie, Erasmus MC, Rotterdam.
1997-1998	AGNIO pediatrische intensive care, Sophia Kinderziekenhuis, Rotterdam.
1992-1995	Coördinator weefseltransplantaties; Bio Implant Services / Eurotransplant, Leiden.

Onderzoek

1998-2005	Promotieonderzoek. ‘*Staphylococcus aureus* infections. Lead by the nose.’ Erasmus MC, Rotterdam (verdediging op 17 juni 2005).
2001	Proteomics of *Staphylococcus aureus* nasal carriage, UCLA, Los Angeles, U.S.A.
1998	Septische complicaties van enteraal versus parenteraal gevoede neonaten aan de hart-long machine, Sophia Kinderziekenhuis, Rotterdam.
1995	Testen nieuwe bottransplantaten in ratten, Osteotech Inc., New Jersey, U.S.A.
1994-1995	Preventie van bacteriële contaminatie van non-heart beating donor hartkleppen, Hartkleppenbank, Rotterdam.

Cursussen

2004	Management Cursus, St. Management Scholing Medische Specialisten, Utrecht.
2003	Molecular Medicine, Josephine Nefkens Institute, Rotterdam.
2003	Medical Mycology, Centraalbureau voor Schimmelcultures, Utrecht.
2002	Virologie, Erasmus MC, Rotterdam.
2002	Medische Parasitologie, Boerhaave Commissie, Leiden.
2001	(Na)scholingscursus Infectieziekten. Boerhaave Commissie, Leiden.

Nevenactiviteiten:

2004-heden	Lid werkgroep "MRSA in de openbare gezondheidszorg", Landelijk Coördinatiecentrum Infectieziekten (LCI).
2004-heden	Redactielid Nederlands Tijdschrift voor Medische Microbiologie.
2003-2004	Organisatie eerste "get out of your lab days", Molecular Medicine Postgraduate School, Rotterdam.
2003-2004	Lid Postdoctorale student commissie Molecular Medicine Postgraduate School, Rotterdam.
2001-2003	Lid Visitatie Commissie voor de opleiding Medische Microbiologie.
2001-2003	Lid Concilium Microbiologicum.
2001-2003	Voorzitter Nederlandse Vereniging voor Arts-assistenten Medische Microbiologie (NVAMM).
2000-2001	Bestuurslid NVAMM.
1996-1997	Bestuurslid Leidse Vereniging van Co-assistenten.

Wetenswaardigheden:

2004-heden	Oprichter werkgroep 'Stront aan de Knikker', aanpak hondenpoep overlast Den Haag.
2004-heden	Fietser ROPARUN, Parijs-Rotterdam.
1999-heden	Drumles van de 'beste drumleraar in de Benelux'.
1994-1995	Producent musical *Anatevka, Fiddler on the Roof*; acht voorstellingen in de Leidse Schouwburg.