Prevention of Healthcare Associated *Staphylococcus aureus* Infections

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Prevention of Healthcare Associated *Staphylococcus aureus* Infections

Preventie van ziekenhuisgerelateerde *Staphylococcus aureus* infecties

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Contents

Chapter 1

General introduction

General introduction

Staphylococcus aureus (S. aureus) belongs to the phylum *Firmicutes* (Latin: *firmus,* strong, and *cutis,* skin, referring to the cell wall). In 1880 in Aberdeen, Scotland, Sir Alexander Ogston was the first to identify Staphylococci in pus from a surgical abscess in a knee joint, which he described as micrococci with an aspect of grape clusters.[1] Rosenbach in 1884 gave the bacterium its name as we nowadays know it: *Staphylococcus* (from the Greek word *Staphylé,* a bunch of grapes) *aureus* (Latin: golden, the colour of the colonies on solid culture media).

Carriage of S. aureus

The human nose harbours many bacteria, predominantly gram-positive cocci.[2] Carriage of these bacteria is usually not of clinical importance. *S. aureus* however, is a more virulent organism than the other gram-positives. It colonizes the skin and mucosae of only a proportion of the general population. The nose is the primary niche, but other body sites are frequently colonized as well.[3,4] It is not fully understood why only a proportion of the human population is colonized with *S. aureus*. Probably, host factors and bacterial factors both play a role and co-determine the *S. aureus* carrier state.[5, 6]

Individuals who consistently carry *S. aureus* are termed persistent carriers; those who experience periods of carriage alternating with periods of non-carriage are termed intermittent carriers.[7] A consistent definition of carrier states does not exist, and as a result, reported carriage rates may vary.[8] In healthy subjects, carriage rates are approximately 20% (range 12-30%) for persistent carriage, about 30% (range 16-70%) for intermittent carriage, and about 50% (range 16-69%) for non-carriage.[9] However, the existence of the intermittent carriage state as a single entity has been subject of discussion. Based on similar antibody profiles and the comparable risk of developing *S. aureus* infections, it is now believed that intermittent carriers are actually non-carriers who transiently contracted *S. aureus*, which was accidentally detected at the moment of nasal screening.[10, 11] This assumption is supported by studies showing that intermittent carriers usually return back into their non-carriage state by themselves after artificial inoculation of *S. aureus* into their nose.[12, 13] This probably imitates the real-life situation in intermittent carriage. The distinction between the different carrier states is of clinical importance, since persistent carriers have higher numbers of staphylococci in their noses, and are at higher risk of infection than non-carriers.[14] Therefore, a test to assess *S. aureus* nasal carriage should detect high-level (persistent) carriers, and not low-level carriers.

Elimination of *S. aureus* nasal carriage can be achieved by a course of mupirocin, a topical antibiotic that has to be administered to the nose.[15, 16] This strategy also eradicates *S. aureus* from extranasal body sites, although less effective.[17, 18] Nevertheless, the effect of a five-day course is often only temporary.[15, 16] Recolonization occurs in a large proportion of decolonized subjects, either with the same isolate that resided in the nose before treatment, or with another strain.[15,16] The latter may be the result of transfer from extranasal sites or the environment.[18]

S. aureus and the immune system

The core genome of *S. aureus* makes up approximately 75% of the total *S. aureus* genome. The accessory genome accounts for the other 25%, consisting of mobile genetic elements (MGE), of which many encode for virulence or resistance functions.[19] There is also a marked difference in gene expression between strains, resulting in a large difference in the secretion of proteins on the surface of *S. aureus*.[20] This leads to a highly heterogeneous pathogenic behaviour.[20, 21] *S. aureus* can produce a wide variety of diseases, ranging from relatively benign folliculitis to severe and life-threatening sepsis and endocarditis. Infections can be of endogenous origin, i.e. caused by a strain carried by the patient; or exogenous, i.e. caused by a strain that was not residing on the skin or mucosa of the patient before. *S. aureus* colonization is thought to be the start of the sequence of events leading to infection in the majority of cases, thereby causing endogenous infections.[22] Infections often arise from a breach of the skin or mucosa, although community acquired bacteremia frequently develops in the absence of a primary focus of infection.[23] Whether an infection is contained and leads to, for example, skin and soft tissue infections, or spreads to the bloodstream, depends on strain and host factors.[24]

The innate immune system plays an important role in the host defense against *S. aureus* infections, supported by the adaptive (humoral) immune system.[25, 26] Many different anti-staphylococcal antibodies can be produced, directed against the numerous different bacterial components of *S. aureus*. The levels of antistaphylococcal antibodies differ greatly between healthy individuals.[26, 27] They also differ significantly between carriers and non-carriers of *S. aureus*.[26, 28, 29] The latter observation may explain the observed better outcome of *S. aureus* bacteremia in carriers compared to non-carriers.[30] Carriers could have a more adequate immune response to infection, as they could be immunologically adapted to the strain carried in their nose.[30]

Healthcare associated infections

The aim of hospital admissions generally is to cure, or at least to support the patient, without doing any harm. Nevertheless, hospital admissions pose patients to the risk of complications of medical interventions. One of these complications is the development of healthcare associated infections. After urinary tract infections and lower respiratory tract infections, surgical site infections are the third most frequently reported healthcare associated infections.[31] Surgical site infections may involve only the skin and subcutaneous tissue (superficial SSI), or may involve deeper soft tissues (deep SSI).[32] *Staphylococcus aureus* is the leading cause of surgical site infections, accounting for approximately 20% of these infections.[31, 32] Bloodstream infections are also frequently caused by *S. aureus.*[31]

Healthcare associated infections lead to increased morbidity, mortality, length of stay, and hospital costs.[33] Societal costs rise as well, and consequences of infections for patients can be devastating, for example in the case of infection of prosthetic joint infections, due to permanent functional impairment.[34, 35]

The risk of developing healthcare associated infections is influenced by a number of factors. Patient characteristics include age, nutritional status, diabetes, and many more. For surgical patients, operation characteristics that influence the risk of surgical site infections are, for example, the duration of the procedure, administration of antibiotic prophylaxis, foreign material in the surgical site, the surgical technique used, and general infection prevention measures.[32]

In 1959, Weinstein reported that *S. aureus* nasal carriers are at increased risk of developing "infectious complications", mainly attributable to *S. aureus*.[36] A review of the literature in 1995 showed that the incidence of *S. aureus* wound infections in nasal carriers of *S. aureus* ranged from 5 to 19%, while in non-carriers, the reported incidence was 2-10%.[22] Also, *S. aureus* infections in patients on continuous ambulatory peritoneal dialysis (CAPD) and on hemodialysis occur significantly more frequently in carriers than in non-carriers.[37-39] Approximately 80% of healthcare associated infections (range 30-100%) are of endogenous origin, i.e. caused by the patient's own *S. aureus* strain.[22, 30, 37, 40]

Methicillin-resistant Staphylococcus aureus (MRSA)

Beta-lactam antibiotics are the treatment of first choice for *S. aureus*. They interact with penicillin-binding proteins (PBPs) present in the bacterial cell wall, thereby halting the growth of bacteria. Already soon after the introduction of penicillin and methicillin, resistance to these

antibiotics was reported.[41] Methicillin resistance is mediated by *mecA,* a horizontally acquired gene which encodes for penicillin-binding protein 2A (PBP2A)[42]. PBP2A has a low affinity for methicillin and most other beta-lactam drugs, resulting in antibiotic class resistance to penicillins, cephalosporins, and carbapenems.[41, 43] Resistance to this class of antibiotics is therefore highly problematic, making therapy for infections more difficult.[24] Furthermore, many MRSA strains are resistant to other classes of antibiotics as well. Compared to MSSA, infections with MRSA are associated with increased morbidity and mortality, and add to the total number of healthcare associated infections.[44, 45] Therefore, it is important to keep the prevalence of MRSA as low as possible. Nevertheless, MRSA is endemic in many hospitals worldwide.[46] Except for the Nordic countries and The Netherlands, the prevalence of MRSA is at least 5%, with reported frequencies over 50%.[46] Thus, only a few countries in the world have successfully controlled MRSA levels. Measures that these countries have taken from the start of the emergence of resistance, are stringent barrier precautions, cultures of patients and personnel to identify sources of transmission, and pre-emptive isolation of patients who are at high risk of MRSA colonization until confirmed to be MRSA negative. [47] Furthermore, these countries advocate a stringent policy for the use of antibiotics.[47] These measures have kept the proportion of *S. aureus* bloodsteam isolates that are methicillin resistant in Denmark, Sweden, Norway and The Netherlands below 1.5%.[48] In The Netherlands, this Search-and-Destroy policy is defined by the Dutch Working Party on Prevention of Infections (Werkgroep Infectiepreventie, WIP).[49] To avert an endemic situation, it is important to continuously update the guidelines for this policy, and to monitor the levels of MRSA in the population.

Aim and outline of this thesis

The general aim of this thesis is to add to the prevention of healthcare associated *Staphylococcus aureus* infections. Carriers of *S. aureus* have a higher risk of developing a healthcare associated infection with *S. aureus* than non-carriers, and the majority of these infections are of endogenous origin. In **Chapter 2**, we aim to prevent endogenous infections by decolonization of *S. aureus* carriers. In a randomized, placebo-controlled multicenter trial, patients are screened for *S. aureus* carriage with rapid diagnostics upon admission. Carriers are treated with a five-day course of mupirocin nasal ointment and chlorhexidine gluconate medicated soap, or placebo ointment and placebo soap, starting within 24 hours after admission to hospital. The primary outcome is the incidence of healthcare associated *S. aureus* infections. In this trial, non-surgical as well as surgical patients were enrolled. In **Chapter 3** we explore which groups of patients benefit most from a screen-and-treat strategy, by comparing mortality rates of both treatment groups (mupirocin and chlorhexidine versus placebo) in surgical patients, as well as in different surgical

subgroups. In **Chapter 4** we establish the mean hospital costs for cardiothoracic and orthopaedic surgery patients who undergo the screen-and-treat strategy, and compare those to patients who are not screened nor treated. In **Chapter 5** we show the amounts of mupirocin that have been dispersed to the clinical wards of the Erasmus MC in the past ten years, and the proportion of mupirocin resistant *S. aureus* and coagulase negative staphylococci isolated from patients on these wards.

In **Chapter 6**, we aim to gain more insight into the epidemiology of endogenous and exogenous healthcare associated *S. aureus* infections. We assess the proportion of non-carriers developing a *S. aureus* infection. Furthermore, we compare nasal and infecting isolates by using Raman spectroscopy as a typing method, to identify possible transmission, and to assess the proportions of exogenous and endogenous *S. aureus* infections in carriers. In **Chapter 7**, the immune response and the infecting strains of twelve patients with *S. aureus* bacteremia are analyzed in detail. The extracellular proteins of the infecting strain are identified and the developing antibody reponse is studied, to compare the immune response in carriers and noncarriers of *S. aureus*.

Finally, in **Chapter 8**, the prevalence of methicillin-resistant *S. aureus* (MRSA) carriage in patients upon admission to hospital in 2005-2007 is reported. The results of these studies, as well as future perspectives, are discussed in **Chapter 9.**

References

1. Ogston A. Report upon Micro-Organisms in Surgical Diseases. Br Med J. 1881; **1**(1054): 369 b2-75.
2. Stefansdottir A, Johansson A, Lidgren L, Wagner P, A WD. Bacterial colonization and resistance patterns in 133 patients undergoing a primary hip- or knee replacement in Southern Sweden. Acta Orthop. 2013; **84**(1): 87-91.
3. Solberg CO. A study of carriers of *Staphylococcus aureus* with special regard to quantitative bacterial estimations. Acta Med Scand Suppl. 1965; **436**: 1-96.
4. White A, Smith J. Nasal Reservoir as the Source of Extranasal Staphylococci. Antimicrobial Agents Chemother (Bethesda). 1963; **161**: 679-83.
5. Peacock SJ, de Silva I, Lowy FD. What determines nasal carriage of Staphylococcus aureus? Trends in microbiology. 2001; **9**(12): 605-10.
6. Nouwen JL, van Belkum A, Verbrugh HA. Determinants of Staphylococcus aureus nasal carriage. Neth J Med. 2001; **59**(3): 126-33.
7. Nahmias AJ, Eickhoff TC. Staphylococcal infections in hospitals. Recent developments in epidemiologic and laboratory investigation. N Engl J Med. 1961; **265**: 177-82 concl.
8. VandenBergh MF, Yzerman EP, van Belkum A, Boelens HA, Sijmons M, Verbrugh HA. Follow-up of Staphylococcus aureus nasal carriage after 8 years: redefining the persistent carrier state. Journal of clinical microbiology. 1999; **37**(10): 3133-40.
9. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 2005; **5**(12): 751-62.
10. van Belkum A, Verkaik NJ, de Vogel CP, Boelens HA, Verveer J, Nouwen JL, et al. Reclassification of Staphylococcus aureus nasal carriage types. J Infect Dis. 2009; **199**(12): 1820-6.
11. Nouwen JL, Fieren MW, Snijders S, Verbrugh HA, van Belkum A. Persistent (not intermittent) nasal carriage of Staphylococcus aureus is the determinant of CPD-related infections. Kidney Int. 2005; **67**(3): 1084-92.
12. Ehrenkranz NJ. Person-to-Person Transmission of Staphylococcus Aureus. Quantitative Characterization of Nasal Carriers Spreading Infection. N Engl J Med. 1964; **271**: 225-30.
13. Nouwen J, Boelens H, van Belkum A, Verbrugh H. Human factor in Staphylococcus aureus nasal carriage. Infect Immun. 2004; **72**(11): 6685-8.
14. White A. Increased infection rates in heavy nasal carriers of coagulase-positive staphylococci. Antimicrobial Agents Chemother (Bethesda). 1963; **161**: 667-70.
15. Casewell MW, Hill RL. Elimination of nasal carriage of Staphylococcus aureus with mupirocin ('pseudomonic acid')--a controlled trial. J Antimicrob Chemother. 1986; **17**(3): 365-72.
16. Bulanda M, Gruszka M, Heczko B. Effect of mupirocin on nasal carriage of Staphylococcus aureus. J Hosp Infect. 1989; **14**(2): 117-24.
17. Reagan DR, Doebbeling BN, Pfaller MA, Sheetz CT, Houston AK, Hollis RJ, et al. Elimination of coincident Staphylococcus aureus nasal and hand carriage with intranasal application of mupirocin calcium ointment. Ann Intern Med. 1991; **114**(2): 101-6.
18. Wertheim HF, Verveer J, Boelens HA, van Belkum A, Verbrugh HA, Vos MC. Effect of mupirocin treatment on nasal, pharyngeal, and perineal carriage of Staphylococcus aureus in healthy adults. Antimicrob Agents Chemother. 2005; **49**(4): 1465-7.
19. Lindsay JA, Holden MT. Staphylococcus aureus: superbug, super genome? Trends in microbiology. 2004; **12**(8): 378-85.
20. Ziebandt AK, Kusch H, Degner M, Jaglitz S, Sibbald MJ, Arends JP, et al. Proteomics uncovers extreme heterogeneity in the Staphylococcus aureus exoproteome due to genomic plasticity and variant gene regulation. Proteomics. 2010; **10**(8): 1634-44.

21. Lindsay JA, Holden MT. Understanding the rise of the superbug: investigation of the evolution and genomic variation of Staphylococcus aureus. Functional & integrative genomics. 2006; **6**(3): 186-201.
22. Wenzel RP, Perl TM. The significance of nasal carriage of Staphylococcus aureus and the incidence of postoperative wound infection. J Hosp Infect. 1995; **31**(1): 13-24.
23. Finkelstein R, Sobel JD, Nagler A, Merzbach D. Staphylococcus aureus bacteremia and endocarditis: comparison of nosocomial and community-acquired infection. Journal of medicine. 1984; **15**(3): 193-211.
24. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998; **339**(8): 520-32.
25. Fournier B, Philpott DJ. Recognition of Staphylococcus aureus by the innate immune system. Clin Microbiol Rev. 2005; **18**(3): 521-40.
26. Dryla A, Prustomersky S, Gelbmann D, Hanner M, Bettinger E, Kocsis B, et al. Comparison of antibody repertoires against Staphylococcus aureus in healthy individuals and in acutely infected patients. Clin Diagn Lab Immunol. 2005; **12**(3): 387-98.
27. Holtfreter S, Nguyen TT, Wertheim H, Steil L, Kusch H, Truong QP, et al. Human immune proteome in experimental colonization with Staphylococcus aureus. Clin Vaccine Immunol. 2009; **16**(11): 1607-14.
28. Verkaik NJ, de Vogel CP, Boelens HA, Grumann D, Hoogenboezem T, Vink C, et al. Anti-staphylococcal humoral immune response in persistent nasal carriers and noncarriers of Staphylococcus aureus. J Infect Dis. 2009; **199**(5): 625-32.
29. Holtfreter S, Roschack K, Eichler P, Eske K, Holtfreter B, Kohler C, et al. Staphylococcus aureus carriers neutralize superantigens by antibodies specific for their colonizing strain: a potential explanation for their improved prognosis in severe sepsis. J Infect Dis. 2006; **193**(9): 1275-8.
30. Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, et al. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet. 2004; **364**(9435): 703-5.
31. Emori TG, Gaynes RP. An overview of nosocomial infections, including the role of the microbiology laboratory. Clin Microbiol Rev. 1993; **6**(4): 428-42.
32. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. Infect Control Hosp Epidemiol. 1999; **20**(4): 250-78; quiz 79-80.
33. Salehi Omran A, Karimi A, Ahmadi SH, Davoodi S, Marzban M, Movahedi N, et al. Superficial and deep sternal wound infection after more than 9000 coronary artery bypass graft (CABG): incidence, risk factors and mortality. BMC Infect Dis. 2007; **7**: 112.
34. Perencevich EN, Sands KE, Cosgrove SE, Guadagnoli E, Meara E, Platt R. Health and economic impact of surgical site infections diagnosed after hospital discharge. Emerg Infect Dis. 2003; **9**(2): 196-203.
35. Barrack RL, Engh G, Rorabeck C, Sawhney J, Woolfrey M. Patient satisfaction and outcome after septic versus aseptic revision total knee arthroplasty. J Arthroplasty. 2000; **15**(8): 990-3.
36. Weinstein HJ. The relation between the nasal-staphylococcal-carrier state and the incidence of postoperative complications. N Engl J Med. 1959; **260**(26): 1303-8.
37. Yu VL, Goetz A, Wagener M, Smith PB, Rihs JD, Hanchett J, et al. Staphylococcus aureus nasal carriage and infection in patients on hemodialysis. Efficacy of antibiotic prophylaxis. N Engl J Med. 1986; **315**(2): 91-6.
38. Luzar MA, Coles GA, Faller B, Slingeneyer A, Dah GD, Briat C, et al. Staphylococcus aureus nasal carriage and infection in patients on continuous ambulatory peritoneal dialysis. N Engl J Med.

1990; **322**(8): 505-9.

39. Wanten GJ, van Oost P, Schneeberger PM, Koolen MI. Nasal carriage and peritonitis by Staphylococcus aureus in patients on continuous ambulatory peritoneal dialysis: a prospective study. Perit Dial Int. 1996; **16**(4): 352-6.
40. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. N Engl J Med. 2001; **344**(1): 11-6.
41. Chambers HF, Deleo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nature reviews. 2009; **7**(9): 629-41.
42. Ubukata K, Nonoguchi R, Matsuhashi M, Konno M. Expression and inducibility in Staphylococcus aureus of the mecA gene, which encodes a methicillin-resistant S. aureus-specific penicillin-binding protein. J Bacteriol. 1989; **171**(5): 2882-5.
43. Chambers HF, Hartman BJ, Tomasz A. Increased amounts of a novel penicillin-binding protein in a strain of methicillin-resistant Staphylococcus aureus exposed to nafcillin. J Clin Invest. 1985; **76**(1): 325-31.
44. Ammerlaan HS, Harbarth S, Buiting AG, Crook DW, Fitzpatrick F, Hanberger H, et al. Secular trends in nosocomial bloodstream infections: antibiotic-resistant bacteria increase the total burden of infection. Clin Infect Dis. 2013; **56**(6): 798-805.
45. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. Clin Infect Dis. 2003; **36**(1): 53-9.
46. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public-health threat. Lancet. 2006; **368**(9538): 874-85.
47. Muto CA, Jernigan JA, Ostrowsky BE, Richet HM, Jarvis WR, Boyce JM, et al. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of Staphylococcus aureus and enterococcus. Infect Control Hosp Epidemiol. 2003; **24**(5): 362-86.
48. Antimicrobial resistance surveillance in Europe 2011. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: European Center for Disease Prevention and Control; 2012.
49. WIP (Dutch Working Party on Infection Prevention) Policy for methicillin-resistant *Staphylococcus aureus*. 2012 [cited August 2013]; Available from: www.wip.nl

Chapter 2

Preventing healthcare associated infections in nasal carriers of Staphylococcus aureus

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Abstract

Background

Nasal carriers of *Staphylococcus aureus* are at increased risk for healthcare associated infections with this organism. Decolonization of nasal and extranasal sites on hospital admission may reduce this risk.

Methods

In a randomized, double-blind, placebo-controlled, multicenter trial, we assessed whether rapid identification of *S. aureus* nasal carriers by means of a real-time polymerase chain-reaction (PCR) assay, followed by treatment with mupirocin nasal ointment and chlorhexidine soap, reduces the risk of healthcare associated *S. aureus* infection.

Results

From October 2005 through June 2007, a total of 6771 patients were screened on admission. A total of 1270 nasal swabs from 1251 patients were positive for *S. aureus*. We enrolled 917 of these patients in the intention-to-treat analysis, of whom 808 (88.1%) underwent a surgical procedure. All the *S. aureus* strains identified on PCR assay were susceptible to methicillin. The rate of *S. aureus* infection was 3.4% (17 of 504 patients) in the mupirocin/chlorhexidine group, as compared with 7.7% (32 of 413 patients) in the placebo group (relative risk (RR) of infection, 0.42; 95% CI [0.23-0.75]). The effect of mupirocin/chlorhexidine treatment was most pronounced for deep surgical-site infections (RR 0.21; 95% CI [0.07-0.62]). There was no significant difference in all-cause in-hospital mortality between the two groups. The time to the onset of nosocomial infection was shorter in the placebo group than in the mupirocin/chlorhexidine group ($P = 0.005$).

Conclusions

The number of surgical-site *S. aureus* infections acquired in the hospital can be reduced by rapid screening and decolonizing of nasal carriers of *S. aureus* on admission.

(Current Controlled Trials number, ISRCTN56186788.)

Introduction

Nasal carriers of high numbers of *Staphylococcus aureus* organisms have a risk of healthcare associated infection with this microorganism that is three to six times the risk among non-carriers and low-level carriers.[1-3] More than 80% of healthcare associated *S. aureus* infections are endogenous.[4-6]

Intranasal application of mupirocin has been shown to be effective for the decolonization of this microbe and the prevention of invasive *S. aureus* infections in patients receiving long-term dialysis treatment.[7-10] However, in other nonsurgical patients, mupirocin had no effect on the rate of healthcare associated *S. aureus* infections.[11] Mupirocin nasal ointment was reported to be effective in preventing surgical-site infections in cardiothoracic surgery, but this study used a historical control group.[12] Two randomized, controlled trials failed to show a reduction in rates of surgical-site infection in orthopaedic and general-surgery populations, although a subgroup analysis in one of these studies suggested that intranasal mupirocin may be effective in preventing healthcare associated *S. aureus* infections in carriers of this organism.[13, 14]

Several explanations have been offered for these failures. In some studies, failure of decolonization may have been due to the timing of treatment. If decolonization is started only after the results of screening cultures become available, healthcare associated infections may already be incubating and may therefore be difficult to prevent. With the development of rapid screening tests for *S. aureus*, the carrier status can be assessed within hours after admission.[15-17] Another explanation could be that nasal carriers of *S. aureus* are also colonized at extra-nasal sites.[18] It is unlikely that nasal application of mupirocin will directly affect these sites. However, decolonization of the skin can be achieved by washing with disinfecting soap, such as chlorhexidine gluconate products.[19]

We conducted a randomized, double-blind, placebo-controlled, multicenter clinical trial in which we rapidly identified nasal carriers of *S. aureus* by real-time polymerase-chain-reaction (PCR) assay on admission. In *S. aureus* carriers only, we assessed whether decolonization of the nostrils with mupirocin ointment and of the skin with chlorhexidine gluconate soap could prevent healthcare associated infections with *S. aureus*.

Methods

Study design

The study was a randomized, double-blind, placebo-controlled clinical trial, conducted at three university hospitals and two general hospitals in The Netherlands. From October 2005 through June 2007, we screened patients who were admitted to the departments of surgery and internal medicine, where the risk for *S. aureus* infection is high. The primary outcome of the trial was the cumulative incidence of healthcare associated *S. aureus* infections. Secondary outcome measures included all-cause in-hospital mortality, duration of hospitalization, and time from admission to the onset of healthcare associated *S. aureus* infections. The institutional ethics committee at each center approved the protocol. Oral informed consent was obtained at the time of screening. Once a patient was randomly assigned to decolonization with either mupirocin and chlorhexidine or placebo, written informed consent was obtained. The manufacturers of the products provided the trial medications and placebo at no cost but did not influence the study design, data collection, analysis, writing, or decision to submit the results for publication.

Inclusion and exclusion criteria

Patients were screened by trained nursing staff for nasal carriage of *S. aureus* either immediately on admission or during the week before admission, with decolonization therapy begun at the time of admission. The inclusion criterion for screening was the expectation that a patient would remain hospitalized for at least 4 days in one of the participating departments (internal medicine, cardiothoracic surgery, vascular surgery, orthopaedics, gastrointestinal surgery, or general surgery). The exclusion criterion for screening was an age of less than 18 years. Inclusion criteria for randomization were nasal carriage of *S. aureus* as determined by real-time PCR and the ability to start the intervention within 24 hours after the patient's admission to a participating ward. The expected duration of hospitalization was estimated again immediately before randomization and had to be at least 4 days. Exclusion criteria for randomization were the presence of active infection with *S. aureus* at the time of randomization, known allergy to mupirocin or chlorhexidine, pregnancy, breast-feeding, use of mupirocin in the preceding four weeks, and the presence of a nasal foreign body.

Randomization

Patients were randomly assigned in a 1:1 ratio to either active treatment with mupirocin ointment 2% (Bactroban, GlaxoSmithKline) in combination with chlorhexidine gluconate soap, 40 mg per milliliter (Hibiscrub, Mölnlycke), or placebo ointment in combination with placebo soap. Placebo soap and ointment were identical to the active treatment except for the active ingredients. A single list of random numbers with a permuted-block design was generated by an independent statistician and distributed to all participating centers.

Enrollment and follow-up

Patients were asked to participate by a member of the trial team. Immediately after providing written informed consent, the patient was assigned to either the active treatment or the placebo according to the randomization list, and the first dose of nasal ointment was applied. Nasal ointment was applied twice daily, and the soap was used daily for a total-body wash. The duration of the study treatment was 5 days, irrespective of the timing of any interventions. Patients who were still hospitalized after 3 weeks and those still hospitalized after 6 weeks received a second and third course of the same trial medication, respectively.

The follow-up period for *S. aureus* infection was the first 6 weeks after discharge. We defined the time to infection as the time from randomization to the onset of infection. Data were censored when follow-up for *S. aureus* infection ended or at the time of death. Time periods for the end points of length of hospital stay and mortality were measured from the primary admission until 6 weeks after discharge from the primary admission. If a patient was readmitted to the hospital within 6 weeks after discharge from the primary admission, the number of hospital days during the subsequent admission was included in the calculation of length of stay.

Patients were monitored for healthcare associated *S. aureus* infection by means of microbiologic cultures. Attending physicians were encouraged to obtain culture samples if infection was suspected. If a culture grew *S. aureus*, the patient's medical record was reviewed to distinguish infection from colonization and to determine whether the infection was healthcare associated according to criteria established by the Centers for Disease Control and Prevention.[20] The results of all clinical cultures performed during the follow-up period were also documented. In surgical patients, standard presurgical prophylactic antimicrobial therapy was given according to the local hospital guidelines.

Microbiologic results

To screen patients for *S. aureus* carriage, a dry, sterile rayon swab (Becton Dickinson) was rotated four times in each nostril. The swab was placed in 100 µl of saline and centrifuged. Part of the sample was processed for real-time PCR. For DNA extraction, the S.E.T.S. II kit (Roche Diagnostics, Almere, The Netherlands) was used. DNA amplification and detection were performed with the LightCycler Staphylococcus kit® (for research use only, Roche Diagnostics, Almere, The Netherlands) as recommended by the manufacturer. A peak-height cut-off of the *S. aureus* meltingcurve was used to avoid false-positive results. High numbers of *S. aureus* (>100 CFU in the sample) are detected reliably with this kit with a sensitivity of approximately 97%. Ten microliters of the remaining sample was inoculated onto a blood agar plate and incubated for 48 hours. After processing, the swab itself was placed in phenol-red mannitol salt broth (PHMB) and incubated for three days. If the directly inoculated blood agar plate showed no growth, the PHMB was subcultured onto a second culture plate. Identification of *S. aureus* was performed by a latex agglutination test (Slidex, BioMérieux, France). Culture results were not used to assess eligibility for randomization. Cultured strains were genotyped by means of pulsed-field gel electrophoresis to allow nasal and infecting strains to be compared, in order to determine whether an infection was endogenous or exogenous, and results were evaluated according to standard criteria.[21]

Statistical analysis

On the basis of previous studies, the estimated cumulative incidence of healthcare associated *S. aureus* infections in carriers of *S. aureus* is 6%. We originally planned to enroll 1800 subjects for randomization to achieve a power of 80% with a two-tailed type I error rate of 0.05 and a reduction of 50% in healthcare associated *S. aureus* infections. After 860 patients had been enrolled, a perceived change in the cumulative incidence of serious *S. aureus* infections was reported in one of the participating centers. On request, the institutional ethics committee at each center approved a sequential analysis of the accumulated data set by an independent statistician. A group sequential analysis was conducted as a double triangular test on the cumulative dataset by sequentially entering data in the statistical program PEST4 of each consecutively enrolled group of 100 patients.[22, 23] Assumptions regarding the a priori incidence of healthcare associated *S. aureus* infections, the expected effect size, the type I error rate, and power were identical to the original design of the study. The assumptions determine the boundaries of the triangular test, as depicted in Figure 2. Analysis of the data from the first 400 patients showed that the upper boundary was crossed; thus, there was sufficient evidence to conclude that the difference in outcomes between the two study groups was significant. At the time of the sequential analysis,

additional patients had already undergone randomization, and the data for these patients were added to the final analysis. The analysis was stratified according to center and was based on the intention-to-treat principle. The relative risk for healthcare associated infections was calculated using PEST4, adjusted for multiple analyses on the same data, and for immunocompromised state.

Differences in patient characteristics and outcomes between the two trial groups were analyzed by a Chi square, Mann Whitney U, *t* test, log-rank test or regression analysis with the statistical program SPSS 15.0.

Results

Study population

In total, 6771 patients were screened for the presence of *S. aureus* in the nasal passages. Results were positive for *S. aureus* on real-time PCR in 1270 samples (18.8%) obtained from 1251 patients. Of the 918 patients who underwent randomization, one withdrew consent and was excluded from the analysis (Figure 1). Six patients in the mupirocin/chlorhexidine group and 11 patients in the placebo group received a second or third course of treatment. Table 1 shows the baseline characteristics of the patients.

Study outcomes

Figure 2 shows the results of the sequential analysis of the cumulative data. The data cross the upper boundary, indicating that there is sufficient evidence that the difference in outcome between the two treatment groups is significant ($P = 0.008$).

The cumulative incidence of healthcare associated *S. aureus* infection was significantly lower in the mupirocin/chlorhexidine group than in the placebo group (Table 2). Among the 917 patients who underwent randomization, 49 had healthcare associated *S. aureus* infections: 17 (3.4%) in the mupirocin/chlorhexidine group and 32 (7.7%) in the placebo group (relative risk (RR) with mupirocin/chlorhexidine, 0.42; 95% confidence interval (CI) [0.23-0.75]). In the sequential analysis we corrected for the imbalance between the groups with respect to the proportion of immunocompromised patients, but this did not affect the outcome. The number of patients who would need to be screened and the number of *S. aureus* carriers who would need to be treated to prevent one healthcare associated *S. aureus* infection were 122 and 23, respectively.

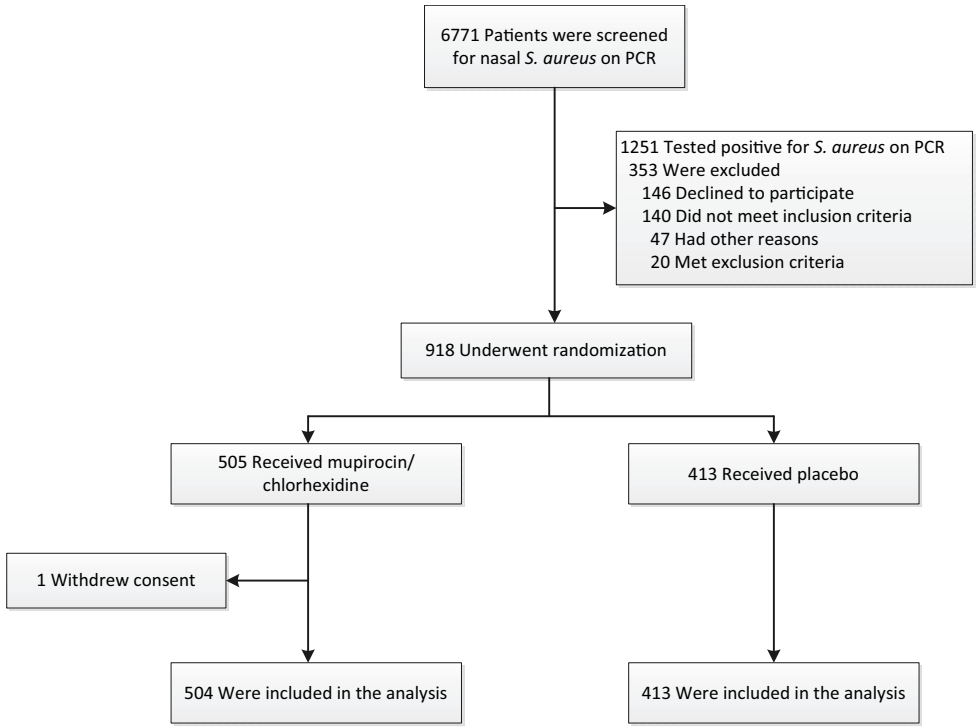

Figure 1. Study enrolment and randomization.
Of the 918 patients who underwent randomization, 1 was inadvertently assigned to treatment with mupirocin/ chlorhexidine despite a nasal swab that was negative for *S. aureus* on PCR assay.

Logistic-regression analysis showed no significant difference in the primary outcome between surgical and nonsurgical patients. The number of nonsurgical patients was small (109 of the 917 patients included in the analysis (11.9%)). Deep surgical-site infections were most frequent (Table 2). Among the surgical patients, this type of infection occurred significantly less frequently in the 441 patients in the mupirocin/chlorhexidine group than in the 367 patients in the placebo group (4 infections (0.9%) vs. 16 (4.4%); RR 0.21; 95% CI [0.07-0.62]). Outcomes for the surgical and nonsurgical patients are presented in Table 3.

Of the 49 strains causing infection, 47 were available for molecular typing to determine whether the infection had an endogenous or exogenous source. The results of molecular typing are shown in Table 2.

The time to infection with *S. aureus* was significantly shorter in the placebo group than in the mupirocin/chlorhexidine group ($P = 0.005$ by the log-rank test). Figure 3 shows the cumulative hazard of healthcare associated *S. aureus* infection in both study groups.

Characteristic	Mupirocin/ Chlorhexidine (N=504)	Placebo (N=413)	*P* value
Mean (±SD) age – yr	61.8 ± 13.9	62.8 ± 13.3	0.25
Male sex – no. (%)	331 (65.7)	251 (60.8)	0.13
Hospital service – no. (%)			
Surgery	441 (87.5)	367 (88.9)	0.53
Internal medicine	63 (12.5)	46 (11.1)	0.53
Admission during month before current admission – no./total no. (%)	86 (17.1)	67 (16.3)	0.76
McCabe score at admission*			
Median	1	1	
Inter quartile range	1 - 2	1 - 2	
Underlying disorder – no./total no. (%)			
Diabetes mellitus type I or II	112 (22.3)	71 (17.2)	0.06
Disorder requiring continuous ambulatory peritoneal dialysis	7 (1.4)	4 (1.0)	0.57
Renal insufficiency	24 (4.8)	23 (5.6)	0.57
Immunodeficiency	19 (3.8)	31 (7.5)	0.01
Liver-function disorder	25 (5.0)	22 (5.3)	0.80
Malignant condition	63 (12.5)	46 (11.2)	0.54
Skin disease	52 (10.4)	58 (14.2)	0.08
Antibiotic therapy – no./total no. (%)			
At time of admission	17 (3.4)	16 (3.9)	0.69
During month before admission	41 (8.2)	28 (6.9)	0.46

Table 1. Baseline characteristics of the 917 study patients.
*We used the McCabe score, as modified by Doern et al.,[24] to classify the severity of the underlying disease as follows: 1, nonfatal; 2, possibly fatal; 3, ultimately fatal; and 4, rapidly fatal.

The mean duration of hospitalization was significantly shorter in the mupirocin/chlorhexidine group than in the placebo group (crude estimate, 12.2 vs. 14.0 days; $P = 0.04$). Crude estimates of the median duration of hospitalization were 9 days in the mupirocin/chlorhexidine group and 10 days in the placebo group ($P = 0.08$). All-cause in-hospital mortality did not differ significantly between the groups (2.6% in the mupirocin/chlorhexidine group and 3.1% in the placebo group; relative risk with mupirocin/chlorhexidine, 0.82; 95% CI [0.37-1.78]). Of the 13 patients in the mupirocin/chlorhexidine group who died, 1 had a healthcare associated *S. aureus* infection. Of the 13 patients in the placebo group who died, 3 had a healthcare associated *S. aureus* infection. These three patients had undergone cardiothoracic surgery, whereas none of the patients in the mupirocin/chlorhexidine group who underwent cardiothoracic surgery died.

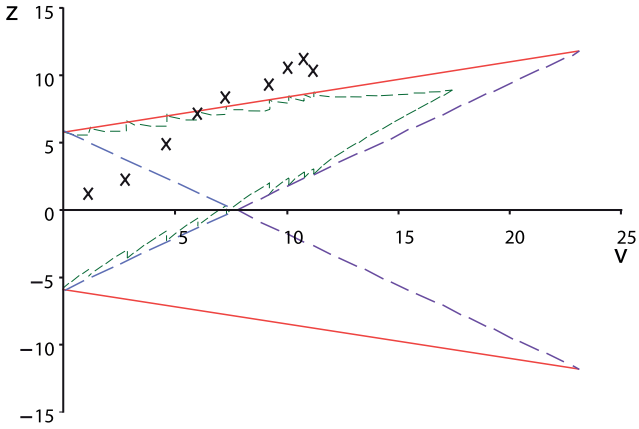

Figure 2. Results of group sequential analysis.

This analysis was conducted as a double-triangular test, in which the horizontal axis (V) represents the cumulative amount of information available and the vertical axis (Z) represents the cumulative effect size. Each point (X) represents a group of 100 patients. Assumptions regarding certain variables determine the boundaries of the test (shown in orange). If the upper boundary is crossed, the intervention can be said to have a beneficial effect; if the lower boundary is crossed, the placebo is more beneficial. If one of the purple dashed lines is crossed, there is no significant difference between the intervention and the placebo. The blue dashed lines are part of the purple boundaries for futility (i.e., equivalence between placebo and intervention); if both blue inner boundaries are crossed, futility would be concluded. The green dashed lines are boundaries that act as a continuity correction (so-called Christmas tree correction), and are the real stopping boundaries. Z represents the difference between the number of infections observed and the number theoretically expected. V represents the variance of Z under the null hypothesis (i.e., no difference between intervention and placebo). In this case, mupirocin/chlorhexidine significantly reduced the cumulative incidence of hospital-acquired *S. aureus* infection ($P = 0.008$).

Variable	Mupirocin/ Chlorhexidine (N=504)		Placebo (N=413)		Relative Risk (95% CI)*
	no.(%)				
S. aureus infection	17	(3.4)	32	(7.7)	0.42 (0.23-0.75)
Source of infection†					
Endogenous	12	(2.4)	25	(6.1)	0.39 (0.20-0.77)
Exogenous	4	(0.8)	6	(1.5)	0.55 (0.16-1.92)
Unknown	1	(0.2)	1	(0.2)	
Localization of infection					
Deep surgical site‡	4	(0.9)	16	(4.4)	0.21 (0.07-0.62)
Superficial surgical site‡	7	(1.6)	13	(3.5)	0.45 (0.18-1.11)
Lower respiratory tract	2	(0.4)	2	(0.5)	0.82 (0.12-5.78)
Urinary tract	1	(0.2)	0	(0)	
Bacteremia	1	(0.2)	1	(0.3)	
Soft tissue	2	(0.4)	0	(0)	

Table 2. Relative risk of healthcare associated *S. aureus* infection and characteristics of infections (Intention-to-Treat Analysis).

*Relative risks are for *S. aureus* infection in the mupirocin/chlorhexidine group.

†The source of the *S. aureus* infections was determined by comparing nasal strains with strains isolated from the infection site by pulsed-field gel electrophoresis.

‡Data are for surgical patients only: 441 in the mupirocin/chlorhexidine group and 367 in the placebo group.

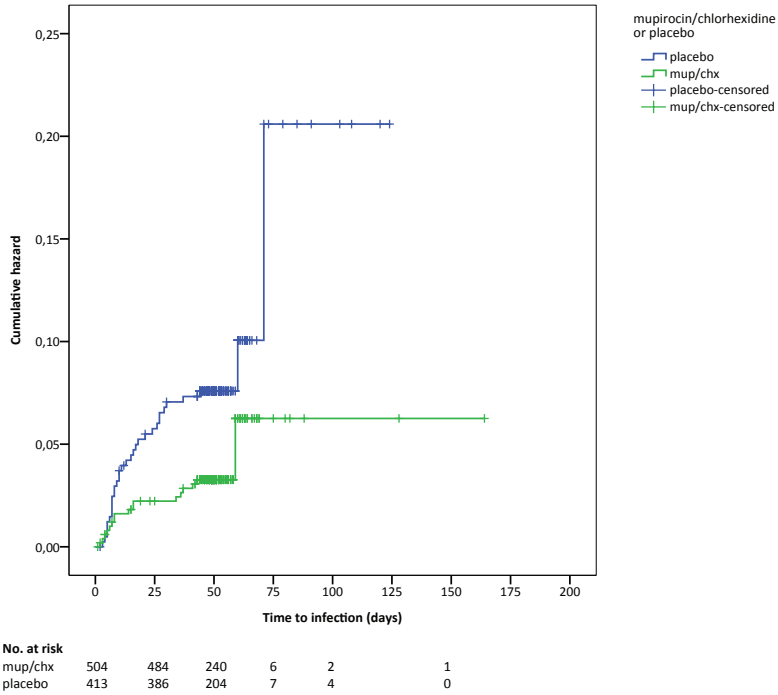

Figure 3. Kaplan-Meier curves showing cumulative hazard of healthcare associated *S. aureus* infection in the study groups.

Microbiologic results

We screened 6771 swabs obtained from 6496 patients to identify nasal carriers of *S. aureus*. The real-time PCR was positive for 1270 samples (18.8%). In 1143 (90%) of these samples, *S. aureus* was also cultured. All *S. aureus* strains that caused healthcare associated infections were susceptible to methicillin and mupirocin. The number of cultured microorganisms and the distribution of species other than *S. aureus* did not differ significantly between the mupirocin/chlorhexidine group and the placebo group.

Adverse reactions

All reported adverse reactions were due to local irritation of the nose or skin and resolved after the study treatment was discontinued.

	Mupirocin and Chlorhexidine		Placebo		RR (95% CI)*	*P* value
All patients (n=917)	**n=504**		**n=413**			
Healthcare-associated *S. aureus* infections – no (%)	17	(3.4)	32	(7.7)	0.42 (0.23-0.75)	
All-cause mortality – no (%)	13	(2.6)	13	(3.1)	0.82 (0.37-1.78)	
Mortality among patients with *S. aureus* infection – no (%)	1	(5.9)	3	(9.4)	0.60 (0.06-6.30)	
Duration of hospitalization – median (IQR)	9	(7-12)	10	(7-15)		0.08
Duration of hospitalization – mean	12.2		14.0			0.04
Surgical patients (n=808)	**n=441**		**n=367**			
Healthcare-associated *S. aureus* infections – no (%)	16	(3.6)	31	(8.4)	0.41 (0.22-0.76)	
All-cause mortality – no (%)	7	(1.6)	12	(3.3)	0.48 (0.19-1.23)	
Mortality among patients with *S. aureus* infection – no (%)	1	(6.3)	3	(9.7)	0.62 (0.06-6.51)	
Duration of hospitalization – median (IQR)	9	(7.5-12)	10	(7-14)		0.04
Duration of hospitalization – mean	11.8		14.0			0.01
Non-surgical patients (n=109)	**n=63**		**n=46**			
Healthcare-associated *S. aureus* infections – no (%)	1	(1.6)	1	(2.2)	0.73 (0.04-11.92)	
All-cause mortality – no (%)	6	(9.5)	1	(2.2)	4.74 (0.55-40.78)	
Mortality among patients with *S. aureus* infection – no (%)	0	(0.0)	0	(0.0)		
Duration of hospitalization – median (IQR)	11	(6-20)	10.5	(5.75-19.25)		0.58
Duration of hospitalization – mean	15.2		14.0			0.63

Table 3. Primary and secondary outcomes of intention-to-treat analysis in all patients, and in surgical and nonsurgical patients.
IQR, interquartile range
*Relative risks are for *S. aureus* infection in the mupirocin/chlorhexidine group.

Discussion

This study shows that rapid detection of *S. aureus* nasal carriage followed by immediate decolonization of nasal and extranasal sites with mupirocin nasal ointment and chlorhexidine gluconate soap significantly reduced the risk of healthcare associated *S. aureus* infections in patients at risk. This intervention also significantly reduced the mean hospital stay by almost 2 days.

In a recent meta-analysis of clinical trials that assessed the effect of nasal mupirocin treatment in surgical patients who were *S. aureus* carriers, the eradication of *S. aureus* reduced the rate of healthcare associated infection with this pathogen by an estimated 45%, but the authors concluded that final proof would be needed from a prospective, randomized clinical trial.[25] A pooled analysis of eight studies showed that intranasal mupirocin application was associated with a significant reduction in the infection rate.[26] The results of our trial provide solid evidence of the preventive effect of *S. aureus* decolonization and a good estimate of the size of this effect: the risk of healthcare associated *S. aureus* infections was reduced by nearly 60%.

Our study differs from previous prospective, randomized trials in several respects. First, nasal carriage of *S. aureus* was detected rapidly by means of real-time PCR at the time of hospital admission. We believe that the rapidity of this assay contributed significantly to the outcome, since it allows targeted decolonization treatment to be initiated within 24 hours of admission - that is, before patients have been exposed to risk factors for healthcare associated *S. aureus* infections. A second important factor in reducing risk was the decontamination of both the nasal passages and the skin. It is well known that nasal carriers are likely to have extranasal sites that are contaminated with the same strain and that carriers are at increased risk for endogenous *S. aureus* infections. [18, 27, 28] We suggest that the use of chlorhexidine for simultaneous elimination of *S. aureus* from extranasal sites is needed to achieve the level of prophylaxis observed in this trial. Although this additional precaution might not lead to complete eradication of the organism, bacterial loads would probably be sufficiently reduced to prevent infection.[29] Third, in our study, treatment was continued for 5 days even when surgery was performed during the course of treatment. Also, these treatments were repeated 3 and 6 weeks after admission for patients who were still in the hospital.

A modification in the study design was necessary because of a perceived change in the overall cumulative incidence of *S. aureus* infections. Since an independent statistician designed and analyzed the data with no foreknowledge, the switch to a sequential design probably did not influence the outcomes of the study.

No significant difference in the cumulative incidence of healthcare associated *S. aureus* infections was found between surgical and nonsurgical patients. However, the reduction in these infections that was achieved with this intervention was most evident among the surgical patients. For such patients, screening and decolonization of carriers provide a clear benefit. Since the proportion of nonsurgical patients in this trial was only 11.9%, and the cumulative incidence of *S. aureus* infections was only 2.2% in the nonsurgical patients who received placebo, inferences about nonsurgical patients are difficult to make. Further research involving larger cohorts at risk is required to assess the benefit of this strategy among nonsurgical patients.

Since mortality was defined in this study as all-cause mortality, excess mortality due to *S. aureus* infections had to be very high to result in significant differences between the study groups. Of the 26 patients who died, 4 had a healthcare associated *S. aureus* infection; 3 of these 4 patients received placebo and underwent cardiothoracic surgery. In contrast, none of the patients who received mupirocin/chlorhexidine and underwent cardiothoracic surgery died. A total of 6 nonsurgical patients in the mupirocin/chlorhexidine group died versus 1 in the placebo group, but none of these deaths were associated with *S. aureus* infections. However, since the numbers are small and the subgroups were not predefined, these data should be interpreted with caution.

Mupirocin and chlorhexidine are considered to be relatively safe. However, since *S. aureus* strains can become resistant to mupirocin, we recommend restricting the use of this agent to known carriers who are at risk for infection.[30] For screening purposes, priority should be given to tests with high specificity, thus limiting the number of false positive results and the unnecessary use of mupirocin and chlorhexidine.

The prevalence of methicillin-resistant *S. aureus* carriage in The Netherlands is only 0.03%.[31] Although this trial was designed to identify and eradicate both methicillin-sensitive and methicillin-resistant *S. aureus,* we did not encounter the latter. Biologically speaking, however, it is plausible that this strategy would also be effective in carriers of methicillin-resistant strains of *S. aureus* that are susceptible to mupirocin. Since carriage patterns may be different for the methicillin-resistant strains, throat swabs in combination with nasal swabs can be considered for identifying carriers of *S. aureus*.[32, 33]

The intervention we describe did not protect patients from all healtcare associated *S. aureus* infections. As we anticipated, it had no or limited effect on exogenous infections. Our intention was to prevent infections with endogenous strains by eradicating these strains from nasal and extranasal sites. However, in some of the patients who received mupirocin and chlorhexidine, endogenous infections developed, and it is unclear why treatment failed in these patients. More

insight into the pathogenesis of endogenous infections would allow preventive strategies to be further enhanced. Also, addressing the problem of cross-infection from exogenous sources of *S. aureus* remains a challenge.

In conclusion, healthcare associated infections with *S. aureus*, especially among surgical patients, can be prevented by rapid screening of patients to identify those who are nasal carriers and initiation of decolonization treatment in confirmed carriers immediately after admission.

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References

1. Luzar MA, Coles GA, Faller B, Slingeneyer A, Dah GD, Briat C, et al. Staphylococcus aureus nasal carriage and infection in patients on continuous ambulatory peritoneal dialysis. N Engl J Med. 1990; **322**(8): 505-9.
2. Kluytmans JA, Mouton JW, Ijzerman EP, Vandenbroucke-Grauls CM, Maat AW, Wagenvoort JH, et al. Nasal carriage of *Staphylococcus aureus* as a major risk factor for wound infections after cardiac surgery. J Infect Dis. 1995; **171**(1): 216-9.
3. Nouwen J, Schouten J, Schneebergen P, Snijders S, Maaskant J, Koolen M, et al. Staphylococcus aureus carriage patterns and the risk of infections associated with continuous peritoneal dialysis. Journal of clinical microbiology. 2006; **44**(6): 2233-6.
4. Weinstein HJ. The relation between the nasal-staphylococcal-carrier state and the incidence of postoperative complications. N Engl J Med. 1959; **260**(26): 1303-8.
5. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. N Engl J Med. 2001; **344**(1): 11-6.
6. Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, et al. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet. 2004; **364**(9435): 703-5.
7. Laupland KB, Conly JM. Treatment of *Staphylococcus aureus* colonization and prophylaxis for infection with topical intranasal mupirocin: an evidence-based review. Clin Infect Dis. 2003; **37**(7): 933-8.
8. Kluytmans JA, Manders MJ, van Bommel E, Verbrugh H. Elimination of nasal carriage of *Staphylococcus aureus* in hemodialysis patients. Infect Control Hosp Epidemiol. 1996; **17**(12): 793-7.
9. Boelaert JR, Van Landuyt HW, Godard CA, Daneels RF, Schurgers ML, Matthys EG, et al. Nasal mupirocin ointment decreases the incidence of *Staphylococcus aureus* bacteraemias in haemodialysis patients. Nephrol Dial Transplant. 1993; **8**(3): 235-9.
10. Nasal mupirocin prevents *Staphylococcus aureus* exit-site infection during peritoneal dialysis. Mupirocin Study Group. J Am Soc Nephrol. 1996; **7**(11): 2403-8.
11. Wertheim HF, Vos MC, Ott A, Voss A, Kluytmans JA, Vandenbroucke-Grauls CM, et al. Mupirocin prophylaxis against nosocomial *Staphylococcus aureus* infections in nonsurgical patients: a randomized study. Ann Intern Med. 2004; **140**(6): 419-25.
12. Kluytmans JA, Mouton JW, VandenBergh MF, Manders MJ, Maat AP, Wagenvoort JH, et al. Reduction of surgical-site infections in cardiothoracic surgery by elimination of nasal carriage of *Staphylococcus aureus*. Infect Control Hosp Epidemiol. 1996; **17**(12): 780-5.
13. Perl TM, Cullen JJ, Wenzel RP, Zimmerman MB, Pfaller MA, Sheppard D, et al. Intranasal mupirocin to prevent postoperative *Staphylococcus aureus* infections. N Engl J Med. 2002; **346**(24): 1871-7.
14. Kalmeijer MD, Coertjens H, van Nieuwland-Bollen PM, Bogaers-Hofman D, de Baere GA, Stuurman A, et al. Surgical site infections in orthopedic surgery: the effect of mupirocin nasal ointment in a double-blind, randomized, placebo-controlled study. Clin Infect Dis. 2002; **35**(4): 353-8.
15. Paule SM, Pasquariello AC, Hacek DM, Fisher AG, Thomson RB, Jr., Kaul KL, et al. Direct detection of *Staphylococcus aureus* from adult and neonate nasal swab specimens using real-time polymerase chain reaction. J Mol Diagn. 2004; **6**(3): 191-6.
16. Hacek DM, Robb WJ, Paule SM, Kudrna JC, Stamos VP, Peterson LR. Staphylococcus aureus Nasal Decolonization in Joint Replacement Surgery Reduces Infection. Clinical orthopaedics and related research. 2008; **466**(6): 1349-55.
17. Choudhurry RS, Melles DC, Eadie K, Vos M, Wertheim HF, Verbrugh HA, et al. Direct detection

of human *Staphylococcus aureus* carriage in the nose using the Lightcycler Staphylococcus kit. J Microbiol Methods. 2006; **65**(2): 354-6.

18. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 2005; **5**(12): 751-62.
19. Kaiser AB, Kernodle DS, Barg NL, Petracek MR. Influence of preoperative showers on staphylococcal skin colonization: a comparative trial of antiseptic skin cleansers. The Annals of thoracic surgery. 1988; **45**(1): 35-8.
20. Horan TC, Gaynes RP. Hospital Epidemiology and Infection Control. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2004.
21. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. Journal of clinical microbiology. 1995; **33**(9): 2233-9.
22. Whitehead J. The design and analysis of sequential clinical trials. Revised Second Edition ed. Chichester: John Wiley & Sons; 1997.
23. PEST4. Operating manual. UK: The University of Reading; 2000.
24. Doern GV, Vautour R, Gaudet M, Levy B. Clinical impact of rapid in vitro susceptibility testing and bacterial identification. Journal of clinical microbiology. 1994; **32**(7): 1757-62.
25. van Rijen MM, Bonten M, Wenzel RP, Kluytmans JA. Intranasal mupirocin for reduction of *Staphylococcus aureus* infections in surgical patients with nasal carriage: a systematic review. J Antimicrob Chemother. 2008; **61**(2): 254-61.
26. van Rijen M, Bonten M, Wenzel R, Kluytmans J. Mupirocin ointment for preventing Staphylococcus aureus infections in nasal carriers. Cochrane database of systematic reviews (Online). 2008; (4): CD006216.
27. Solberg CO. A study of carriers of *Staphylococcus aureus* with special regard to quantitative bacterial estimations. Acta Med Scand Suppl. 1965; **436**: 1-96.
28. Williams RE. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. Bacteriol Rev. 1963; **27**: 56-71.
29. Wendt C, Schinke S, Wurttemberger M, Oberdorfer K, Bock-Hensley O, von Baum H. Value of whole-body washing with chlorhexidine for the eradication of methicillin-resistant Staphylococcus aureus: a randomized, placebo-controlled, double-blind clinical trial. Infect Control Hosp Epidemiol. 2007; **28**(9): 1036-43.
30. Deshpande LM, Fix AM, Pfaller MA, Jones RN. Emerging elevated mupirocin resistance rates among staphylococcal isolates in the SENTRY Antimicrobial Surveillance Program (2000): correlations of results from disk diffusion, Etest and reference dilution methods. Diagnostic microbiology and infectious disease. 2002; **42**(4): 283-90.
31. Wertheim HF, Vos MC, Boelens HA, Voss A, Vandenbroucke-Grauls CM, Meester MH, et al. Low prevalence of methicillin-resistant Staphylococcus aureus (MRSA) at hospital admission in the Netherlands: the value of search and destroy and restrictive antibiotic use. J Hosp Infect. 2004; **56**(4): 321-5.
32. Miller LG, Diep BA. Clinical practice: colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant Staphylococcus aureus infection. Clin Infect Dis. 2008; **46**(5): 752-60.
33. Mertz D, Frei R, Periat N, Zimmerli M, Battegay M, Fluckiger U, et al. Exclusive Staphylococcus aureus throat carriage: at-risk populations. Arch Intern Med. 2009; **169**(2): 172-8.

Chapter 3

Decreased one-year mortality after rapid screening and decolonization of S. aureus carriers undergoing clean surgical procedures

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Submitted

Abstract

Objective

To identify patients who benefit most from *S. aureus* screening and decolonization treatment upon admission.

Summary Background Data

In *S. aureus* carriers, the risk of surgical-site infections with *S. aureus* is increased. Previously, we demonstrated in a randomized, placebo-controlled trial (RCT) that these infections can largely be prevented by detection of carriage and decolonization treatment upon admission. In the present study, we use one- and three-year mortality rates in both treatment arms of the RCT to identify patient groups that should be targeted when implementing the screen-and-treat strategy.

Methods

The municipal personal records database was checked for mortality dates of all surgical patients three years after enrolment in the RCT. One- and three-year mortality rates were calculated for all patients, for subgroups according to type of surgery, and for patients with clean procedures.

Results

Of the 808 patients enrolled, 793 patients were included in the analysis. After three years, 44/431 (10.2%) and 43/362 (11.9%) had died in the mupirocin/chlorhexidine and placebo groups, respectively. No significant differences in mortality rates were observed between the treatment groups or the subgroups according to type of surgery. In the subgroup of 666 patients with clean procedures (382 cardiothoracic, 167 orthopedic, 61 vascular, and 56 other procedures), mupirocin/chlorhexidine significantly reduced one-year mortality: 11/365 (3.0%) died in the mupirocin/chlorhexidine group versus 21/301 (7.0%) in the placebo group (RR 0.38, 95% CI [0.18 – 0.81]; P=0.012).

Conclusion

Detection and decolonization of *S. aureus* carriage not only prevents healthcare associated *S. aureus* infections, but also significantly reduces one-year mortality in surgical patients undergoing clean procedures. This subgroup should be the primary target when implementing the screen-and-treat strategy in clinical practice.

Introduction

Staphylococcus aureus (S. aureus) colonizes the nares and skin of a substantial proportion of the human population. Carriage rates range from about 20 to 50%, depending on the population and the definitions used.[1, 2] Colonization with *S. aureus* is usually harmless in healthy individuals. However, carriage is known to be a risk factor for the development of healthcare associated *S. aureus* infections.[3, 4] Prospective studies demonstrate that approximately 80% of *S. aureus* strains isolated from healthcare associated infections are identical to the nasal strains found in these patients upon admission. [5-7]

Healthcare associated infections are associated with increased morbidity, mortality, length of stay and hospital costs.[8-10] Recently, we demonstrated in a multicenter, randomized, placebo-controlled trial (RCT) that approximately 60% of healthcare associated infections with *S. aureus* can be prevented by rapid screening of patients for *S. aureus* carriage upon admission, and subsequent decolonization treatment with mupirocin nasal ointment and washing with chlorhexidine gluconate medicated soap: 17/504 (3.4%) patients in the mupirocin/chlorhexidine group, and 32/413 (7.7%) patients in the placebo group developed *S. aureus* infections.[6] The effect of the intervention was most prominent in surgical patients and largely due to the prevention of endogenous deep surgical site infections (SSI): 4/441 (0.9%) surgical patients in the mupirocin/chlorhexidine group, and 16/367 (4.4%) in the placebo group developed a deep *S. aureus* SSI infection.

The implementation of the screen-and-treat strategy poses a logistic challenge to clinical practice. The results of *S. aureus* screening have to be available within a few hours after admission to be able to start treatment with mupirocin and chlorhexidine in time. This requires participation of the patient, nurses, doctors, and laboratory personnel, including timely communication between the laboratory and the patient's attending physician and nurses. Results of reliable rapid diagnostic screens should be reported rapidly. The use of mupirocin without screening for carriage is discouraged, since it would not provide any benefit for the (*S. aureus* non-carrying) majority of patients, and increases the risk of emergence of mupirocin resistance.[11, 12] Therefore, it is very important to identify those patient groups that benefit most from a screen-and-treat strategy.

We hypothesized that preventing deep surgical site infections would have a beneficial effect on mortality beyond the initial follow up period of the RCT. Thus, in the present study, we compared one- and three-year mortality rates of surgical patients treated with mupirocin nasal ointment and chlorhexidine gluconate soap, with the rates of those that had received placebo medication. Since the majority of deep SSI developed after cardiac, orthopedic, and vascular surgery, we also assessed mortality rates in different subgroups of patients.

Methods

In a randomized, double blind, placebo-controlled, clinical multicenter trial, approved by the medical ethics committees of the participating hospitals, surgical and non-surgical patients (n=6771) were screened for nasal *S. aureus* carriage by real-time PCR. Of those, 1251 tested positive for *S. aureus* nasal carriage. Carriers of *S. aureus* who met the inclusion criteria and gave informed consent (n=917) were randomized to receive either mupirocin nasal ointment 2% and chlorhexidine gluconate soap 40 mg per milliliter, or placebo ointment and placebo soap, as previously described.[6] The duration of the study treatment was five days. Patients who were still hospitalized after three weeks and those still hospitalized after six weeks received a second and third course of the same trial medication, respectively. Follow-up for the development of healthcare associated *S. aureus* infections was until six weeks after discharge from the hospital. For each surgical patient enrolled in the RCT (n=808), three years after the date of enrolment the municipal personal records database was checked for the presence of a mortality date by using the patient's birth name, date of birth and zip code, which was done by a data manager who was blinded for the intervention. The municipal personal records database contains the personal details of all inhabitants of The Netherlands. Among other purposes it is used by the government to collect taxes.

We used univariate Kaplan Meier and the Mantel-Cox log-rank test to assess whether the use of mupirocin and chlorhexidine was associated with one- and three-year mortality rates. Analyses were performed for all surgical patients, as well as for the following subgroups: five subgroups according to the type of surgery (cardiothoracic surgery; orthopedic surgery; vascular surgery; abdominal surgery; and other type of surgery), and two subgroups based on the CDC wound classification system[13]: patients with clean procedures and patients with clean-contaminated, contaminated or dirty procedures. If the Mantel-Cox log-rank *P*-value was less or equal to 0.1, the following determinants were analyzed by Kaplan Meier and Mantel-Cox log-rank tests to be identified as a possible confounder for mortality rates: gender; diabetes mellitus; CAPD; renal insufficiency; end stage liver disease; solid or hematological malignancy; immune-compromised state; use of immunosuppressive medication; modified McCabe score[14]; and, if applicable, the surgical department where patients were admitted. Subsequently, a Cox-regression survival analysis was performed including age, and those determinants with a Mantel-Cox log-rank *P*-value less or equal to 0.1, to identify factors associated with mortality at one and three years after the date of inclusion.

Results

Of the 808 surgical patients enrolled in the multicenter RCT, 15 patients (1.9%) were lost to follow up because birth dates and zip codes did not match. Of the remaining 793 patients, 22/431 (5.1%) patients in the mupirocin/chlorhexidine treated group, and 29/362 (8.0%) in the placebo treated group had died within the first year after enrolment. After three years, 44/431 (10.2%) and 43/362 (11.9%) had died in the mupirocin/chlorhexidine and placebo groups, respectively. Table 1 shows the results of the univariate and, if applicable, multivariate analyses for all surgical patients together, as well as for the different subgroups. Figure 1 shows the cumulative hazard of mortality in both treatment groups. The median time to mortality did not significantly differ between the two treatment groups (mupirocin/chlorhexidine vs. placebo: median 99 vs. 135 days (P=0.49) after 1 year, and 365 vs. 273 days (P=0.11) after 3 years).

Of the 87 patients who died, 14 (16.1%) had a documented healthcare associated *S. aureus* infection according to CDC definitions within the follow-up period of the randomized controlled trial.[15] Eight of these infections were deep surgical site infections (SSI): two in the mupirocin/chlorhexidine group (2 out of 44 patients who died, 4.5%) and six in the placebo group (6/43 = 14.0%); P=0.13. Other healthcare associated infections were superficial SSIs (1 in the mupirocin/chlorhexidine group, 1 in the placebo group), lower respiratory tract infections (1 in the mupirocin/chlorhexidine group, 2 in the placebo group), and bacteremia (1 in the mupirocin/chlorhexidine group).

In the RCT, a total of 47 surgical patients had developed a healthcare associated *S. aureus* infection within its follow-up period (16 in the mupirocin/chlorhexidine group , 31 in the placebo group). Twenty patients had a documented deep SSI (4 in the mupirocin/chlorhexidine group , 16 in the placebo group). Of these patients, six (30%) had died within a year (1 in the mupirocin/chlorhexidine group , 5 in the placebo group), and eight (40.0%) had died within three years after enrolment (2 in the mupirocin/chlorhexidine group , 6 in the placebo group). Of the 746 patients who had not developed any health-care associated *S. aureus* infection within the follow-up period, 73 (9.8%) had died after three years (39/415 (9.4%) in the mupirocin/chlorhexidine group, and 34/331 (10.3%) in the placebo group).

One-year mortality rates

In the univariate and multivariate analyses of all surgical patients together, no significant difference in one-year survival was found between the two treatment groups (Table 1). When in the univariate analysis the patients were stratified according to their type of surgery (orthopedics, cardiothoracic surgery, vascular surgery, abdominal surgery, or other types of surgery), one-

	Mupirocin / chlorhexidine					Placebo						
	N=	*Deaths year 1 no (%)*		*Deaths year 3 no (%)*		*N=*	*Deaths year 1 no (%)*		*Deaths year 3 no (%)*		*P-value year 1*	*Adjusted RR yr 1 [95% CI]*	*P-value year 3*
All surgical patients	431	22	(5.1)	44	(10.2)	362	29	(8.0)	43	(11.9)	0.099	0.65 [0.37-1.14]	0.435
Cardiac surgery	231	6	(2.8)	14	(6.6)	171	13	(7.6)	18	(10.5)	0.032	0.38 [0.13-1.06]	0.153
Orthopedic surgery	85	1	(1.2)	6	(7.1)	85	2	(2.4)	5	(5.9)	0.561	NA	0.745
Vascular surgery	52	5	(9.6)	7	(13.5)	39	6	(15.4)	9	(23.1)	0.394	NA	0.229
Abdominal surgery	21	5	(23.8)	7	(33.3)	21	4	(19.0)	5	(23.8)	0.721	NA	0.514
Other procedures	60	5	(8.3)	10	(16.7)	46	4	(8.7)	6	(13.0)	1.000	NA	0.592
Clean procedures	365	11	(3.0)	27	(7.4)	301	21	(7.0)	30	(10.0)	0.017	0.38 [0.18-0.81]	0.221
Non-clean procedures	48	8	(16.7)	11	(22.9)	51	6	(11.8)	9	(17.6)	0.483	NA	0.499

Table 1. Effect of treatment on one-year and three-year mortality in surgical patients, and in different subgroups.
NA, not applicable.

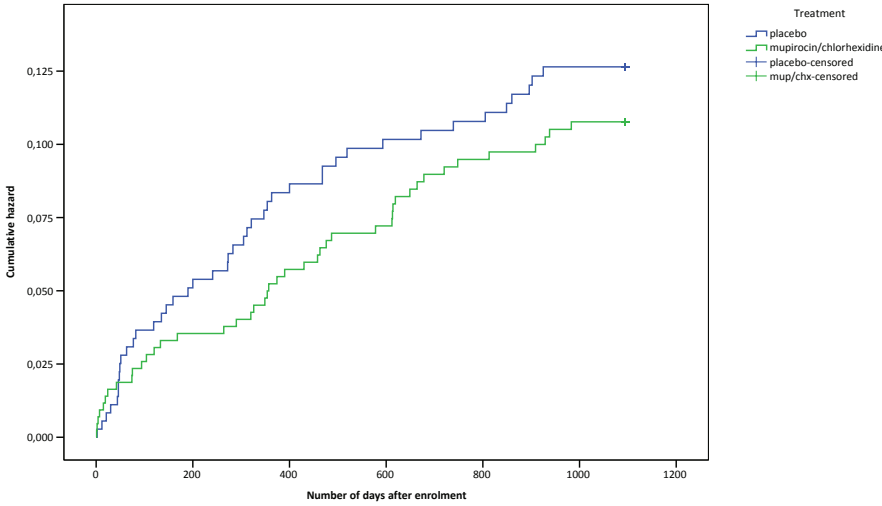

Figure 1. Kaplan–Meier curves showing cumulative hazard of mortality in the two treatment groups.

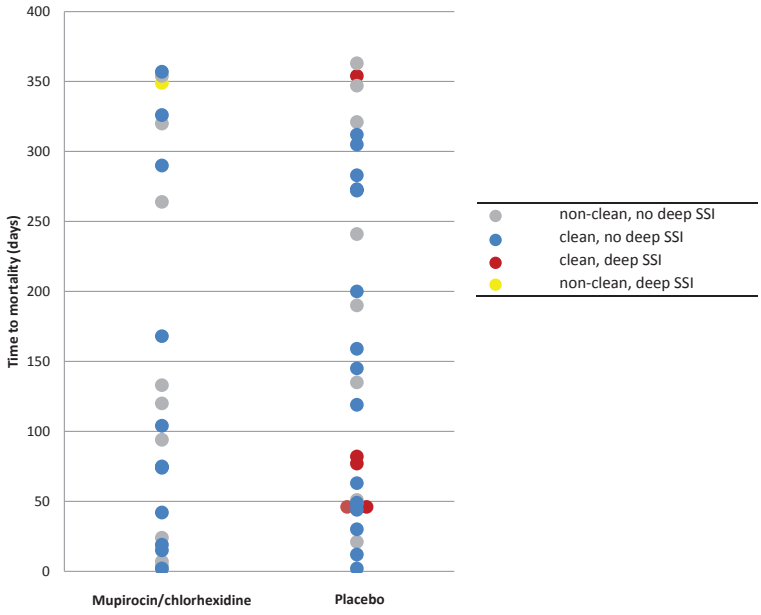

Figure 2. Scatterplot showing the time from enrolment to mortality for all patients who died within the first year of follow-up. Patients who underwent a clean procedure are depicted in blue (no deep SSI, n=11 in the mup/chx group and n=16 in the placebo group) or red (deep SSI, n=5 in the placebo group). Patients who underwent a non-clean procedure are depicted in grey (no deep SSI, n= 7 in the mup/chx group, and n=6 in the placebo group) or yellow (deep SSI, n=1 in the mup/chx group).

year mortality was significantly decreased in the mupirocin/chlorhexidine group compared to the placebo treated group in cardiothoracic patients (6/213 patients (2.8%) died in the mupirocin/chlorhexidine group versus 13/171 patients (7.6%) in the placebo group, *P*=0.032). In the multivariate analysis, the effect of mupirocin/chlorhexidine on the mortality rate was not significant (adjusted relative risk (RR) 0.38; 95% CI 0.13 to 1.06; *P*=0.064).

Of the 793 surgical patients, 666 patients underwent a clean surgical procedure (including 382 cardiothoracic, 167 orthopedic, 61 vascular, and 56 other procedures). In this group both the univariate and multivariate analysis showed that mupirocin/chlorhexidine significantly reduced one-year mortality. According to the univariate analysis, 11/365 (3.0%) died in the mupirocin/chlorhexidine group versus 21/301 (7.0%) in the placebo group, *P*=0.017. In the multivariate analysis, the adjusted relative risk for mortality in the mupirocin/chlorhexidine group was 0.38 (95% CI [0.18 - 0.81]; *P*=0.012). Other factors significantly associated with mortality in the multivariate analysis were the presence of a solid or hematological malignancy (adjusted RR 4.65; 95%CI [1.58 - 13.70]; *P*=0.005), and age (adjusted RR per year 1.08, 95%CI [1.03 - 1.12]; P=0.001). The median time to mortality did not significantly differ between the two treatment groups (mupirocin/chlorhexidine vs. placebo: 75 vs. 82 days (*P*=0.82)). Fifteen patients who had underwent a clean procedure had developed a deep SSI. In the placebo group, 5/12 (41.7%) of these patients had died within a year after enrolment. In the mupirocin/chlorhexidine group, 0/3 (0%) of these patients had died. In other words, 5/21 (23.8%) patients who had died in the placebo group, and 0/11 (0%) patients in the mupirocin/chlorhexidine group, had suffered from a deep surgical site infection within the follow-up period of the randomized controlled trial.

The effect of mupirocin/chlorhexidine on one-year mortality in patients who did not undergo clean procedures (including clean-contaminated, contaminated, and dirty-infected wounds) was not significant (*P*=0.483, Table 1).

Figure 2 shows the time to mortality for all patients in the two treatment groups who died within the first year.

Three-year mortality rates

No significant differences in mortality rates were found between the two treatment groups at three years after randomization (Table 1). After excluding the first year, the mortality rates for the second and third years after randomization were not significantly different either (data not shown).

Discussion

With the present study, we show that one- and three-year mortality rates of *S. aureus* carriers undergoing surgery, and treated prophylactically with mupirocin and chlorhexidine, did not significantly differ from these rates in patients treated with placebo. However, in the large subgroup of patients who underwent clean surgery (cardiothoracic, orthopedic, vascular and other procedures), one-year mortality was nearly three times lower in the mupirocin and chlorhexidine group than in the placebo group (adjusted relative risk 0.38, 95% CI [0.18 - 0.81]).

Therefore, the reduction in the *S. aureus* infection rate that was previously reported clearly has a beneficial effect beyond 6 weeks after discharge from hospital.[6] Our findings can be useful to select those patients, which benefit most from the screen-and-treat strategy for *S. aureus* carriage. In the preceding RCT nonsurgical patients as well as patients undergoing cardiothoracic, orthopedic, vascular, abdominal and miscellaneous other types of surgery were enrolled.[6] Within these categories, many different types of surgery were performed, ranging from prosthetic joint replacement to trauma surgery, and from coronary artery bypass surgery to low anterior resection of the rectum. However, for reasons of logistics and costs, it may not be needed, nor may it be feasible, to screen every surgical patient entering the hospital. Indiscriminately treating all patients is discouraged, since the use of mupirocin should be restricted to those that may benefit, and to avoid development of resistance.[16, 17] Furthermore, without screening, the potential emergence of mupirocin resistant *S. aureus* clones cannot be detected at an early stage. Therefore, it is important to identify patient groups in which the screen-and-treat strategy for *S. aureus* carriage has the best preventive effect on morbidity and mortality. This study demonstrates that patients undergoing clean procedures should be targeted for this screen-and-treat strategy.

An important drawback of our study is that the causes of death were not available in the municipal records. Although a causal relationship with mortality cannot be demonstrated, the number of patients with a documented deep SSI who had died, markedly differed between the two treatment groups in favor of mupirocin and chlorhexidine. This provides further argument that the screen-and-treat strategy has a beneficial impact on mortality in clean surgery by preventing health-care associated *S. aureus* infections.

From the US Centers for Disease Control definition of a clean (class I) wound, it follows that these surgical procedures do not enter colonized tracts, but usually breach the skin only.[18] Clean wound infections are, therefore, most likely caused by skin flora, particularly *S. aureus*. Thus, it is not surprising that the effect of the screen-and-treat strategy for *S. aureus* carriage on mortality is most evident in patients who undergo clean procedures.

The size of the subgroups in separate surgical procedures was too small to have sufficient power to show any effect on mortality. Also, mortality was low in several surgical specialties, e.g. in the orthopedic subgroup, where only 1/85 (1.2%) and 2/85 (2.4%) patients died in the mupirocin/chlorhexidine and placebo treated group, respectively. Even though it seems that patients who underwent an orthopedic procedure do not die from *S. aureus* infections, these infections frequently have devastating consequences for the patient. We did not, however, analyze morbidity or quality of life. In another study we found a significant difference in costs between treated and non-treated patients that had undergone cardiothoracic and orthopedic procedures.[19] The average cost to the hospital was € 2,841 lower for a cardiothoracic patient, and € 955 lower for an orthopaedic patient who had been treated with mupirocin/chlorhexidine, as compared to placebo-treated patients.

We did not only compare one-year, but also three-year mortality rates between mupirocin/chlorhexidine and placebo treated patients. After three years, no significant differences were found. The mortality rates during the second and third years after enrolment were, however, statistically not different. The screen and treat strategy, thus, only affects mortality within the first year after surgery; after three years this effect naturally wanes since patients will die from causes other than from deep surgical wound infections which occurred more than one year before.

Most studies on *S. aureus* screening in the last decade have focused on MRSA only.[20-22] Our results question this selected approach, since it neglects methicillin susceptible *S. aureus* (MSSA), which remains the leading cause of invasive infections in most countries.[23] For prevention of infections in surgical patients, screening for *S. aureus*, including both MRSA and MSSA, would be the preferred strategy. Meanwhile, the US Centers for Disease Control have now included this strategy in their top recommendations for safer health care. It has more impact on patient safety, and for the laboratory, it is technically simpler since only the presence of *S. aureus*-specific targets have to be covered. Our results support limiting the indication for the *S. aureus* screen-and-treat strategy to clean surgical procedures.

In conclusion, rapid detection and decolonization of *S. aureus* carriers not only reduces the incidence of healthcare associated *S. aureus* infections, but also significantly reduces one-year mortality in surgical patients who undergo clean operations. We identified the patient group that benefits most from this screen and treat strategy in terms of one-year survival, and this group should thus be targeted when implementing a *S. aureus* screen-and-treat strategy in clinical practice. The results of this study provide an important stimulus to opinion leaders and decision makers to change current pre-operative protocols and allocate resources for this prophylactic strategy.

References

1. Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis* 2005; 5(12):751-62.
2. Williams RE. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. *Bacteriol Rev* 1963; 27:56-71.
3. Weinstein HJ. The relation between the nasal-staphylococcal-carrier state and the incidence of postoperative complications. *N Engl J Med* 1959; 260(26):1303-8.
4. White A. Increased infection rates in heavy nasal carriers of coagulase-positive staphylococci. *Antimicrobial Agents Chemother (Bethesda)* 1963; 161:667-70.
5. von Eiff C, Becker K, Machka K, et al. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. *N Engl J Med* 2001; 344(1):11-6.
6. Bode LG, Kluytmans JA, Wertheim HF, et al. Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. *N Engl J Med* 2010; 362(1):9-17.
7. Wertheim HF, Vos MC, Ott A, et al. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. *Lancet* 2004; 364(9435):703-5.
8. Kirkland KB, Briggs JP, Trivette SL, et al. The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs. *Infect Control Hosp Epidemiol* 1999; 20(11):725-30.
9. Hollenbeak CS, Murphy DM, Koenig S, et al. The clinical and economic impact of deep chest surgical site infections following coronary artery bypass graft surgery. *Chest* 2000; 118(2):397-402.
10. Jarvis WR. Selected aspects of the socioeconomic impact of nosocomial infections: morbidity, mortality, cost, and prevention. *Infect Control Hosp Epidemiol* 1996; 17(8):552-7.
11. Shrestha NK, Banbury MK, Weber M, et al. Safety of targeted perioperative mupirocin treatment for preventing infections after cardiac surgery. *Ann Thorac Surg* 2006; 81(6):2183-8.
12. Bathoorn E, Hetem DJ, Alphenaar J, et al. Emergence of high level mupirocin resistance in coagulase-negative staphylococci associated with increased short-term mupirocin use. *J Clin Microbiol* 2012.
13. Mangram AJ, Horan TC, Pearson ML, et al. Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. *Infect Control Hosp Epidemiol* 1999; 20(4):250-78; quiz 279-80.
14. Doern GV, Vautour R, Gaudet M, et al. Clinical impact of rapid in vitro susceptibility testing and bacterial identification. *J Clin Microbiol* 1994; 32(7):1757-62.
15. Horan TC, Gaynes RP. Hospital Epidemiology and Infection Control. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2004.
16. Patel JB, Gorwitz RJ, Jernigan JA. Mupirocin resistance. *Clin Infect Dis* 2009; 49(6):935-41.
17. Deshpande LM, Fix AM, Pfaller MA, et al. Emerging elevated mupirocin resistance rates among staphylococcal isolates in the SENTRY Antimicrobial Surveillance Program (2000): correlations of results from disk diffusion, Etest and reference dilution methods. *Diagn Microbiol Infect Dis* 2002; 42(4):283-90.
18. Mangram AJ, Horan TC, Pearson ML, et al. Guideline for Prevention of Surgical Site Infection, 1999. Centers for Disease Control and Prevention (CDC) Hospital Infection Control Practices Advisory Committee. *Am J Infect Control* 1999; 27(2):97-132; quiz 133-4; discussion 96.
19. van Rijen MM, Bode LG, Baak DA, et al. Reduced Costs for Staphylococcus aureus Carriers Treated Prophylactically with Mupirocin and Chlorhexidine in Cardiothoracic and Orthopaedic Surgery. *PLoS One* 2012; 7(8):e43065.

20. Reilly JS, Stewart S, Christie P, et al. Universal screening for meticillin-resistant Staphylococcus aureus in acute care: risk factors and outcome from a multicentre study. *J Hosp Infect* 2012; 80(1):31-5.
21. Murthy A, De Angelis G, Pittet D, et al. Cost-effectiveness of universal MRSA screening on admission to surgery. *Clin Microbiol Infect* 2010; 16(12):1747-53.
22. Robicsek A, Beaumont JL, Paule SM, et al. Universal surveillance for methicillin-resistant Staphylococcus aureus in 3 affiliated hospitals. *Ann Intern Med* 2008; 148(6):409-18.
23. ECDC. Annual Epidemiological Report 2011. Reporting on 2009 surveillance data and 2010 epidemic intelligence data. European Centre for Disease Prevention and Control 2011.
24. Top CDC Recommendations to Prevent Healthcare-Associated Infections. Available at: http://www.cdc.gov/HAI/prevent/top-cdc-recs-prevent-hai.html. Accessed April 24, 2013.

Chapter 4

Reduced hospital costs for Staphylococcus aureus nasal carriers treated prophylactically with mupirocin and chlorhexidine soap

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Abstract

Background

A multi centre double-blind randomized-controlled trial (M-RCT), carried out in The Netherlands in 2005-2007, showed that hospitalized patients with *S. aureus* nasal carriage who were treated prophylactically with mupirocin nasal ointment and chlorhexidine gluconate medicated soap, had a significantly lower risk of health-care associated *S. aureus* infections than patients receiving placebo (3.4% vs. 7.7%, RR 0.42, 95% CI [0.23-0.75]). The objective of the present study was to determine whether treatment of patients undergoing elective cardiothoracic or orthopaedic surgery with mupirocin/chlorhexidine (screen-and-treat strategy) affected the costs of patient care.

Methods

We compared hospital costs of patients undergoing cardiothoracic or orthopaedic surgery (n=415) in one of the participating centers of the M-RCT. Data from the 'Planning and Control' department were used to calculate total hospital costs of the patients. Total costs were calculated including nursing days, costs of surgery, costs for laboratory and radiological tests, functional assessments and other costs. Costs for personnel, materials and overhead were also included. Mean costs in the two treatment arms were compared using the t-test for equality of means (two-tailed). Subgroup analysis was performed for cardiothoracic and orthopaedic patients.

Results

An investigator-blinded analysis revealed that costs of care in the treatment arm (mupirocin/chlorhexidine, n=210) were on average €1911 lower per patient than costs of care in the placebo arm (n=205) (€8602 vs. €10513, *P*=0.01). Subgroup analysis showed that mupirocin/chlorhexidine treated cardiothoracic patients cost €2841 less (n=280, €9628 vs. €12469, *P*=0.006) and orthopaedic patients €955 less than non-treated patients (n=135, €6097 vs. €7052, *P*=0.05).

Conclusions

In conclusion, in patients undergoing cardiothoracic or orthopaedic surgery, screening for *S. aureus* nasal carriage and treating carriers with mupirocin/chlorhexidine results in a substantial reduction of hospital costs.

Introduction

Staphylococcus aureus (S. aureus) nasal carriage rates range from about 20 to 50%, depending on the population and the definitions used.[1-3] Infections with *S. aureus* can develop after disruption of the skin barrier, for example after an incision has been made during surgery. It has been shown that in surgical patients, healthcare associated *S. aureus* infections are mainly caused by their own *S. aureus* strain (endogenous infection).[4-7] *S. aureus* nasal carriage is now considered to be a well-defined risk factor for subsequent infection in various groups of patients, especially those on dialysis; with cirrhosis of the liver; undergoing surgery; and with intravascular devices or in intensive care.[3, 8] This raised the hypothesis that eradication of *S. aureus* from the nose would result in fewer *S. aureus* infections in these groups of patients. Many studies have evaluated this effect in the past decades. Until 2010, only a few studies were double-blind randomized-controlled trials (RCT).[5, 9-16] In these studies various patient populations were treated intranasally with mupirocin, an antibiotic nasal ointment. None of these studies found a significantly reduced number of *S. aureus* infections compared to placebo treatment. However, in most of these studies, both *S. aureus* nasal carriers and non-carriers were treated. Perl *et al*. were the first to perform a subgroup analysis on carriers only, and showed that 4.0% of mupirocin treated patients with nasal carriage of *S. aureus* suffered from healthcare associated *S. aureus* infections, compared to 7.7% of those who received placebo (P=0.02).[15] Subsequently, all data pertaining to carriers in the above mentioned RCTs were combined in a systematic review, which showed that carriers who were treated with mupirocin before surgery had 44% less chance of developing a healthcare associated *S. aureus* infections than patients receiving placebo.[17]

Based on these findings a multi-centre double-blind randomized-controlled trial (M-RCT) was performed in which only *S. aureus* nasal carriers were included.[6] This study showed that patients treated with mupirocin and chlorhexidine gluconate medicated soap had a significantly lower risk of healthcare related *S. aureus* infections than patients receiving placebo (3.4% vs. 7.7%, RR 0.42, 95% CI [0.23-0.75]).

The objective of the present study was to compare hospital costs of patients treated with mupirocin/chlorhexidine (screen-and-treat strategy) to those of patients treated with placebo (comparable to a non-screen-and-treat strategy), in patients undergoing elective cardiothoracic or orthopaedic surgery.

Methods

In the M-RCT, performed in three university hospitals and two teaching hospitals, patients who were admitted to departments of surgery and internal medicine were screened for *S. aureus* nasal carriage.[6] The present cost analysis was carried out for patients of only the Amphia hospital, a teaching hospital which serves a population of approximately 440,000 inhabitants. During the study period, on average 41,534 patients were admitted annually to this hospital with 271,528 in-patient days per year (mean number over the period 2005 to 2007, excluding day care).

A total of 415 patients admitted for elective cardiothoracic and orthopaedic surgery in this hospital participated in the M-RCT. Cardiothoracic patients (n=280) underwent Coronary Artery Bypass Grafting (CABG) operations with or without valve replacement (n=88 and n=150, respectively) or other cardiothoracic surgery (n=3). In 39 patients the nature of cardiothoracic surgery was not further specified. Orthopaedic patients (n=135) underwent knee replacement (n=45), hip replacement (n=50), spinal surgery (n=28) or other orthopaedic procedures (n=12).

An investigator-blinded analysis was carried out to compare all hospital costs incurred between start of admission and the end of follow-up (42 days after discharge) for patients in both treatment groups (mupirocin/chlorhexidine vs. placebo). Costs were analyzed for the total follow up period, as well as per admission (categorized as the first, second, third admission etc) during this period. Actual total hospital costs per included patient were retrieved from the data files of 'Planning and Control' (P&C) department of the hospital (Figure 1). Since the study medication (mupirocin/chlorhexidine) was supplied for free during the study, the cost of this medication was added to the costs of patients treated with mupirocin/chlorhexidine. Screening costs were already included in the laboratory tests performed; for the placebo group, screening costs were subtracted from total costs because this study arm represents the strategy without screening or treatment. For the period between discharge and the end of follow-up, all costs made during readmissions or costs for outpatient visits were included. Community costs were not estimated. All costs for readmissions and secondary surgical procedures in this period were included. Physicians' fees were not registered in the P&C data file, so these costs could not be included in this analysis.

Mean costs in both treatment arms were compared using the *t*-test for equality of means (two-tailed). Statistical significance was accepted when $P<0.05$. Subgroup analysis was performed for cardiothoracic and orthopaedic patients.

The average Euro to US dollar exchange rate during the study period was 1.35.

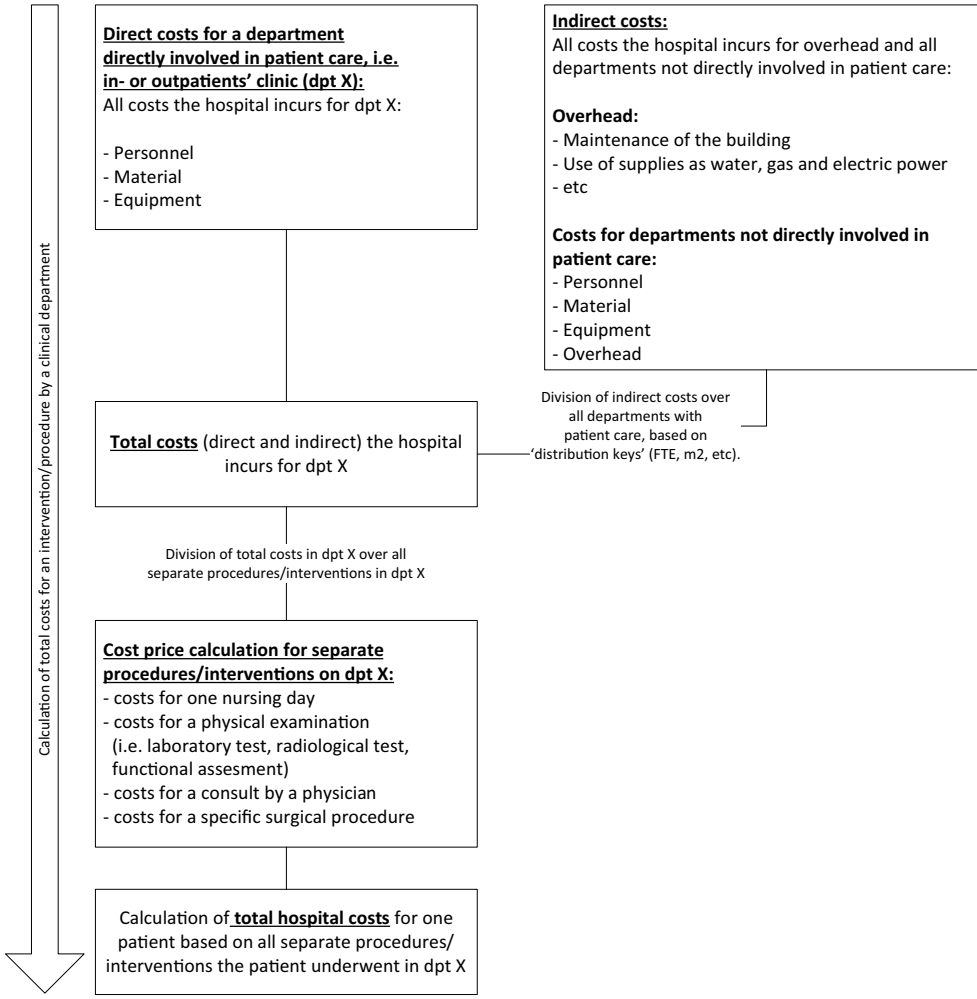

Figure 1. Calculation of total costs incurred by the hospital for an individual patient in a particular department.

Results

Mean total hospital costs for a mupirocin/chlorhexidine treated patient undergoing cardiothoracic or orthopaedic surgery were significantly lower than costs for a placebo treated patient (€8602 vs. €10513, *P*=0.01) (Table 1). Table 1 shows that mean costs per patient for all individual categories, i.e. costs for nursing days, surgery, functional assessments, and laboratory and radiological tests during the first two admissions combined, were higher in the placebo group than in the mupirocin/chlorhexidine treated group. During the first admission significant differences between the treatment groups were found only in costs for nursing days. For the

	Mean costs per patient (€)		
	MUP/CHX (N=210)	**Placebo (N=205)**	***P* value**
Total hospital costs	8602.07	10513.33	**0.01**
Total costs admission 1	8445.94	9630.63	0.073
Costs for nursing days (excl. IC) during admission 1	2867.69	3214.21	**0.023**
Costs for nursing days IC during admission 1	1472.2	2094.84	0.259
Costs for surgery during admission 1	3388.82	3496.11	0.293
Costs for laboratory tests during admission 1	301.33	333.05	0.200
Costs for radiodiagnostics and functional assessments during admission 1	66.02	83.33	0.082
Other costs (consults of physicians etc.) during admission 1	349.97	409.09	**0.018**
Total costs admission 2	77.00	849.96	**0.015**
Costs for nursing days (excl. IC) during admission 2	70.56	320.78	**0.029**
Costs for nursing days IC during admission 2	0	350.56	0.134
Costs for surgery during admission 2	6.24	111.02	**0.013**
Costs for laboratory tests during admission 2	0	27.2	**0.013**
Costs for radiodiagnostics and functional assessments during admission 2	0	5.5	**0.038**
Other costs (consults of physicians etc.) during admission 2	0.2	34.9	**0.011**
Total costs admission 3	40.21	0.7	0.292
Total costs for examinations and laboratory tests performed in outpatient departments during the follow-up period	38.91	32.03	0.437

Table 1. Mean hospital costs (€) for patients treated with mupirocin/chlorhexidine or placebo.
Data for cardiothoracic and orthopaedic patients are combined.
MUP/CHX, mupirocin/chlorhexidine

second admission, costs for nursing days, costs made during surgery, costs for laboratory and radiological tests, and functional assessments were found to be significantly lower in the treatment arm. Subgroup analysis showed that the mean expenses for mupirocin/chlorhexidine treated cardiothoracic patients were €2841 lower than for non-treated cardiothoracic patients (€9628 vs. €12469, *P*=0.006) and €955 lower for mupirocin/chlorhexidine treated orthopaedic patients compared to non-treated orthopaedic patients (€6097 vs. €7052, *P*=0.05) (Figure 2).

The distribution of costs depicted in the box plot (Figure 2) shows that the difference in costs between the two treatment groups is mainly caused by a number of patients with higher costs in the placebo group compared to the mupirocin/chlorhexidine group. This holds true for both the cardiothoracic and the orthopaedic patients. Four of these patients suffered from a deep endogenous *S. aureus* infection.

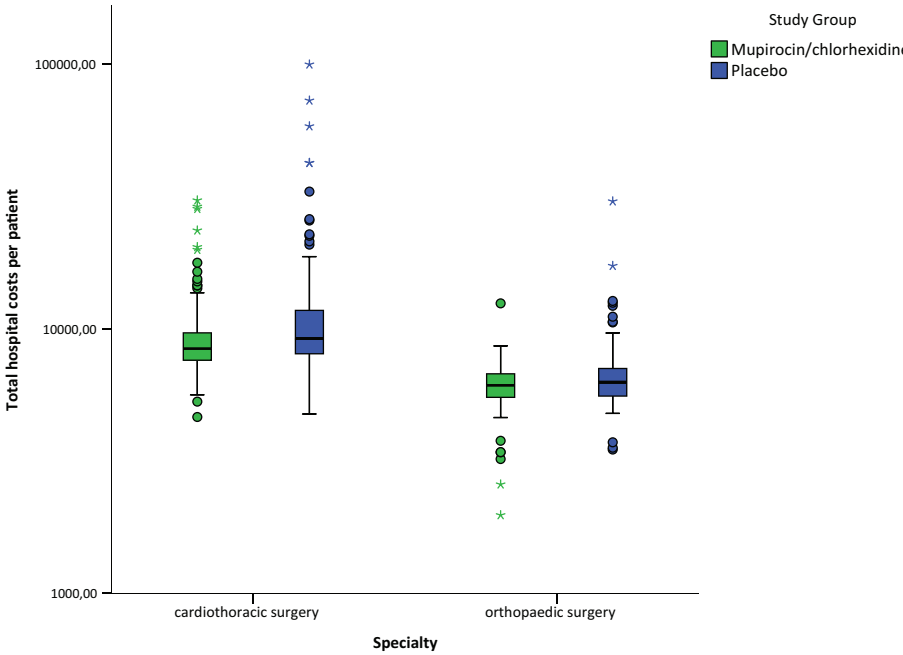

Figure 2. Box plot of total hospital costs for patients treated prophylactically with mupirocin/chlorhexidine or placebo. Total costs were estimated for the period between the dates of admission and the end of follow-up. Data are shown for cardiothoracic patients and orthopaedic surgical patients, separately. Patients with highest costs are shown in bullets • (between 1,5 and 3 times the interquartile range) and asterisks * (more than 3 times the interquartile range).

In the placebo group, 13 of 205 patients acquired a *S. aureus* infection in the hospital, compared to 3 of 210 patients in the mupirocin/chlorhexidine group (P=0.01). The hospital costs for uninfected patients varied between €1986 and €72704, with a mean of €8834 and a median of €7898. For infected patients these ranged between €3693 and €99512, with a mean of €27313 and a median of €19707 (P<0.001).

Discussion

This study shows that mean hospital costs for nasal *S. aureus* carriers undergoing elective cardiothoracic or orthopaedic surgery receiving treatment with mupirocin/chlorhexidine were significantly lower than for patients without treatment (placebo). This was caused by significantly higher hospital costs for *S. aureus* infected patients ($P<0.001$) in combination with significantly more *S. aureus* infected patients in the placebo group ($P=0.01$). It must be noted that for cardiothoracic surgery, nine of twenty patients with highest costs suffered from a deep *S. aureus* infection, i.e. eight cases of mediastinitis after CABG with/without valve replacement, and one case of pericarditis after pericardiectomy. Thus, almost half of the patients incurring the highest costs suffered from a deep *S. aureus* infection. This explains why prevention of these infections by application of mupirocin/chlorhexidine results in a significant cost reduction. In orthopaedic surgery, two deep-seated infections developed, one after total knee replacement and one after total hip revision. Costs of these two patients were found in the group of 25 patients with highest costs.

To put these results into perspective, this screen-and-treat strategy for *S. aureus* nasal carriers undergoing cardiothoracic or orthopaedic surgery would save the Amphia hospital approximately € 1,500,000 per year.

The *S. aureus* screen-and-treat strategy was already shown to result in a higher quality of patient care by reducing the number of *S. aureus* infections.[6] Lower costs and safer patient care were also found in the subgroups of patients undergoing cardiothoracic surgery or orthopaedic surgery. Other authors already estimated that introduction of a screen-and-treat strategy would result in lower hospital costs.[18-20] For example, the study by Wassenberg *et al*. was based on the actual hospital costs for patients with deep-seated prosthetic joint and cardiac surgery infections in combination with the evidence-based assumptions that non-carriers have six times less chance of acquisition of such infections than *S. aureus* carriers, and that the relative risk of deep-seated *S. aureus* infections after mupirocin/chlorhexidine treatment was 0.21 compared to placebo.[6, 15] The strength of the present study is, that it is the first to calculate the real hospital costs based on the data files of the P&C department. The analyses of this study were performed in an investigator-blinded fashion and patients were randomly assigned to either placebo or treatment arms.[6]

The results of the present study are useful for hospitals that are planning to implement the screen-and-treat strategy, but which need more evidence to convince their financial management. Some hospitals prefer to implement the treat-all strategy instead of the screen-and-treat strategy, mainly for two reasons. They argue that first, treating all patients is cheaper

than screening all patients and subsequently treating nasal *S. aureus* carriers, and second, this procedure is more convenient for the HCWs.[16, 18] Both a screen-and-treat and a treat-all strategy have been proven cheaper for the hospital than no screening or treatment at all. Of course, this treat-all strategy is cheaper than the screen-and-treat strategy, because the costs of the screening test, which are more expensive than the costs for mupirocin ointment and antibacterial soap, can be omitted. Wassenberg showed that treating all patients without screening would result in a saving of €7339 per life year gained, as compared to €3330 if only identified carriers were treated.[18] The low price and safety of mupirocin will easily lead to non-prudent use of this important antimicrobial agent. However, this treat-all strategy is associated with a high rate of unnecessary and thus unethical treatments that increase the likelihood of the development of resistance.[21] Mupirocin resistance will obviously lead to failure of *S. aureus* decolonization strategies. Cautious use of mupirocin is likely to maintain mupirocin resistance at a low level, thus preserving its efficacy. The aim of the prophylactic treatment is not to eradicate *S. aureus* forever but to result in short-term *S. aureus* eradication of approximately a month to prevent postoperative *S. aureus* wound infections. It was shown that combined low-level mupirocin and genotypic chlorhexidine resistance significantly increases the risk of persistent methicillin-resistant *S. aureus* (MRSA) carriage after decolonization therapy.[22] Although the MRSA rates in The Netherlands are still low, it is useful to monitor for mupirocin and chlorhexidine resistance in hospitals using a screen-and-treat strategy for *S. aureus* carriage.[23] In the Amphia hospital, mupirocin/chlorhexidine has been used for over 15 years in cardiothoracic surgery and to date, mupirocin resistance after treatment has not been found (unpublished data).

In order to resolve practical issues, patients planned for elective cardiothoracic or orthopaedic surgery should be screened preoperatively in the outpatients department, and for those found to be a carrier, a prescription should be send to the community pharmacy by the physician, so that patients can start treatment at home prior to admission. This treatment can be continued and finished in the hospital. For patients admitted without prior screening, rapid testing using molecular tools is an option, available 24 hours a day for optimal patient care.

The results of this study clearly show a financial benefit associated with the screen-and-treat strategy in elective cardiothoracic and orthopaedic surgery. Based on the nasal *S. aureus* carriage rate of 20% we found in the study, per thousand surgical patients approximately €400,000 could be saved. Worldwide millions of surgical procedures are performed each year, so huge numbers of patients would benefit from this strategy, and this would be accompanied by large savings. The US Centers for Disease Control have now included this strategy in their top recommendations for safer health care (http://www.cdc.gov/HAI/prevent/top-cdc-recs-prevent-hai.html). For other surgical procedures or non-surgical hospitalizations, debate is still open on the economic impact of such a strategy.

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References

1. Williams RE. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. Bacteriol Rev. 1963 Mar;27:56-71.
2. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 2005 Dec;5(12):751-62.
3. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev. 1997 Jul;10(3):505-20.
4. Kluytmans JA, Mouton JW, Ijzerman EP, Vandenbroucke-Grauls CM, Maat AW, Wagenvoort JH, et al. Nasal carriage of *Staphylococcus aureus* as a major risk factor for wound infections after cardiac surgery. J Infect Dis. 1995 Jan;171(1):216-9.
5. Wertheim HF, Vos MC, Ott A, Voss A, Kluytmans JA, Vandenbroucke-Grauls CM, et al. Mupirocin prophylaxis against nosocomial *Staphylococcus aureus* infections in nonsurgical patients: a randomized study. Ann Intern Med. 2004 Mar 16;140(6):419-25.
6. Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, Roosendaal R, et al. Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. N Engl J Med. 2010 Jan 7;362(1):9-17.
7. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. N Engl J Med. 2001 Jan 4;344(1):11-6.
8. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. Infect Control Hosp Epidemiol. 1999 Apr;20(4):250-78; quiz 79-80.
9. Boelaert JR, De Smedt RA, De Baere YA, Godard CA, Matthys EG, Schurgers ML, et al. The influence of calcium mupirocin nasal ointment on the incidence of Staphylococcus aureus infections in haemodialysis patients. Nephrol Dial Transplant. 1989;4(4):278-81.
10. Garcia AM, Villa MV, Escudero ME, Gomez P, Velez MM, Munera MI, et al. [Use of nasal mupirocin for Staphylococcus aureus: effect on nasal carriers and nosocomial infections]. Biomedica. 2003 Jun;23(2):173-9.
11. Harbarth S, Dharan S, Liassine N, Herrault P, Auckenthaler R, Pittet D. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 1999 Jun;43(6):1412-6.
12. Kalmeijer MD, Coertjens H, van Nieuwland-Bollen PM, Bogaers-Hofman D, de Baere GA, Stuurman A, et al. Surgical site infections in orthopedic surgery: the effect of mupirocin nasal ointment in a double-blind, randomized, placebo-controlled study. Clin Infect Dis. 2002 Aug 15;35(4):353-8.
13. Konvalinka A, Errett L, Fong IW. Impact of treating Staphylococcus aureus nasal carriers on wound infections in cardiac surgery. J Hosp Infect. 2006 Oct;64(2):162-8.
14. Nasal mupirocin prevents *Staphylococcus aureus* exit-site infection during peritoneal dialysis. Mupirocin Study Group. J Am Soc Nephrol. 1996 Nov;7(11):2403-8.
15. Perl TM, Cullen JJ, Wenzel RP, Zimmerman MB, Pfaller MA, Sheppard D, et al. Intranasal mupirocin to prevent postoperative *Staphylococcus aureus* infections. N Engl J Med. 2002 Jun 13;346(24):1871-7.
16. Perez-Fontan M, Rosales M, Rodriguez-Carmona A, Moncalian J, Fernandez-Rivera C, Cao M, et al. Treatment of Staphylococcus aureus nasal carriers in CAPD with mupirocin. Adv Perit Dial. 1992;8:242-5.
17. van Rijen M, Bonten M, Wenzel R, Kluytmans J. Mupirocin ointment for preventing Staphylococcus aureus infections in nasal carriers. Cochrane database of systematic reviews (Online).

2008(4):CD006216.

18. Wassenberg MW, de Wit GA, Bonten MJ. Cost-effectiveness of preoperative screening and eradication of Staphylococcus aureus carriage. PLoS One. 2011;6(5):e14815.
19. Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Jacobson C, et al. Budget impact analysis of rapid screening for *Staphylococcus aureus* colonization among patients undergoing elective surgery in US hospitals. Infect Control Hosp Epidemiol. 2008 Jan;29(1):16-24.
20. Courville XF, Tomek IM, Kirkland KB, Birhle M, Kantor SR, Finlayson SR. Cost-effectiveness of preoperative nasal mupirocin treatment in preventing surgical site infection in patients undergoing total hip and knee arthroplasty: a cost-effectiveness analysis. Infect Control Hosp Epidemiol. 2012 Feb;33(2):152-9.
21. Cookson BD. The emergence of mupirocin resistance: a challenge to infection control and antibiotic prescribing practice. J Antimicrob Chemother. 1998 Jan;41(1):11-8.
22. Lee AS, Macedo-Vinas M, Francois P, Renzi G, Schrenzel J, Vernaz N, et al. Impact of combined low-level mupirocin and genotypic chlorhexidine resistance on persistent methicillin-resistant Staphylococcus aureus carriage after decolonization therapy: a case-control study. Clin Infect Dis. 2011 Jun 15;52(12):1422-30.
23. Bode LG, Wertheim HF, Kluytmans JA, Bogaers-Hofman D, Vandenbroucke-Grauls CM, Roosendaal R, et al. Sustained low prevalence of meticillin-resistant Staphylococcus aureus upon admission to hospital in The Netherlands. J Hosp Infect. 2011 Nov;79(3):198-201.

Chapter 5

Emerging mupirocin resistance in staphylococci

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Abstract

We obtained data on the dispension of mupirocin, and laboratory data on mupirocin resistance in staphylococci per hospital ward. In wards where large amounts of mupirocin were used, a significantly higher proportion of coagulase negative staphylococci (CoNS) isolates were high-level resistant to mupirocin compared to wards where mupirocin was used less frequently.

Introduction

Treatment with Pseudomonic acid A, or mupirocin, is effective in eliminating *S. aureus* carriage.[1,2] Eliminating *S. aureus* from carriers results in a reduction of exit-site infections and peritonitis in renal dialysis patients, and in a reduction of surgical site infections.[3, 4]

Mupirocin is well-tolerated and cheap.[2] Although non-carriers do not benefit from *S. aureus* decolonization treatment, many hospitals do not screen patients for *S. aureus* carriage but prophylactically treat all surgical patients with a five-day course of mupirocin nasal ointment, with or without chlorhexidine body washes.

Resistance to mupirocin has been reported since the mid-1980's. Low-level mupirocin resistance is mediated by mutations in the native tRNA synthetase, resulting in minimum inhibitory concentrations (MICs) of 8-64 µg/mL and leading to rapid recolonization after stopping treatment.[5, 6] High-level resistance, mediated by the acquisition of the novel tRNA synthetase *mupA*, results in MICs ≥512 µg/mL and treatment failure.[6] High- and low-level mupirocin resistance is emerging, particularly in methicillin-resistant *S. aureus* (MRSA) and coagulase negative staphylococci (CoNS).[7-10] Recently, Bathoorn *et al*. reported that emerging high-level mupirocin resistance in CoNS in a tertiary center was associated with an increase in the use of mupirocin.[10]

In the Erasmus MC, a university hospital in The Netherlands, mupirocin has been widely used without screening for *S. aureus* carriage in the cardiothoracic ward and the dialysis center. On most other wards, mupirocin is used only infrequently.

We hypothesized that in wards where mupirocin is frequently used, the proportion of methicillin-susceptible *S. aureus* (MSSA) and CoNS isolates that are resistant to mupirocin would be significantly higher than in wards where mupirocin is used only occasionally. Furthermore, we hypothesized that mupirocin high-level resistance emerges in time when its use increases.

Methods

The Erasmus MC pharmacy provided data on the dispensing of 3 and 15 g tubes of mupirocin ointment 20 mg/g (2%) for nasal/topical use per ward from 2000 to June 2013. From the laboratory of medical microbiology, data on MSSA and CoNS for which an MIC for mupirocin was determined were available from June 2010 to June 2013. Susceptibility testing was performed either by Vitek 2 (bioMérieux) or E-test (bioMérieux). For *S. aureus*, EUCAST breakpoints are MIC

≤1 µg/mL susceptible, MIC >256 µg/mL resistant. However, the detection range of the Vitek 2 is ≤2 µg/mL and >256 µg/mL. Since 2 µg/mL is a very rare MIC for MSSA, and since for CoNS no breakpoints are available, we considered MIC ≤2 µg/mL susceptible, MICs 4-256 µg/mL low-level resistant, and MIC >256 µg/mL high-level resistant to mupirocin. For the analysis, we selected one isolate per patient, or more isolates when antibiograms differed between isolates.

Results

From 2000 to 2006, the pharmacy annually dispensed up to 4000 g of mupirocin ointment 2% per year within the hospital (range 2076-3850, median 3435 g ointment 2%/year). From 2007 to 2012, more than 4000 g of mupirocin ointment 2% was annually delivered (range 4365-5085, median 4743 g/year), with the largest amount in 2012. From January to June 2013, 2403 g of mupirocin ointment 2% was dispensed.

The largest amounts of mupirocin were dispensed to two cardiothoracic wards, two cardiology wards, and the dialysis ward: >200 g ointment 2%/year was delivered to each of these wards (range 232-1564, median 443 g/year). Two non-cardiac ICUs, a pulmonary medicine ward, the gastroenterology ward and one haematology ward used 50-200 g ointment 2%/year each (range 59-130, median 74 g/year). To all other wards less than 50 g ointment 2%/year was dispensed (range 0-36 g/year).

In total, 3686 MSSA isolates were selected for analysis (wounds n=944; lower respiratory tract n=840; nose n=394; blood n=151; other n=1357). Of these, 11 (0.3%) were high-level resistant, and 3 (0.08%) were low-level resistant to mupirocin. No increase of resistance in time was observed, and based on antibiograms, resistant isolates were unrelated.

A total of 1608 CoNS isolates were available for the analysis (blood n=897; other n=711). Of those, 283 (17.6%) were high-level resistant, 72 (4.5%) were low-level resistant (4.5%) and 1253 (77.9%) were susceptible to mupirocin.

In 2010, 40/283 (14.1%) CoNS were high-level resistant to mupirocin. In 2011, 2012 and 2013, 76/531 (14.3%), 112/541 (20.7%) and 55/253 (21.7%) isolates were high-level mupirocin resistant, respectively. The increase in the proportion of resistant isolates from 2011 to 2012 was significant (P=0.006; Chi-square test). Since changing management may have influenced the indication of taking cultures over the years, we also analyzed the proportion of resistance in blood isolates only. In 2010, 2011, 2012 and 2013, 20/145 (13.8%), 34/277 (12.3%), 61/315 (19.4%) and 38/160 (23.8%) of blood culture CoNS isolates were high-level mupirocin resistant,

respectively. This increase from 2011 to 2012 was also significant (*P*=0.02).

On the five wards where >200 g ointment 2%/year was delivered, 84 CoNS were isolated from blood. Of those, 38 (45.2%) were high-level mupirocin resistant. On wards where 50-200g ointment 2%/year and <50 g ointment 2%/year was delivered, the proportion of mupirocin high-level resistant CoNS blood isolates was 54/300 (18.0%) and 61/513 (11.9%), respectively (see Figure 1 for *P*-values).

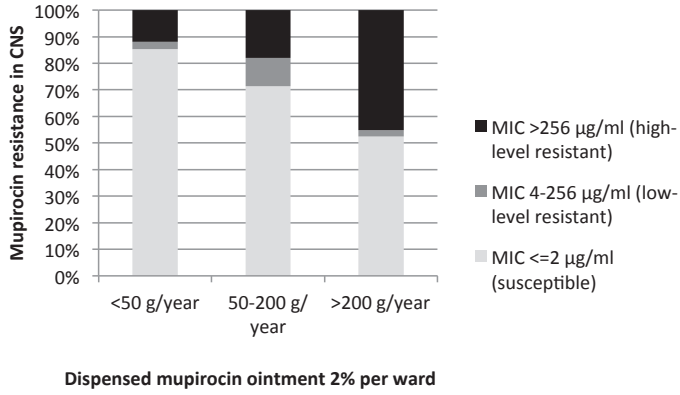

Figure 1. Mupirocin resistance in CoNS in relation to the dispensed amounts of mupirocin.
(*) denotes P<0.001, (**) denotes P=0.02 for the difference between the proportions of mupirocin high-level resistant isolates (Chi-square test).

Discussion

On the cardiothoracic, cardiology, and dialysis wards, where >200 g mupirocin ointment 2% per year was dispensed, a significantly higher proportion of the CoNS isolated was high-level resistant to mupirocin than on wards where less mupirocin was delivered. Based on antimicrobial susceptibility patterns, no large clusters of CoNS were found. From cardiothoracic and cardiology wards for example, 70 high-level mupirocin resistant isolates with at least 33 different antibiograms were cultured. The largest possible cluster consisted of ten isolates from all four different wards, isolated between June 2010 and November 2012. Therefore, dissemination of a mupirocin resistant clone does not seem to be responsible for the high proportion of mupirocin resistance observed.

Acquisition of resistance by isolates during or after mupirocin therapy is also not a very likely explanation for the observed high level of resistance in CoNS on wards with frequent use of mupirocin, since high-level resistance is plasmid-mediated, and not induced by mupirocin. A more likely explanation may be that patients are already colonized with mupirocin resistant isolates upon admission to hospital. After elimination of mupirocin sensitive CoNS and *S. aureus* clones by routine application of mupirocin, resistant isolates colonize the nose and body of patients, and cause infections during hospital stay. Approximately half of the patients admitted to the cardiothoracic wards of the Erasmus MC are referred for surgery from other hospitals, and may therefore be colonized with more resistant pathogens than patients from the community.

In CoNS we also observed a significant increase in the proportion of high-level resistant isolates between 2011 and 2012. Since the mid-1990s, all cardiothoracic patients in the Erasmus MC receive a course of mupirocin pre-surgically, irrespective of *S. aureus* carriage state. Since the cardiothoracic wards of the Erasmus MC are located in a separate building, these patients are unlikely to mix with other patient groups. From 2006 onwards, the number of cardiothoracic procedures has increased from approximately 800 per year, to 1100 per year nowadays, which has accordingly resulted in increased dispension of mupirocin to these wards. The amount of antibiotic used per patient however has not increased. In general though, over the past few years, mupirocin use has increased in Dutch hospitals, e.g. in orthopedic, vascular and general surgery, as part of the perioperative strategy to prevent *S. aureus* infections in carriers.[10] The increase of high-level resistance may therefore be associated with the increased use of mupirocin in general.

Since mupirocin susceptibility was not routinely measured before June 2010 in our laboratory, no earlier data on resistance are available. Although it is unknown whether the rate of development of resistance we observed here is stable or increasing, it is certainly alarming. With the increase of resistance in CoNS, the risk of transfer of the plasmid to *S. aureus* also increases, which has been observed in a clinical situation yet.[12] To preserve mupirocin as an effective therapeutic and preventive agent against *S. aureus*, development of resistance in CoNS should be avoided. Therefore, antibiotic stewardship programs of hospitals should not only focus on broad-spectrum antibiotics, but also on topical, small-spectrum antibiotics, like mupirocin.

In conclusion, mupirocin resistance in MSSA is observed only sporadically. In CoNS, high-level resistance is observed frequently on wards where mupirocin is often used, and may be the result of selection of resistant clones by mupirocin. This hypothesis should be investigated in a patient centered trial. Furthermore, resistance seems to emerge in CoNS, and may be associated with the increased use of mupirocin for prevention of surgical site infections in several types of surgery. As this is not the first study observing an association between mupirocin use and development of resistance,[6, 10] the indications for the use of this antibiotic have to be considered

carefully. From the perspective of prevention of antibiotic resistance, the use of mupirocin as antibiotic prophylaxis should be restricted to patients colonized with *S. aureus* who will undergo invasive procedures with a high risk of surgical site infections with *S. aureus*.

References

1. Scully BE, Briones F, Gu JW, Neu HC. Mupirocin treatment of nasal staphylococcal colonization. Arch Intern Med. 1992; **152**(2): 353-6.
2. Doebbeling BN, Breneman DL, Neu HC, Aly R, Yangco BG, Holley HP, Jr., et al. Elimination of Staphylococcus aureus nasal carriage in health care workers: analysis of six clinical trials with calcium mupirocin ointment. The Mupirocin Collaborative Study Group. Clin Infect Dis. 1993; **17**(3): 466-74.
3. Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, Roosendaal R, et al. Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. N Engl J Med. 2010; **362**(1): 9-17.
4. van Rijen M, Bonten M, Wenzel R, Kluytmans J. Mupirocin ointment for preventing Staphylococcus aureus infections in nasal carriers. Cochrane database of systematic reviews (Online). 2008; (4): CD006216.
5. Harbarth S, Dharan S, Liassine N, Herrault P, Auckenthaler R, Pittet D. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 1999; **43**(6): 1412-6.
6. Patel JB, Gorwitz RJ, Jernigan JA. Mupirocin resistance. Clin Infect Dis. 2009; **49**(6): 935-41.
7. Deshpande LM, Fix AM, Pfaller MA, Jones RN. Emerging elevated mupirocin resistance rates among staphylococcal isolates in the SENTRY Antimicrobial Surveillance Program (2000): correlations of results from disk diffusion, Etest and reference dilution methods. Diagnostic microbiology and infectious disease. 2002; **42**(4): 283-90.
8. Desroches M, Potier J, Laurent F, Bourrel AS, Doucet-Populaire F, Decousser JW, et al. Prevalence of mupirocin resistance among invasive coagulase-negative staphylococci and methicillin-resistant Staphylococcus aureus (MRSA) in France: emergence of a mupirocin-resistant MRSA clone harbouring mupA. J Antimicrob Chemother. 2013; **68**(8): 1714-7.
9. Lepainteur M, Royer G, Bourrel AS, Romain O, Duport C, Doucet-Populaire F, et al. Prevalence of resistance to antiseptics and mupirocin among invasive coagulase-negative staphylococci from very preterm neonates in NICU: the creeping threat? J Hosp Infect. 2013; **83**(4): 333-6.
10. Bathoorn E, Hetem DJ, Alphenaar J, Kusters JG, Bonten MJ. Emergence of high level mupirocin resistance in coagulase-negative staphylococci associated with increased short-term mupirocin use. Journal of clinical microbiology. 2012.
11. www.eucast.org. [cited July 14, 2013]; Available from: www.eucast.org
12. Hurdle JG, O'Neill AJ, Mody L, Chopra I, Bradley SF. In vivo transfer of high-level mupirocin resistance from Staphylococcus epidermidis to methicillin-resistant Staphylococcus aureus associated with failure of mupirocin prophylaxis. J Antimicrob Chemother. 2005; **56**(6): 1166-8.

Chapter 6

Healthcare associated methicillin-sensitive Staphylococcus aureus infections: incidence, sources, and the role of nasal carriage

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Submitted

Abstract

Background

To develop measures to prevent healthcare related *S. aureus* infections, more insight into the epidemiology of exogenous infections is needed.

Methods

Patients were screened for nasal *S. aureus* carriage upon admission to a university hospital in The Netherlands. Those developing a *S. aureus* infection within 42 days after screening were included in the analysis. Raman spectroscopy was used for typing of isolates.

Results

In 48/1738 (2.8%) patients screened, a *S. aureus* infection developed. Infections developed significantly more frequent in carriers than in non-carriers (OR 2.05, 95% CI [1.15-3.68]). Thirty-four infections were of exogenous origin (28 in non-carriers, 6 in carriers). Raman spectroscopy showed that seven (20.6%) of these infections clustered in four small clusters.

For the total hospital population, the proportion of patients acquiring exogenous infections (1.9-2.2%) was significantly different from the proportion developing endogenous *S. aureus* infections (0.6-0.9%) ($P \leq 0.01$).

Conclusions

S. aureus nasal carriers are at increased risk of developing a *S. aureus* infection as compared to non-carriers, but the majority of healthcare associated *S. aureus* infections are of exogenous origin. Furthermore, small clusters of identical *S. aureus* strains cause infections, without being recognized. Prevention of *S. aureus* infections should therefore focus on both endogenous and exogenous infections.

Introduction

Staphylococcus aureus (S. aureus) colonizes the nares, throat, and skin of a significant part of the human population.[1] *S. aureus* carriers have a higher risk of developing healthcare related infections with this pathogen than non-carriers.[2] Furthermore, 80% of *S. aureus* bloodstream infections that develop in carriers are of endogenous origin.[1] Several studies show that the incidence of *S. aureus* infections in various study populations can be reduced by eradicating the pathogen from the nares and skin of carriers.[3, 4]

Little is known about the incidence and sources of healthcare related *S. aureus* infections in non-carriers. Outbreaks of methicillin-susceptible *S. aureus* have been described,[5, 6] but knowledge on the clonal relationship of strains causing exogenous in-hospital MSSA infections in non-outbreak settings is only scarce.[7, 8] To develop measures to prevent healthcare related *S. aureus* infections, more insight into the epidemiology of exogenous infections is needed.

The objectives of the present study were to estimate the incidence of *S. aureus* infections in carriers and non-carriers, to estimate the proportion of endogenous and exogenous infections, and to gain insight into the clonality of strains causing *S. aureus* infections.

Methods

Patients from surgical and non-surgical wards were screened upon admission to the Erasmus University Medical Center in Rotterdam to assess nasal carriage of *S. aureus*. Informed consent was obtained orally. A dry swab was rotated in both nares, and processed for real-time PCR as well as for culture with broth enrichment. Outcome of screening assessed by PCR was an eligibility criterion for enrolment in a randomized, controlled trial for the prevention of hospital-acquired *S. aureus* infections.[3] Patients enrolled in the randomized controlled trial received either mupirocin nasal ointment and chlorhexidine gluconate medicated soap, or placebo ointment and placebo soap. Since treatment with mupirocin/chlorhexidine significantly decreased the incidence of healthcare associated *S. aureus* infections as compared to placebo treatment, those patients who received mupirocin and chlorhexidine were excluded from analysis for the present study.[3] All other patients screened were included in the present study, and defined as carrier or non-carrier based on culture results. The laboratory database was checked for clinical cultures from which *S. aureus* was isolated between two days and six weeks after screening. The medical charts of these patients were reviewed to distinguish between colonization and infection with *S. aureus,* using definitions established by the Centers for Disease Control and Prevention.[9] Patients

with a documented infection were included in the analysis.

Raman spectroscopy was used for typing of the isolates and to establish the presence of clonal relationship between strains. The methods used were as described before.[10] More than half of the isolates available were typed in duplicate to assess reproducibility. We defined an infection as endogenous if the isolate from the nose and the isolate from the site of infection were found in the same cluster, or if the correlation coefficient of these isolates was higher than the lowest correlation coefficient of the duplicates. In all other cases, infections were defined as exogenous.

An epidemiological link was considered if patients had been admitted to the same ward in the same time period; if patients had doctors or anaesthesiologists in common; or if patients had the same zip code.

Results

A total of 1834 patients were screened for the presence of *S. aureus* in the nares (Figure 1). The carriage rate was 551/1834 = 30.0%, as determined by culture. Ninety-six patients received mupirocin/chlorhexidine as study treatment in the RCT, and were therefore excluded from the analysis in the present study. In total, 1738 patients received either placebo medication or no study medication, and thus met the inclusion criteria for the present study. Of them, 456 patients carried *S. aureus* (456/1738=26.2%). In 48 patients a *S. aureus* infection developed within 42 days after screening (48/1738=2.8%). In *S. aureus* carriers, the incidence of *S. aureus* infection was 4.4% (20 infections in 456 carriers). In non-carriers, this incidence was 2.2% (28 infections in 1282 non-carriers). The odds ratio of infection for carriers compared to non-carriers was 2.05 (95% CI [1.15-3.68], *P*=0.014, Pearsons Chi-square).

Raman spectroscopy was performed for all infecting isolates and accompanying nasal isolates if available (Figure 2). Two nasal isolates were lost, as well as eight infecting isolates (three from carriers, five from non-carriers). In total, 58 isolates were available for typing. To assess the reproducibility and the cut-off for the correlation coefficient, 34 isolates were typed in duplicate. The cut-off was set at 0.999706.

For 15 out of 20 carriers who developed an infection with *S. aureus*, both the nasal and infection isolates were available for typing. In nine cases, the nasal and infecting isolates had correlation coefficients above the cut-off, and the infections were thus defined as endogenous.

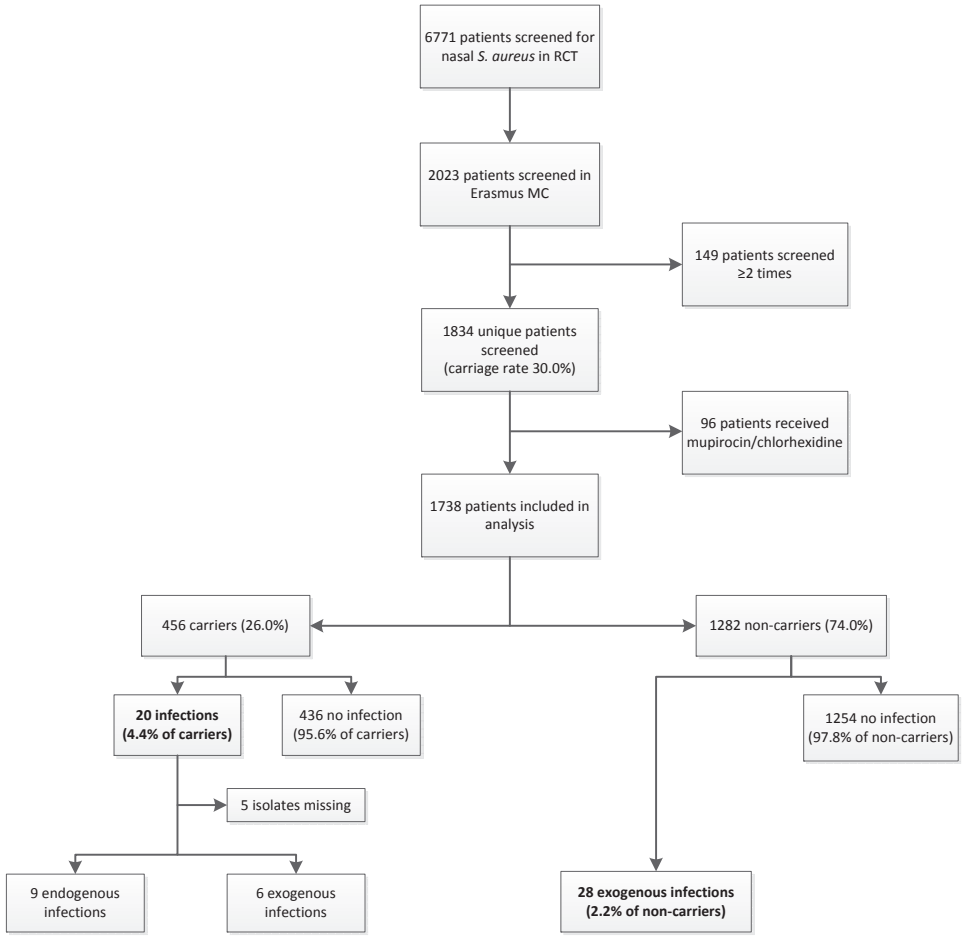

Figure 1. Flowchart of the patients screened, and included in the analysis.

In the other six cases, the nasal and infecting strains were markedly different in Raman patterns, and the infections were therefore defined as of exogenous origin. If the five missing isolates are taken into account, the proportion of endogenous infections is between 45 and 70%; the proportion of exogenous infections in carriers is between 30 and 55%. In total, 34 infections could be classified as exogenous (28 in non-carriers, 6 in carriers).

Seven of the 34 exogenous infections (20.6%) clustered with other isolates according to Raman spectroscopy. Five infecting strains of non-carriers were lost. The other 22 exogenous infections showed unique Raman patterns (Figure 2). Cluster 20 covered three isolates: the

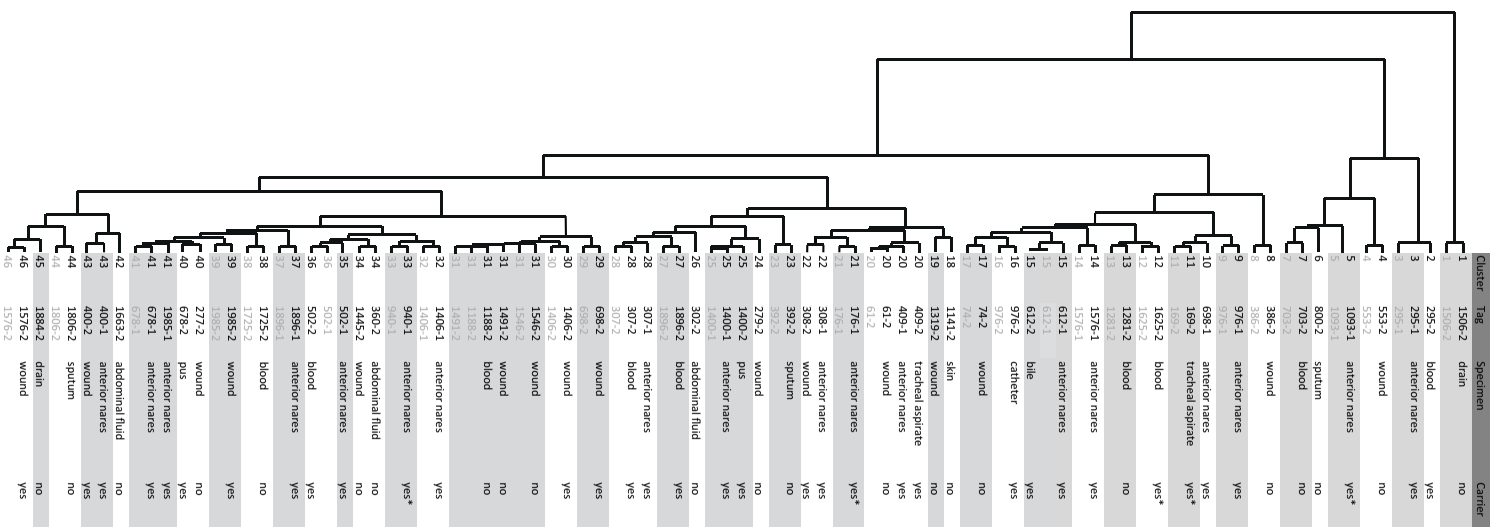

Figure 2. Hierarchical cluster analysis of the nasal and infecting *S. aureus* isolates.
Isolates in grey are duplicates and used for establishing the correlation coefficient cut-off. The tag is composed of the patient number and either -1 for a nasal isolate, or -2 for an infecting isolate. Based on the correlation coefficient (not shown), the infections of patients 295, 307, 308, 400, 409, 502, 612, 678, and 1400 are defined as endogenous.
*Accompanying nasal or infection isolate of this patient lost

nasal and infecting isolate of a carrier, and the infecting isolate of a non-carrier. Patient 61, the non-carrier, developed an infection several months before the carrier was screened. An epidemiological link could not be found. Cluster 31 covered three infecting isolates from three non-carriers admitted on the same surgical ward over a period of three months. Two patients in cluster 31 (1188 and 1546) developed a surgical site infection after amputation of the foot, but different surgeons and anesthesiologists were involved in surgery. The third patient in this cluster (1491) developed cellulitis after kidney transplantation. Patients 1491 and 1546 had overlapping admission periods; Patient 1188 was already discharged from hospital before the other two were admitted. It was not possible to find out whether the patients shared rooms or health-care workers, or if there were other situations where transmission could have taken place, other than the shared department and thus inanimate environment. A third cluster (cluster 34) covered two infecting isolates of non-carriers, isolated one year apart on different wards. No epidemiological link could be detected between these two patients. The fourth cluster (cluster 40) contained an infecting isolate of a non-carrier, and an infecting isolate of a carrier. The first patient developed a surgical-site infection after colorectal resection. The second patient was admitted for one day on the day of discharge of the first patient, but on another ward. He was not screened, neither had an infection, at that time. Three months later he was screened upon re-admission, and subsequently developed an endogenous surgical-site infection following treatment for esophageal stenosis. Surgeons and anesthesiologists were different and no other epidemiological link could be found between these patients.

Based on the carriage rate, the incidence of infections in the groups of carriers and non-carriers, and the proportion of endogenous infections, we calculated the proportion of patients admitted to hospital who developed exogenous and endogenous *S. aureus* infections (Figure 3). The proportion of exogenous infections was 1.9-2.2%, the proportion of endogenous infections was 0.6-0.9% (*P*=0.01 for 1.9% vs 0.9% (*z*-test for proportions)).

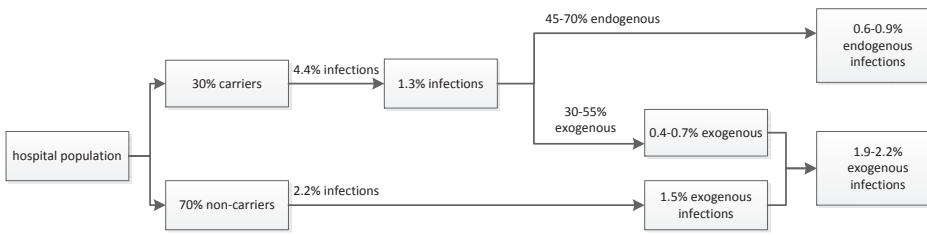

Figure 3. Proportions of endogenous and exogenous infections and calculated total risk of *S. aureus* infection in the population of patients admitted to hospital.
Proportions are as measured in the current study. For the population screened in this study, these proportions differ significantly (hospital population screened: n=1834; *P*=0.01 (*z*-test for proportions).
* Result of calculation without rounding off

Discussion

In this study, carriers of *S. aureus* developed a healthcare associated *S. aureus* infection significantly more frequently than non-carriers. In *S. aureus* carriers, the majority of infections were of endogenous origin, but for the hospital population in general, the proportion of exogenous infections was significantly higher than the proportion of endogenous *S. aureus* infections (1.9-2.2 vs 0.6-0.9%). Typing of the strains isolated in carriers and non-carriers revealed three small clonal clusters.

S. aureus is one of the leading causes of health-care related infections. Prevention of *S. aureus* infections is therefore an important aim to reduce costs, prolonged hospital stay, morbidity and mortality. The incidence of endogenous infections can be reduced by screening patients for *S. aureus* carriage upon admission, and treating proven carriers with mupirocin nasal ointment and chlorhexidine gluconate medicated soap.[3] However, this strategy does not apply to non-carriers or exogenous infections in carriers. The majority of patients are not colonized with *S. aureus*, and the proportion of exogenous infections was in our population even higher than the proportion of endogenous infections. Prevention of *S. aureus* infections should therefore not only focus on endogenous infections and thus on eradication of *S. aureus* carriage, but also on the avoidance of acquisition of pathogens from exogenous sources. We advocate the existence of a 'horizontal' approach, with a broad program attempting to reduce the rates of all infections due to all pathogens, next to the 'vertical' approach focusing on the single pathogen *S. aureus,* by screening and treating carriers upon admission to hospital.[11]

This study was performed on a selection of patients. First, we only included patients who were screened for nasal carriage of *S. aureus*. Patients who were not eligible for the randomized trial, for example if they were expected to be admitted for up to three days, were not screened. Possibly, more patients were infected or colonized with these strains but not cultured. Second, a proportion of the carriers were not included in this study as they were randomized to mupirocin and chlorhexidine treatment. It is alarming that we could identify clusters of identical strains in such a small selection of the in-hospital patient population. It is likely that these small clusters represent just the tip of the iceberg.

We can only speculate about the transmission route of the infecting *S. aureus* strains, since we screened only a small proportion of the hospital population, and we did not screen health-care workers, medical equipment or other possible environmental sources for the presence of *S. aureus*. This study shows the importance of general preventive measures to avoid spread of pathogens throughout the hospital. Hand hygiene compliance in a Dutch study conducted in 24 hospitals was less than 20%, with hand hygiene before patient contact at merely 2%

(unpublished data: V. Erasmus PhD; M. Vos MD PhD, J.H. Richardus MD PhD, P. van Empelen PhD, H.A. Verbrugh MD PhD, A. Oenema PhD, T.J. Daha, E.W. Steyerberg PhD, E.F. van Beeck PhD; April 2012). Compliance in the Erasmus MC, that participated in this study, was not significantly different from the other hospitals. Attention should therefore be given to improvement of compliance, since awareness of the importance of hand hygiene is lacking, and compliance significantly reduces the rate of health-care associated infections.[12, 13]

We defined an infection as exogenous if a nasal carrier was infected with a strain different from the strain isolated from his nares, or if a non-carrier developed an infection. Since we used broth enrichment to culture *S. aureus* from nasal swabs, low-level and intermittent carriers were also detected. Generally, these patients are considered to be at lower risk of developing endogenous infections.[14] This may not only explain the high proportion of carriers we found, but also the relatively large proportion of exogenous infections in carriers, compared to results of previous studies.[1] Furthermore, although the nares are the primary niche for *S. aureus* in carriers, some carriers are exclusively colonized at extra-nasal sites.[15] It has not been studied sufficiently yet whether extra-nasal strains in carriers are identical to the strain carried in the nose, or whether extra-nasal sites serve as a source for endogenous *S. aureus* infections to the same extent as the nares. Studies on this topic are limited, conflicting, and many are performed in small population samples.[16, 17] Therefore, it is possible that infections classified as exogenous and not clustering with other infecting strains are actually endogenous infections from extra-nasal sites. Future studies should investigate in a large population sample whether nasal and extra-nasal strains in carriers are identical or not, and whether infections in non-nasal carriers are of extra-nasal or exogenous origin. With the outcomes of those studies, prevention should either focus mainly on transmission of strains from true exogenous reservoirs, or also on endogenous infections in non-nasal carriers.

Availability of routine daily typing results makes it possible to recognize transmission of pathogens in hospital at an early stage. Ideally, every newly isolated infecting strain should be compared with previously isolated strains to be able to recognize clusters of strains and possible transmissions early. Raman spectroscopy is a rapid, easy-to-use, high-throughput typing system with a high reproducibility.[10] Furthermore, newly detected and typed strains can easily be compared to already typed strains available in the Raman-dataset. These characteristics make Raman spectroscopy a useful typing system in the diagnostic microbiological laboratory.

In conclusion, this study shows that *S. aureus* nasal carriers are at increased risk of developing a *S. aureus* infection as compared to non-carriers, but the majority of healthcare associated *S. aureus* infections are of exogenous origin. Furthermore, small clusters of identical *S. aureus* strains cause infections, without being recognized. Prevention of *S. aureus* infections, and

healthcare associated infections in general, should therefore be on the top end list of measures to improve patient safety. Next to the implementation of vertical interventions such as *S. aureus* screening and treatment of carriers, emphasis is needed on the horizontal approach: on preventive measures like hand hygiene, strict separation of clean and dirty environments, and avoiding the sharing of utensils and the inanimate environment. In this respect, single patient rooms are to be preferred above multi-bed hospital rooms. Furthermore, future studies should focus on the presence of *S. aureus* on other sites than the nose, which can act as a reservoir and a source for *S. aureus* infections.

References

1. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 2005; 5(12): 751-62.
2. Weinstein HJ. The relation between the nasal-staphylococcal-carrier state and the incidence of postoperative complications. N Engl J Med. 1959; 260(26): 1303-8.
3. Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, Roosendaal R, et al. Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. N Engl J Med. 2010; 362(1): 9-17.
4. Nasal mupirocin prevents *Staphylococcus aureus* exit-site infection during peritoneal dialysis. Mupirocin Study Group. J Am Soc Nephrol. 1996; 7(11): 2403-8.
5. Wilcox MH, Fitzgerald P, Freeman J, Denton M, Gill AB, Hoy C, et al. A five year outbreak of methicillin-susceptible Staphylococcus aureus phage type 53,85 in a regional neonatal unit. Epidemiol Infect. 2000; 124(1): 37-45.
6. Boers SA, van Ess I, Euser SM, Jansen R, Tempelman FR, Diederen BM. An outbreak of a multiresistant methicillin-susceptible Staphylococcus aureus (MR-MSSA) strain in a burn centre: the importance of routine molecular typing. Burns. 2011; 37(5): 808-13.
7. Vriens MR, Fluit AC, Troelstra A, Verhoef J, van der Werken C. Is methicillin-resistant Staphylococcus aureus more contagious than methicillin-susceptible S. aureus in a surgical intensive care unit? Infect Control Hosp Epidemiol. 2002; 23(9): 491-4.
8. Van Dijk Y, Wielders CL, Fluit AC, Paauw A, Diepersloot RJ, Mascini EM. Genotyping of clinical methicillin-susceptible Staphylococcus aureus isolates in a Dutch teaching hospital. Journal of clinical microbiology. 2002; 40(2): 663-5.
9. Horan TC, Gaynes RP. Hospital Epidemiology and Infection Control. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2004.
10. Willemse-Erix DF, Scholtes-Timmerman MJ, Jachtenberg JW, van Leeuwen WB, Horst-Kreft D, Bakker Schut TC, et al. Optical fingerprinting in bacterial epidemiology: Raman spectroscopy as a real-time typing method. Journal of clinical microbiology. 2009; 47(3): 652-9.
11. Wenzel RP, Edmond MB. Infection control: the case for horizontal rather than vertical interventional programs. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2010; 14 Suppl 4: S3-5.
12. Erasmus V, Brouwer W, van Beeck EF, Oenema A, Daha TJ, Richardus JH, et al. A qualitative exploration of reasons for poor hand hygiene among hospital workers: lack of positive role models and of convincing evidence that hand hygiene prevents cross-infection. Infect Control Hosp Epidemiol. 2009; 30(5): 415-9.
13. Kirkland KB, Homa KA, Lasky RA, Ptak JA, Taylor EA, Splaine ME. Impact of a hospital-wide hand hygiene initiative on healthcare-associated infections: results of an interrupted time series. BMJ Qual Saf. 2012.
14. White A. Increased infection rates in heavy nasal carriers of coagulase-positive staphylococci. Antimicrobial Agents Chemother (Bethesda). 1963; 161: 667-70.
15. Mertz D, Frei R, Jaussi B, Tietz A, Stebler C, Fluckiger U, et al. Throat swabs are necessary to reliably detect carriers of Staphylococcus aureus. Clin Infect Dis. 2007; 45(4): 475-7.
16. Noble WC, Valkenburg HA, Wolters CH. Carriage of Staphylococcus aureus in random samples of a normal population. J Hyg (Lond). 1967; 65(4): 567-73.
17. Uemura E, Kakinohana S, Higa N, Toma C, Nakasone N. Comparative chracterization of Staphylococcus aureus isolates from throats and noses of healthy volunteers. Jpn J Infect Dis. 2004; 57(1): 21-4.

Chapter 7

Distinctive patterns in the human antibody response to Staphylococcus aureus bacteremia in carriers and non-carriers

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Proteomics 11 (2011) 3914-3927

Abstract

Staphylococcus aureus is both a prominent cause of nosocomial infections with significant morbidity and mortality and a commensal with nasal carriage in around 30% of the population. The rapid spread of multi-resistant strains necessitates novel therapeutic strategies, a challenging task because the species *S. aureus* and the host response against it are highly variable. In a prospective study among 2023 surgical and non-surgical patients, 12 patients developed *S. aureus* bacteremia. They were analyzed in detail using a personalized approach. For each patient, the extracellular proteins of the infecting *S. aureus* strain were identified and the developing antibody response was assessed on 2-D immunoblots. *S. aureus* carriers showed clear evidence of strain-specific pre-immunization. In all immune-competent bacteremia patients, antibody binding increased strongly, in most cases already at diagnosis. In endogenous infections, the pattern of antibody binding was similar to the pre-infection pattern. In exogenous infections, in contrast, the pre-infection pattern was radically altered with the acquisition of new specificities. These were characteristic for individual patients. Nevertheless, a common signature of 11 conserved *S. aureus* proteins, recognized in at least half of the bacteremic patients, was identified. All patients mounted a dynamic antibody response to a subset of these proteins.

Introduction

Staphylococcus aureus is one of the most prevalent causes of nosocomial infections, the spectrum of which ranges from skin and soft tissue infections and osteomyelitis to life-threatening pneumonia and sepsis.[1-7] The world-wide increase in antibiotic resistance gives additional reason for concern and spurs efforts to extend the therapeutic portfolio and develop active and passive vaccines.[2, 8-10]

This is a challenging task because *S. aureus* is equipped with multiple virulence and immune escape factors, which may concertedly aggravate pathogenesis.[11, 12] An additional layer of complexity results from the genetic and, hence, phenotypic heterogeneity of the species. Two *S. aureus* strains can differ in up to 20% of their genome, even when they share the core genome. The variable genome consists of mobile genetic elements (MGE), such as pathogenicity islands and phages, which encode numerous secreted virulence factors, including toxins, exoenzymes and immune modulators.[13-18] Besides genome diversity, there are remarkable strain differences in the regulation of gene expression resulting in extraordinary heterogeneity of the species *S. aureus* at the protein level. Protein variability is particularly pronounced in the extracellular and cell surface proteomes, which directly contact the host immune system.[18-20]

Besides being a pathogen, *S. aureus* is also a frequent colonizer of the human mucosa and skin. About 20% of the healthy human population is persistently colonized in the nostrils, the throat or even the intestine, but also intermittent carriers and even non-carriers are repeatedly exposed to *S. aureus*.[21-23] Healthy individuals therefore harbour a broad range of anti-staphylococcal antibodies, even though short-term colonization is probably not sufficient to induce a robust antibody response to *S. aureus*.[24-26]

We propose that most anti-*S. aureus* antibody responses are elicited by invasion rather than mere carriage. In fact, average concentrations of antibodies binding to a number of selected *S. aureus* antigens increase during infection.[27-32] Different from non-carriers and intermittent carriers, persistent carriers may be exposed to the same strain for a long time, and it appears likely that they experience repeated minor invasive episodes. We therefore predicted that they possess antibodies to virulence factors harboured by their colonizing strain in addition to those directed against conserved bacterial epitopes. In case of an *S. aureus* bacteremia, which in carriers is mostly caused by the colonizing strain,[33] this pre-immunization would help shape the anti-bacterial immune response.

To test this prediction, we performed a prospective and personalized investigation of the humoral immune response to staphylococcal bacteremia using immune proteomics.[24] Bacterial

proteins released during growth in cell culture were separated by 2-DE, blotted onto membranes and decorated with antibodies according to the concept of Jungblut.[24, 34] Such two-dimensional immunoblots (2-D-IBs) were developed with patient sera that were obtained before onset of infection and during the disease course. This allowed us to follow the antibody response in each individual and match it to the extracellular proteome of the corresponding infecting *S. aureus* strain. Distinct patterns in the immune response to endogenous and exogenous infection were revealed on a background of extraordinary heterogeneity of pathogen and host. In addition, a common signature consisting of eleven conserved *S. aureus* proteins was identified that regularly elicited an antibody response during bacteremia.

Materials and methods

Study design

A total of 6771 patients at elevated risk of *S. aureus* bacteremia were screened for *S. aureus* nasal carriage as part of a prospective clinical trial that aimed to prevent nosocomial *S. aureus* infections. Nasal *S. aureus* strains were isolated and stored.[35] In one of the five participating centers (Erasmus University Medical Center), where 2023 patients were recruited, an admission serum sample was also stored, if available. *S. aureus* bacteremia was diagnosed based on positive blood culture later in the disease course. The invasive strains were isolated from the blood cultures and additional serum samples were collected over the course of infection. From 12 bacteremic patients, a complete set of materials was available for analysis. Minimally, this consisted of a pre-infection nasal swab and serum sample, the blood culture *S. aureus* isolate and a serum sample obtained at the moment of bacteremia diagnosis. In most cases, more serum samples were available from the disease course. Of the 12 patients with *S. aureus* bacteremia, six had an exogenous and six an endogenous infection. Four patients were immune compromised (Table 1).

S. aureus strains and sera

The colonizing and invasive clinical *S. aureus* isolates were stored as glycerol stocks. For antigen preparation, bacteria were inoculated in tryptic soy broth (TSB) to an optical density at 540 nm of 0.05 and cultivated in 100mL cultures of tryptic soy broth at 37°C and 180 rpm. Totally, 3.5h after the bacterial culture entered the stationary phase, cultures were harvested, the extracellular proteins were extracted and protein concentration was determined as previously described.[24]

ID	Age	Sex	Colonization status	Type of infection	Reason for hospitalization	Comorbidities	Immuno suppres	Survival	Cause of death	Days of serum sampling[a]		
										Serum screening	Serum diagnosis	Sera during infection
103	55	M	Carrier	Endo[b]	Septic shock after reconstruction of cicatricial hernia	HIV positive	No	Died at day 7	Multiple organ failure caused by sepsis	**-d231**	**d0**	-
307	66	F	Carrier	Endo	Evaluation of abdominal complaints after surgery		No	Survived		**-d7**	**d0**	**d19**
337	35	M	Carrier	Endo	Implementation of CAPD cathether	Renal transplant	No	Survived		**-d165**	**d0**	**d15**
502	46	F	Carrier	Endo	Liver transplantation	Liver cirrhosis	Yes	Survived		**-d4**	**d0**	**d14**
1328	40	M	Carrier	Endo	Amputation foot	Renal and pancreas transplant	Yes	Survived		**d0**	**d0**	**d10**
1362	72	M	Carrier	Endo	Ischemia foot		No	Survived		**-d10**	**d0**	**d15**
1255	59	M	Carrier	Exo[c]	Ischemia foot		No	Died at day 4	Septic shock by SBP[d]	**-d115, -d3,** -d2	**d0**	d1, d2, **d3**
302	60	F	Non-carrier	Exo	Partial liverresection		No	Survived		**-d10**	**d0**	d1, d2, d4, **d5, d9**
703	87	M	Non-carrier	Exo	General malaise		No	Survived		**-d28**	**d0**	-
771	49	M	Non-carrier	Exo	Abscess after implementation of vascular prosthesis		No	Survived		**-d52**	**d0**	**d7**
1264	55	M	Non-carrier	Exo	Cerebral embolus	Protein loosing enteropathy	Yes	Died at day 0	Multiple organ failure caused by sepsis	**-d114**	**d0**	-
1725	20	F	Non-carrier	Exo	Renal transplantation		Yes	Survived		**-d12**	**d0**	d1-7, d9-13, **d14,** d16, d18-22, **d28,** d35, d38, **d57,** d71-73, d76

Table 1. Patient data

[a] Bold: analyzed samples

[b] Endogenous infection

[c] Exogenous infection

[d] SBP, spontaneous bacterial peritonitis

Extracellular proteins were stored in aliquots at -80°C. Serum samples were obtained from all patients at screening and diagnosis. From most patients, 1-5 additional serum samples were taken during the course of infection (Table 1). Sera were stored in aliquots at -80°C.

S. aureus strain characterization

Virulence gene patterns were determined by multiplex PCR, as previously described.[14] Genotyping was based on sequencing the hypervariable region of the protein A (*spa*) gene. With the BURP algorithm (Ridom), *spa* types were grouped into clonal clusters (calculated cost between members of a group≤5). *Spa* types shorter than five repeats were not grouped.[14]

Protein staining

Hundred microgram extracellular proteins were loaded on 11-cm Immobiline Dry Strips (GE Healthcare, Munich, Germany) with the pH range 6-11. After second dimension protein separation, gels were stained with Flamingo® Fluorescent Gel Stain (BioRad, Munich, Germany) according to the manufacturer's instructions, except for fixation, which was performed twice for 1h. Gels were scanned using a Typhoon 9400 scanner (GE Healthcare) in the fluorescence acquisition mode (532 nm) at a resolution of 100 mm.

Preparative 2-DE and identification of selected proteins by MALDI-TOF-MS

Proteins were separated by preparative 2-DE as described above (11-cm strips; 100 mg extracellular proteins loaded per strip), and peptides were prepared for MALDI-MS by trypsin digestion as previously described.[24] The MALDI-TOF measurement of spotted peptide solutions was carried out on a Proteome-Analyzer 4700/4800 (Applied Biosystems, Foster City, CA, USA) as reported.[24]

Database searches were performed using the GPS explorer software version 3.6 (build 3329) with an organism-specific database. The combined MS and MS/MS peak lists were searched against a *S. aureus* 8325 protein database obtained from the ENTREZ genome database site (ftp://ftp.ncbi.nih.gov/genomes/Bacteria/) and a database containing protein sequences derived from the genome sequences of all completely sequenced *S. aureus* strains and, moreover, all additional protein sequences of *S. aureus* (continuously updated from www.uniprot.org) using the MASCOT search engine version 2.104 (Matrix Science, London, UK). The following search criteria were applied: Carbamidomethylation (C) and oxidation (M) were set as

variable modifications; a peptide mass tolerance of ±50 ppm and a fragment mass tolerance of ±0.55 Da were used; the peptide charge state of +1 was accepted for the precursor peptides; the maximum number of missed cleavages was set to 1. The Mowse score for a significant identification of a protein spot had to exceed a value of 50, which corresponds to a *P*-value of 0.05. The labeled gel images of *S. aureus* isolates inducing bacteremia in immunocompetent patients are available in the database PROTECS (http://microbio1.biologie.uni-greifswald.de/csp/bio/login.csp?JumpURL=showcollection.csp?OBJID=53). The spot labels provide the following information: spot number_protein identification_*S. aureus* isolate (for detailed access information see Supporting Information at the end of the manuscript).

MS information, namely spot name, isoelectric point, molecular mass, sequence coverage, score and mass peaks lists for each identified protein spot are available online in the database PROTECS (see Supporting Information at the end of the manuscript and http://microbio1.biologie.uni-greifswald.de/csp/bio/login.csp?JumpURL=showcollection.csp?OBJID=53).

2-D-IB

2-DE with mini 2-DE gels and 2-D-IBs were performed as described.[24] In short, isoelectric focusing was performed with 7-cm Immobiline Dry Strips (GE Healthcare). The pH range of 6-11 was chosen for analysis because most extracellular proteins resolved in this range, while protein A was excluded so that unspecific IgG binding, which obscured a large part of the blot at a pH range of 4-7, could be avoided.[24] The separated staphylococcal proteins were blotted onto a PVDF membrane (Immobilon-P, Millipore, Billerica, MA, USA) with 1.33 mA/cm^2 for 2 h (graphite blotter MilliBlot; Millipore) and incubated with the corresponding human sera at 1:10,000 dilution. Binding of IgG or IgM was detected by peroxidase-conjugated goat anti-human IgG (Dianova, Hamburg, Germany) or peroxidase-conjugated goat anti-human IgM (Dianova) and visualized with an ECL substrate (SuperSignal West Femto Maximum Sensitivity Substrate, Pierce, Rockford, IL, USA).[24] Serum samples from one patient (screening, diagnosis, infection) were always analyzed in the same experiment; three independent experiments were performed for each patient.

2-D-IB spot detection and quantification

Analysis of the 2-D-IB images was performed with the Delta-2D software package version 4.0 (Decodon, Greifswald, Germany) as described.[24]

A fused image of all 2-D-IBs from one patient was obtained in a two-step procedure. First, all 2-D-IB images from one time course experiment were matched with the most complex 2-D-IB, and a fusion image was obtained using the union fuse option. Second, the fusion images from the three technical replicates were matched and fused. Spots on the fusion image were automatically detected and manually validated by comparing the original blot images with the fusion image. Subsequently, the spot map and the corresponding labels from the fusion image were transferred to all blot images in the project, ensuring uniform analysis throughout the study. Because 2-D-IBs differed strongly in signal intensity, no normalization was performed, but the raw volume data were analyzed instead. For spot detection on 2-D-IBs, the signal intensity threshold was set to 0.5 arbitrary units (Au).

To identify the proteins corresponding to the 2-D-IB spots, the fusion 2-D-IB images were further matched with the Flamingo®-stained gels. All 2-D-IB spot volumes that corresponded to protein species of one protein were summed up to generate the cumulated spot volume, a measure for total IgG binding to this protein.

Statistical analysis

The median spot intensity of the three technical replicates was determined for each spot. To quantify infection-induced changes in the 2-D-IB spot intensities, their median ratios (screening versus latest sample) were calculated from three independent experiments. Student's *t*-test was used to compare antibody binding intensities at different time points.

A multilevel regression model was used to analyze the effects of group and time on measurements.[36] Variation within each level (patients and proteins) was assessed through a random intercept on the patient level and a random intercept and slope for time on the protein level. The logarithm of spot intensities was used as the dependent variable. The model included data from 7 patients and 691 spots at two time points. Group (endogenous versus exogenous), time (continuous), and the interaction between both group and time ($P<0.001$ for interaction, likelihood ratio test) were considered as fixed effects. A patient-specific random intercept and a spot-specific random intercept and slope for time were assumed. The covariance structure on the spot level was assumed to be unstructured. Model assumptions including normality of residuals on each level were assessed and fulfilled. Nested models were tested for significant improvements in model fit by comparing the reduction in the -2log-likelihood statistics and the Akaike information criterion (AIC). Analyses were performed with STATA/SE 10.0 (Stata LP, TX, USA). The statistical package gllamm was used for multilevel analyses. Graphs were created with GraphPadPrism Version 5 (GraphPad Software, CA, USA).

Results

Taking into account the pronounced variability of the species *S. aureus*, we used a prospective and personalized approach to study the development of the human antibody response in *S. aureus* bacteremia. 2-D-IB served to analyze the binding of each patient's serum antibodies to the antigen spectra of the corresponding invasive *S. aureus* strains. This was possible in the context of a randomized, double blinded, placebo-controlled clinical trial by Bode et al., who screened and decolonized *S. aureus* carriers to prevent nosocomial *S. aureus* infections.[35] Colonizing *S. aureus* strains and serum samples were obtained from 2023 patients at hospital admission. Serum samples and strains from 12 patients who subsequently developed *S. aureus* bacteremia were available for detailed investigation. In six patients, the colonizing strain was identical to the infecting strain (endogenous infection). Five patients were not nasally colonized and developed an exogenous infection; in one patient, the colonizing strain was different from the infecting strain (exogenous infection). Four patients received immune suppressive drugs (Table 1).

S. aureus bacteremia isolates

The bacteremia isolates were diverse (Table 2). MLST and *spa*-typing showed that they belonged to different clonal lineages - CC8, CC12, CC15, CC30, CC45 - and harboured variable combinations of superantigen genes. Three strains could not be assigned to a CC. Strains from three patients appeared to be clonally related (CC8, *spa*-type t064, identical virulence gene pattern), 1255inv, 1328 and 1362. Another very similar pair, differing only in a single locus, included the strains 307 and 1264 (CC8, *spa*-types t2238 and t008, similar virulence gene pattern). Most bacteremic carriers had an endogenous infection. In these cases, the genotypic analysis confirmed the clonal identity of the colonizing and infecting isolates. Carrier 1255 was an exception, since he was infected by an exogenous strain (Table 2). The 13 investigated *S. aureus* isolates had highly variable exoproteomes. However, spot patterns were of similar complexity (except for strain 1264) with 122–173 protein spots on the 2-D gels (Supporting Information Figure 1*).

IgG response to exogenous and endogenous infection

Three patients (302, 703 and 771) were non-carriers and developed an exogenous infection. A typical example of their IgG response is illustrated in Figs. 1-3; an overview of all investigated cases is given in the Supporting Information Figure 2*. Before onset of bacteremia and at diagnosis, serum IgG binding to the secreted proteins of the infecting *S. aureus* strain was relatively weak (Fig. 1D). The 2-D-IBs showed only 52 spots, most of which were of low intensity. During

				Virulence genes						Genotype			
ID	Strains	Colonization status	Type of infection	*agr*	*egc*	*non-egc*	*eta, etd*	*mecA*	*pvl*	*spa* type	*spa* repeat	*spa* CC	Deduced MLST CC[a]
103	103inv	Carrier	Endo[b]	1	-	-	*etd*	-	-	t937	08-16-34-24-34-34-17-17	Singleton	
103	103col			1	-	-	*etd*	-	-	t937	08-16-34-24-34-34-17-17	Singleton	
307	307inv	Carrier	Endo	1	-	*d j r*	-	-	-	t2238	11-19-12-02-17-34-24-34-22-25	CC064	CC8
307	307col			1	-	*d j r*	-	-	-	t2238	11-19-12-02-17-34-24-34-22-25	CC064	CC8
337	337inv	Carrier	Endo	2	-	-	-	-	-	t084	07-23-12-34-34-12-12-23-02-12-23	CC084	CC15
337	337col			2	-	-	-	-	-	t084	07-23-12-34-34-12-12-23-02-12-23	CC084	CC15
502	502inv	Carrier	Endo	2	-	*b p*	-	-	-	t160	07-23-21-24-33-22-17	CC160	CC12
502	502col			2	-	*b p*	-	-	-	t160	07-23-21-24-33-22-17	CC160	CC12
1328	1328inv	Carrier	Endo	1	-	*b k q*	-	-	-	t064	11-19-12-05-17-34-24-34-22-25	CC064	CC8
1328	1328col			1	-	*b k q*	-	-	-	t064	11-19-12-05-17-34-24-34-22-25	CC064	CC8
1362	1362-I inv	Carrier	Endo	1	-	*b k q*	-	-	-	t064	11-19-12-05-17-34-24-34-22-25	CC064	CC8
1362	1362-II inv			1	-	*b k q*	-	-	-	t064	11-19-12-05-17-34-24-34-22-25	CC064	CC8
1362	1362col			1	-	*b k q*	-	-	-	t064	11-19-12-05-17-34-24-34-22-25	CC064	CC8
1255	1255inv	Carrier	Exo[c]	1	-	*b k q*	-	-	-	t064	11-19-12-05-17-34-24-34-22-25	CC064	CC8
1255	1255col			1	*g i m n o*	*c l tst*	-	-	-	t026	08-16-34	n.d.	
302	302inv	Non-carrier	Exo	1	*i m n o*	*c l tst*	-	-	-	t015	08-16-02-16-34-13-17-34-16-34	CC015	CC45
703	703inv	Non-carrier	Exo	3	*g i m n o u*	*h tst*	-	-	-	t5337	04-44-31-12-16-34-16-12-25-22-22-34	Singleton	
771	771inv	Non-carrier	Exo	3	*g i m n o u*	*a tst*	-	-	-	t012	15-12-16-02-16-02-25-17-24-24	CC012	CC30
1264	1264inv	Non-carrier	Exo	1	-	*d j r*	-	-	-	t008	11-19-12-21-17-34-24-34-22-25	CC064	CC8
1725	1725inv	Non-carrier	Exo	2	*g i m n o*	-	-	-	-	t209	07-16-12-23-34	Singleton	

Table 2. *S. aureus* isolates

[a] MLST-CC deduced with Ridom software from *spa*-type

[b] Endogenous infection

[c] Exogenous infection

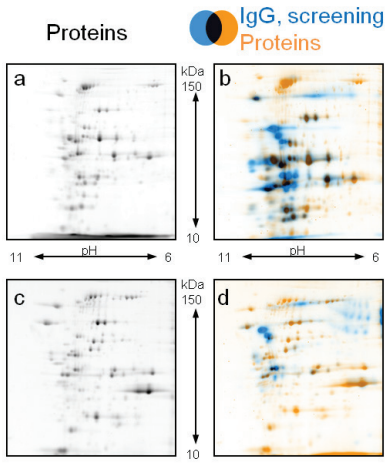

Figure 1. Pre-infection IgG binding to proteins released by *S. aureus*.
The anti-*S. aureus* IgG-binding patterns that preexisted before bacterial invasion are depicted for carrier 307 (endogenous infection; A, B), and for non-carrier 302 (exogenous infection; C, D). The proteins released during post-exponential growth by the two invasive *S. aureus* strains were separated by 2-DE (pH 6-11) and stained with the sensitive protein dye Flamingo® (A, C). The two bacterial isolates had very different exoproteomes. In a parallel approach, the secreted bacterial antigens were blotted onto PVDF membranes and decorated with the corresponding patients' sera obtained before onset of bacteremia. The overlay images (protein - orange; IgG binding - blue) show that the antibody response of the carrier to his colonizing strain was stronger (B) than that of the non-carrier to the exogenous strain, with which later became infected (D). Three technical replicates were prepared in independent experiments; these were very similar, and one of them is shown.

infection, there was a strong increase in the IgG-binding intensity and, in addition, numerous new signals appeared (Figs. 2G-J and 3B). The newly recognized *S. aureus* antigen groups were highly individual (Supporting Information Table 2*).

In contrast, *S. aureus* carriers who went on to develop an endogenous infection (103, 307, 337 and 1362) showed a more complex spot pattern with more spots and higher spot intensities at screening (Fig. 1B and Supporting Information Figure 2*). De novo appearance of spots was rare in endogenous infections; patient 307 did not develop any new signals (Fig. 2A-C and 3A).

Figure 4 summarizes the results of the 2-D-IB analysis of the seven immune-competent patients with endogenous and exogenous *S. aureus* infection. When studied in a prospective manner, *S. aureus* carriers who then developed endogenous bacteremia showed significantly more IgG binding to their invasive *S. aureus* strain (more spots of higher median intensity) than patients who were later confronted with an exogenous strain (Fig. 4A). During the course of infection, IgG binding to antigens of the invasive *S. aureus* strain increased significantly in all patients ($P<0.0001$: pts. 103, 302, 307, 337, 771; $P=0.0035$: pt. 703; $P=0.032$: pt. 1362). In five patients, this was already significant at the time of diagnosis (Fig. 5 and data not shown). The enhancement was more pronounced in cases of exogenous infection, where numerous new antigen-specific IgG species were developed, while in endogenous infection, the IgG-binding patterns were largely conserved and spot intensities doubled on average (Fig. 4B and Supporting Information Table 1*). In the end, IgG binding to the antigens released by the invasive *S. aureus* strains was similar in endogenous and exogenous infection in the immune-competent patients (Table 3 and Supporting Information Figure 2*). To investigate antibody function, we tested

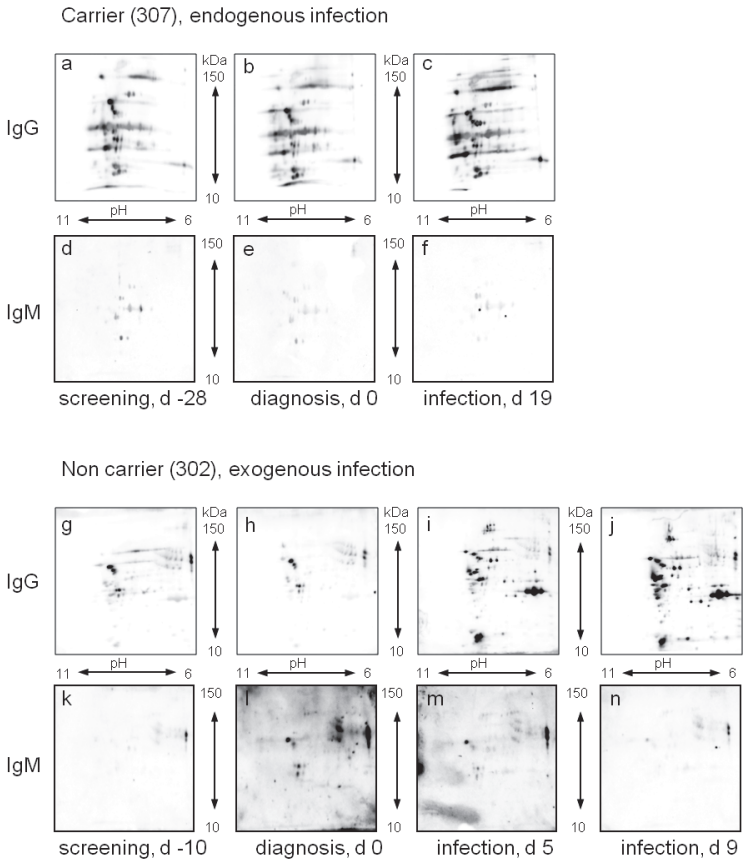

Figure 2. Kinetics of individual antibody responses to *S. aureus* infection.
The time course of the antibody response to *S. aureus* invasion is illustrated for patient 307 with an endogenous infection (A-F), and for patient 302, who developed an exogenous infection (G-N). Secreted antigens from the invasive *S. aureus* strains 307 (A-F) and 302 (G-N) were separated by 2-DE and blotted onto PVDF membranes. They were probed with the corresponding patients' sera obtained at different time points (pt. 307: d -28, d 0, d 19; pt. 302: d -10, d 0, d 5, d 9). In contrast to endogenous bacteremia (A-C), exogenous infection elicited a strong de novo IgG response to numerous *S. aureus* antigens (G-J). The endogenous infection did not elicit an IgM response (D-F). In exogenous infection, strong IgM binding was observed, which preceded the IgG induction and was very transient (K-N). The staphylococcal antigen panels recognized by IgM and IgG overlapped only partially. Three technical replicates were prepared in independent experiments; these were very similar, and one of them is shown.

patient sera for superantigen neutralization as previously described.[25] The results confirmed our findings with IgG binding on 2-D-IBs. The neutralizing capacity for the superantigens of the infecting strain increased strongly during exogenous but not during endogenous infection (data not shown).

In addition, we studied four patients on long-term treatment with immune suppressive drugs; one of them also suffered from a protein-losing enteropathy (Table 1). Most exhibited

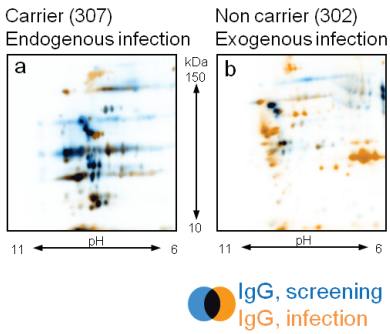

Figure 3. Development of IgG binding in endogenous and exogenous infection.
IgG-binding patterns before onset of infection (blue) and during infection (orange) were superimposed. While in endogenous infection, the antibody binding patterns were largely conserved (patient 307, (A)), new spots appeared during exogenous infection (patient 302, (B)).

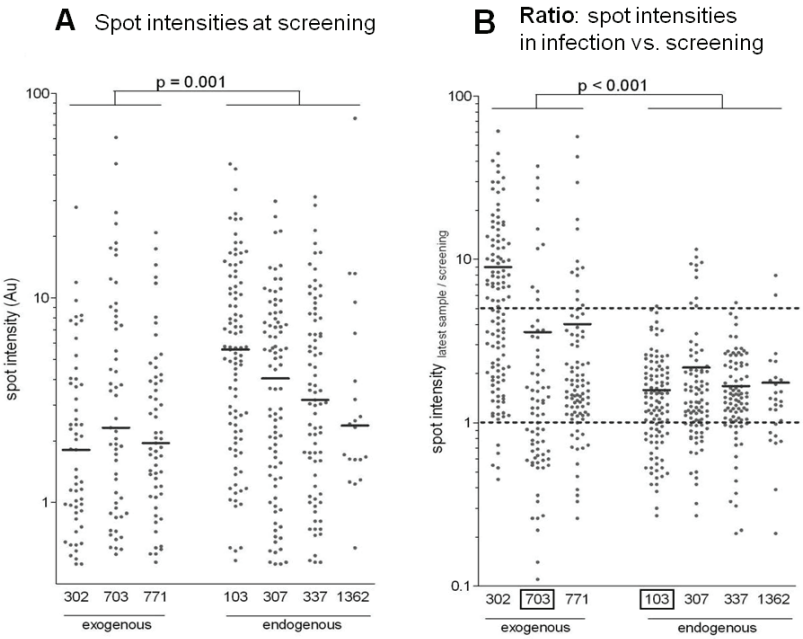

Figure 4. IgG binding to *S. aureus* antigens before onset, at diagnosis and during infection.
The time course of the IgG response to *S. aureus* bacteremia was prospectively analyzed in three non-carriers with an exogenous and four carriers with an endogenous infection. 2-D-IBs were performed to probe patient's serum IgG binding to secreted antigens from the matching invasive *S. aureus* strain. Spot intensities (integrated grey volumes) were determined from three replicate blots and medians are depicted (A) or used for the calculation of ratios (B). The threshold was set at a volume of 0.5 relative units. (A) At screening, before onset of bacteremia, IgG binding to antigens from endogenous strains was significantly stronger than IgG binding to those from exogenous isolates. There were more spots and the median intensities were significantly higher. Median intensities from all spots are indicated. (B) To monitor changes in IgG binding, the ratios of the spot intensities during infection (latest time point) and before infection were calculated for each spot. During infection, IgG binding increased significantly in every patient (*P* values ranged from 0.032 to <0.0001; see text). In exogenous infection, the increase was stronger than in endogenous infection. The overall means are marked. Boxed numbers: diagnosis was the latest time point of serum sampling.

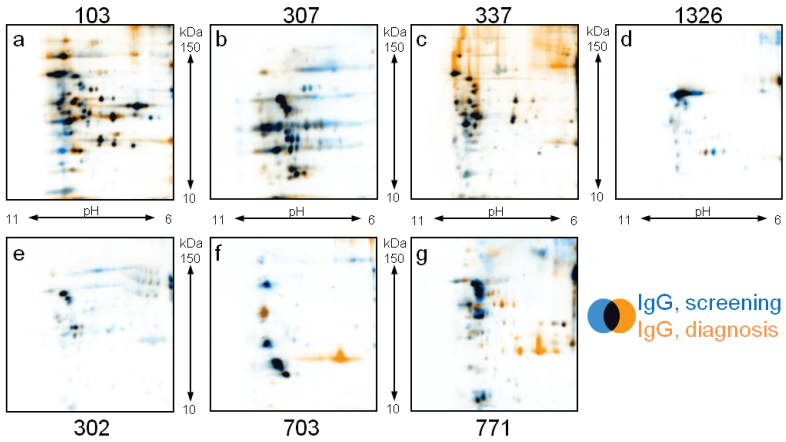

Figure 5. IgG binding at diagnosis.
IgG-binding patterns before onset of infection (blue) and at diagnosis (orange) were superimposed. In most patients, antibody binding had increased at diagnosis. This is more obvious in exogenous infection (D-G) than in endogenous infection (A-C), since in the latter, the antibody binding patterns were more conserved.

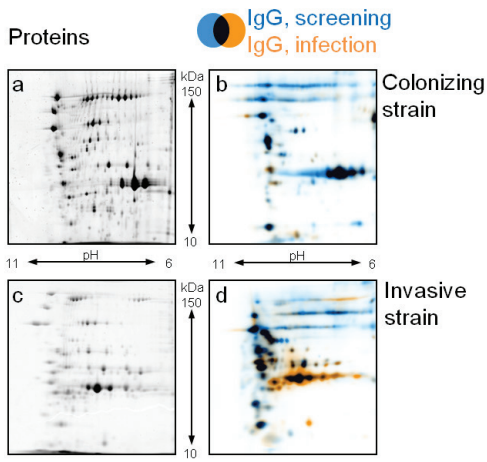

Figure 6. Strain-specificity of the anti-*S. aureus* IgG response.
Patient 1255 is an *S. aureus* carrier, who was infected by an exogenous *S. aureus* strain. The secretomes of the colonizing (A) and invasive (C) strains were different. Overlay images of IgG immunoblots probed with sera obtained before bacterial invasion (blue) and during infection (orange) reveal an increase of IgG binding to antigens from the invasive (D) but not from the colonizing strain (B). One out of three independent experiments is shown.

weak IgG binding before onset of bacteremia and little IgG induction during *S. aureus* infection. The degree of the impairment of their antibody response to *S. aureus* invasion was variable as illustrated in the Supporting Information Figs. 2 and 3*. This suggests that a patient's severely suppressed immune status carries the risk of not being able to mount an antibody response to invasive staphylococcal infection.

S. aureus strain	Type of infection	Immune suppress	Secretome (no. of protein spots)	IgG binding (no. of spots on 2D-IB)		
				Screening[a]	Infection[b]	New spots[c]
302inv	Exo[d]	No	144	52	115	63
703inv	Exo	No	140	55	61	6
771inv	Exo	No	129	64	94	30
103inv	Endo[e]	No	152	94	102	8
307inv	Endo	No	138	85	84	-
337inv	Endo	No	173	79	93	14
1362-I inv	Endo	No	129	20	24	4
1255inv[f]	Exo	No	122	72	75	3
1255col[f]	-	No	135	75	57	-
1264inv	Exo	Yes	75	3	9	6
1725inv	Exo	Yes	160	42	43	1
502inv	Endo	Yes	141	46	43	-
1328inv	Endo	Yes	146	101	100	-

Table 3. Complexity of *S. aureus* secretomes and IgG binding patterns
[a] Serum obtained before onset of bacteremia
[b] Last serum sample obtained during infection
[c] Difference between screening and infection
[d] Exogenous infection
[e] Endogenous infection
[f] Carrier 1255 was colonized by *S. aureus* strain 1255col but exogenously infected by a different *S. aureus* strain, 1255inv

Protein identification

The extracellular proteomes of the seven bacteremia isolates from the patients described in Fig. 4 were analyzed in detail. Around 1200 spots were excised from the 2-D gels (Supporting Information Fig. 1C-I*) and 1006 protein spots were identified by MS. Around 15% of the spots were only weakly stained by the Flamingo® gel stain and could not be reliably identified by MS. Therefore, we did not attempt to identify proteins of very low abundance that were visualized

following antibody binding, but remained undetectable on the stained 2-D protein gels. The identified spots represented 76 staphylococcal proteins (Supporting Information Table 2* and at http://microbio1.biologie.uni-greifswald.de/csp/bio/login.csp?JumpURL=showcollection.csp?OBJID=53). Thirty proteins were present as a single spot, 21 were represented by two spots and 25 by three or more different protein species. As expected, the exoproteomes of the seven clonally unrelated *S. aureus* strains were very heterogeneous. In addition to this diversity, which was mirrored by the IgG-binding patterns on the 2-D-IBs, there were pronounced differences between the patients regarding IgG-binding intensities to individual *S. aureus* antigens and their kinetics during bacteremia (Supporting Information Table 2*). Only 11 staphylococcal proteins elicited antibody binding in more than half of the patients and thus constituted the core immune proteome (Table 4). These comprised extracellular enzymes, two component toxins and α-toxin (Hla). Rarely, proteins were abundant in the extracellular proteome; nevertheless, no

		Patient ID[a,b]						
Protein		**302**	**703**	**771**	**103**	**307**	**337**	**1362I**
Core immune proteome – IgG binding in > 50% of patients								
Aaa	Autolysin	+		+	+		+++	
Atl	Bifunctional autolysin	+++	+++	+++	+++	+++	+++	++
GlpQ	Glycerophosphoryl diester phosphodiesterase	++	+	+		+	++	+
Hla	α-hemolysin	+			+++	+	++	
HlgA/Hlg2	γ-hemolysin component A	+		+	+++	+++		
HlgB/LukF	γ-hemolysin component B	+	+		+++	+	+	
HlgC/LukS	γ-hemolysin component C	+		+	+++	+	+++	
LtaS	Glycerol phosphate lipoteichoic acid synthase	+	+	+	+	+	+	
Nuc	Thermonuclease precursor	+	+		+		+	
Plc	1-Phosphatidylinositol phosphodiesterase	++	+		+++	+	+	
SspP/ScpA	Staphopain thiol proteinase	+	+		+		+	
Proteins present, but no IgG binding[c]								
BlaR1	β-lactamase regulator 1				nb[d]	nb		
Geh	Glycerol ester hydrolase		lb[e]	lb	lb	nb	nb	
Lip	Lipase	nb	nb	nb	nb			
HysA	Hyaluronate lyase					nb	nb	
SACOL2666	*N*-acetylmuramoyl-L-alanine amidase				nb	nb	nb	

Table 4. Protein identification

[a] IgG-binding intensity: **+++**, cumulated spot intensity ≥ 90th percentile; **++**, cumulated spot intensity ≥ 80th percentile; +, cumulated spot intensity < 80th percentile

[b] Grey shading, at least two-fold increase of IgG-binding in bacteremia

[c] Shown if applicable to at least two bacteremia patients

[d] nb, no IgG-binding

[5] lb, low binding protein very abundant, very low intensity IgG-binding

antibody binding was observed (Table 4, Supporting Information Table 2*). This was the case for the enzymes glycerol ester hydrolase (Geh), lipase (lip) and hyaluronate lyase (HysA), all of which are known to be extracellular proteins. β-Lactamase regulator 1 and *N*-acetylmuramoyl-L-alanine amidase (SACOL2666), which did not bind serum IgG either, were present in lower amounts.

Strain-specific IgG response in infection

Patient 1255 was colonized with an *S. aureus* strain belonging to the lineage CC45 (1255col), but was infected with a CC8 strain (1255inv), both exhibiting distinct exoproteomes (Fig. 6A and C). This gave us the opportunity to address the question of strain-specificity of the anti- *S. aureus* antibody response. Before onset of bacteremia, IgG binding to the proteins released by the colonizing and the invasive strain were strong in this patient, comparable to carriers with endogenous infection (Supporting Information Fig. 2*). In infection, however, enhancement of IgG binding and two new spots were observed only when sera were probed with antigens from the invasive but not from the colonizing *S. aureus* strain (Fig. 6B and D).

The IgM response to S. aureus infection

In patient 307 (endogenous infection) and patient 302 (exogenous infection), the IgM response to the exoproteins of the invasive strains was studied to compare binding patterns and induction kinetics with those of IgG. These cases were selected because serum samples from a suitable time window were available and a strong IgG response promised informative results. In exogenous infection (302), there was a strong but very transient IgM response, which reached its maximum at diagnosis, earlier than IgG, but was completely gone 9 days later (Fig. 2K-N). Some antigens were bound by IgM as well as IgG, but most were unique to the different antibody classes. In contrast, no IgM was induced in endogenous infection (307) (Fig. 2D-F).

Discussion

In the complex setting of human *S. aureus* bacteremia, which is characterized by extensive heterogeneity of pathogen and host, the prospective analysis of patients' individual immune response kinetics proved to be a powerful tool. In all immune-competent patients, serum IgG binding to numerous soluble *S. aureus* antigens increased substantially during bacteremia. Among these antigens, a common signature of 11 *S. aureus* exoproteins was identified. In

addition, the personalized approach - each patient's antibody response was tested with antigens from the corresponding *S. aureus* strain - revealed distinctive patterns in the immune response to endogenous versus exogenous infection. To study the development and kinetics of the humoral immune response to *S. aureus*, we selected proteins secreted by the bacteria during post-exponential growth as indicator antigens and chose 2-D immunoblotting for the examination of antibody binding. The soluble exoproteins are a highly variable subproteome of *S. aureus*, which is enriched in virulence factors.[11]

This method does not cover cell wall-associated proteins and some conformational epitopes are denatured, which limits its scope. Furthermore, very strong non-specific IgG binding by protein A prompted us to concentrate our analysis on the pH range 6-11, which was not affected. Unfortunately, this excluded a number of abundantly expressed proteins from the investigation: IsaA, Aur, Aly SspA, SspB, SA 2097.[37, 38] Within these limitations, the approach provides the unique opportunity to directly relate the host immune response to the antigen spectrum that can be expressed by the infecting *S. aureus* strain. In our study, all patients' sera were tested with the antigens of their own invasive *S. aureus* strain. In view of the pronounced variability of the species *S. aureus*,[14, 15] which was also reflected by the invasive strains examined in this study, this is a significant advantage.

The prospective study design allowed (for the first time) the analysis of the pre-existing antibody repertoire against an autologous or heterologous invasive *S. aureus* strain. Carriers usually develop an endogenous infection.[33] In the present study, carriers who went on to develop an endogenous infection showed stronger serum IgG binding before onset of infection than patients with exogenous bacteremia. More antigens were recognized and, on average, baseline antibody binding to the individual proteins was stronger. This corroborates and extends earlier results of an efficient neutralizing antibody response to superantigens in *S. aureus* carriers that were specific for their colonizing strain.[25, 26] The strain-specific pre-immunization of carriers could be explained by a history of repeated exposure of their immune system to its antigens, since persistent carriers typically carry high bacterial loads of a single *S. aureus* clone over a prolonged time period and probably experience multiple subclinical infections.

In contrast, non-carriers and intermittent carriers contact different *S. aureus* strains over time.[23] When confronted with a previously unencountered *S. aureus* strain, their pre-existing antibody repertoire would be expected to possess gaps, especially in the variable proteome, as was shown in this study. Such differences in pre-immunization might put patients at different and probably less favorable starting positions in the case of bacterial invasion. Using a set of eight *S. aureus* antigens, Jacobsson et al. recently showed that patients with a fatal course of *S. aureus* bacteremia had lower initial serum antibody concentrations than patients who recovered.[39]

The variable immune proteome is enriched in virulence factors, many of which are encoded by mobile genetic elements.[15] When confronted with bacterial toxins as in toxic shock syndrome, pre-immunization can be decisive for disease outcome.[40, 41] In a large clinical study performed in The Netherlands, carriers had a better outcome of *S. aureus* bacteremia than non-carriers.[7] It remains to be shown how much the differential pre-immunization status might have contributed to this, but we clearly show here that the immune response during bacteremia is shaped prior to infection during colonization.

All immune-competent patients showed a pronounced antibody response to *S. aureus* invasion. Not surprisingly, immune suppression interfered with this. The prospective approach including screening samples before onset of bacteremia was instrumental, since in many cases, IgG binding to multiple antigens had already increased at diagnosis. This indicates that the immune system had sensed the bacterial invasion very early, which may be relevant for diagnosis and therapy. When comparing septicemia patients with healthy controls, a number of groups have reported higher average concentrations of serum antibodies specific for selected *S. aureus* antigens.[27-29, 39, 42, 43] Most studies used a cross-sectional approach, and all described profound inter-individual heterogeneity in antibody binding, reflecting the individual histories of encounters with *S. aureus*. In contrast, experimental colonization with *S. aureus* did not elicit a comparable IgG reaction, as we have shown with a similar prospective strain-specific 2-D-IB approach.[24]

The immune response patterns for endogenous and exogenous infection differed. Starting from a higher baseline IgG binding, 2-D-IB spot patterns were largely conserved in endogenous infection but increased in intensity. This corresponds to a secondary immune response; the *S. aureus* strain is ''familiar'' to the immune system of its carriers.[25] Exogenous infection of non-carriers enhanced pre-existing IgG specificities as well, but it additionally elicited numerous new spots on the 2-D-IBs, which in conjunction with a strong transient IgM response (pt. 302) supports the idea of a primary immune response to different *S. aureus* antigens. Functional assays for superantigen neutralizing antibodies corroborated these findings (not shown). The newly recognized *S. aureus* antigens differed between patients, indicating that before bacterial invasion, the individuals had distinctive gaps in their anti-*S. aureus* antibody repertoire. More patients need to be investigated to understand how this might influence the disease course.

Protein identification underlined the extensive *S. aureus* strain variability and once more confirmed that the extracellular proteome is strongly enriched in virulence factors, including superantigens and superantigen-like proteins, pore-forming toxins and enzymes.[11] Since there was IgG binding to most proteins of the extracellular proteome, this constitutes a highly relevant subproteome for the investigation of the immune response. Against this background, the

absence of antibody binding to some *S. aureus* proteins was notable. β-Lactamase regulator 1 represents a membrane-bound protein and was released in the supernatant only at very low concentrations. The coverage by the peptidoglycan layer likely explains why it was not immunogenic. Similarly, *N*-acetylmuramoyl-L-alanine amidase levels were low. In contrast, the enzymes lipase, glycerol ester hydrolase and hyaluronate lyase were released in abundance in the bacterial post-exponential growth phase. Antibody binding to the lipases has been reported in other experimental contexts.[43] Whether the lack of antibody binding in our study was due to low protein release during bacteremia in vivo, denaturation of epitopes on the 2-D-IBs, or bacterial interference with the host immune response requires further investigation.

Out of the 76 proteins identified in the exoproteomes of seven unrelated invasive *S. aureus* strains, only one, bifunctional autolysin (Atl), was identified among all strains and bound by serum IgG from all infected patients. In total, 11 immunogenic proteins were identified in more than half of the *S. aureus* strains (at least four), and thus constitute the core immune proteome. The autolysins (Atl and Aaa) and the glycerol phosphate lipoteichoic acid synthase (LtaS) are involved in the bacterial cell wall metabolism; Atl has previously been recognized as an immune dominant antigen by several groups.[24, 42, 44, 45] Included in the core immune secretome were several pore-forming toxins: α-toxin (Hla) and the components A, B and C of the γ hemolysin (HlgA-C), also known as hemolysin γ 2 (Hlg2), leukocidin F and S (LukF, LukS).[46] These toxins are strongly immune reactive, and antibodies are common in healthy adults.[42, 43, 47] In accordance with this, the toxins elicited an increase in IgG binding mainly in exogenous infection. Such antibodies might contribute to protection, as has been shown for neutralizing antibodies against a-toxin in a mouse pneumonia model.[48] The cysteine proteinase staphopain (SspP, also known as ScpA) is also discussed as a virulence factor of *S. aureus* since it interferes with the clotting cascade, degrades collagen and induces vascular leakage.[49, 50] Finally, the extracellular enzymes 1-phosphatidyl inositol phosphodiesterase (Plc) and thermonuclease (Nuc) were commonly recognized by the humoral immune system of bacteremia patients.

It will now be of interest to assess the diagnostic potential of this core immune proteome in investigations with larger patient cohorts and simpler tests, for example, based on the Luminex technique.[26, 51] Such multiplex tests might in the future be used to predict upcoming episodes of bacteremia. However, for the discrimination of the immune response patterns to exogenous and endogenous *S. aureus* bacteremia, it was essential to take into account all bacterial antigens, conserved and variable. Therefore, while being very labor-intensive, the personalized and prospective approach described in this study is a powerful means to elucidate the immunological rules that govern the multifaceted encounters between *S. aureus* and its host.

The strong IgG response to *S. aureus* invasion with its high degree of strain specificity makes

a case for an antigen-driven cognate immune response. This requires T-cell help,[52] and the broad antibody spectrum points to a large *S. aureus*-specific memory T-cell pool. This should be explored in the future because memory T cells of different subtypes, including regulatory T cells, shape the host response to infection, e.g. through effector cytokines, which they rapidly release upon antigen exposure.[53-55] Th1 and Th17 cells are essential mediators of vaccine protection in a mouse model of *S. aureus* infection.[56, 57]

Given the extraordinary variability of *S. aureus*, which is mirrored by the human immune response, effective vaccines and diagnostic tools will have to combine multiple bacterial antigens. The rapid response of the adaptive immune system to *S. aureus* invasion might be exploited for early clinical diagnosis. The proteins representing the core immune proteome shown in this study could serve as a lead for the development of such diagnostic tests. However, a major challenge is posed by the large differences in preexisting antibody patterns, which render the interpretation of single-point measurements difficult if not impossible. Changes in the individual serum response will have to be assessed kinetically, if possible including the comparison with one or more pre-infection samples.

Supporting protein identification data

Supporting Information 1

The bacterial extra-cellular proteomes were separated by 2-DE. Protein spots of the following bacteremia isolates from immune competent patients were subjected to mass spectrometry: *S. aureus* isolates inducing endogenous infections (*S. aureus* 103inv, 307inv, 337inv, 1362Iinv) and *S. aureus* isolates inducing exogenous infections (302inv, 703inv, 771inv). Information on protein spot identification is accessible online (http://microbio1.biologie.uni-greifswald.de/csp/bio/login.csp?JumpURL=showcollection.csp?OBJID=53) using the public log in. Select the gel image of one clinical *S. aureus* isolate to access “Baseinformation” as well as “GelSpots belonging to this GelImage”. “Show on Image” will then open the the 2D gel image with spot labels (protein number as in suppl. data 2 _ protein name _ name of the bacterial isolate according to the text and tables 2 and 3).

Supporting Information 2

Mass peaks lists for each protein are also available online (http://microbio1.biologie.uni-greifswald.de/csp/bio/login.csp?JumpURL=showcollection.csp?OBJID=53). Use the public log in and select the collection “*S. aureus* bacteremia in human patients”. Below the Abstract there is a link to the mass spectrometry data. Save the zipped folder to your hard disc and extract the compressed files. Identified spots of individual *S. aureus* isolates are displayed in separate sheets of the excel file containing: spot

name, iso-electric point, molecular mass, sequence coverage, score and a link to the corresponding peak.

* Supporting information available from http://onlinelibrary.wiley.com/doi/10.1002/pmic.201000760/suppinfo

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References

1. Pittet D, Wenzel RP. Nosocomial bloodstream infections. Secular trends in rates, mortality, and contribution to total hospital deaths. *Arch Intern Med* 1995;155:1177-84.
2. Boucher HW, Corey GR. Epidemiology of methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 2008;46 Suppl 5:S344-9.
3. Concia E, Prandini N, Massari L, Ghisellini F, Consoli V, Menichetti F, Lazzeri E. Osteomyelitis: clinical update for practical guidelines. *Nucl Med Commun* 2006;27:645-60.
4. Dryden MS. Skin and soft tissue infection: microbiology and epidemiology. *Int J Antimicrob Agents* 2009;34 Suppl 1:S2-7.
5. Fluit AC, Wielders CL, Verhoef J, Schmitz FJ. Epidemiology and susceptibility of 3,051 *Staphylococcus aureus* isolates from 25 university hospitals participating in the European SENTRY study. *Journal of clinical microbiology* 2001;39:3727-32.
6. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 1999;29:1128-32.
7. Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, van Keulen PH, Vandenbroucke-Grauls CM, Meester MH, Verbrugh HA. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. *Lancet* 2004;364:703-5.
8. Chambers HF. Community-associated MRSA--resistance and virulence converge. *N Engl J Med* 2005;352:1485-7.
9. Garcia-Lara J, Foster SJ. Anti-*Staphylococcus aureus* immunotherapy: current status and prospects. *Curr Opin Pharmacol* 2009;9:552-7.
10. Schaffer AC, Lee JC. Staphylococcal vaccines and immunotherapies. *Infect Dis Clin North Am* 2009;23:153-71.
11. Foster TJ. Immune evasion by staphylococci. *Nature reviews* 2005;3:948-58.
12. Rooijakkers SH, van Strijp JA. Bacterial complement evasion. *Mol Immunol* 2007;44:23-32.
13. Goerke C, Pantucek R, Holtfreter S, Schulte B, Zink M, Grumann D, Broker BM, Doskar J, Wolz C. Diversity of prophages in dominant *Staphylococcus aureus* clonal lineages. *J Bacteriol* 2009;191:3462-8.
14. Holtfreter S, Grumann D, Schmudde M, Nguyen HT, Eichler P, Strommenger B, Kopron K, Kolata J, Giedrys-Kalemba S, Steinmetz I, Witte W, Broker BM. Clonal distribution of superantigen genes in clinical *Staphylococcus aureus* isolates. *Journal of clinical microbiology* 2007;45:2669-80.
15. Lindsay JA, Holden MT. *Staphylococcus aureus*: superbug, super genome? *Trends in microbiology* 2004;12:378-85.
16. Novick RP, Subedi A. The SaPIs: mobile pathogenicity islands of Staphylococcus. *Chem Immunol Allergy* 2007;93:42-57.
17. Verkaik NJ, Dauwalder O, Antri K, Boubekri I, de Vogel CP, Badiou C, Bes M, Vandenesch F, Tazir M, Hooijkaas H, Verbrugh HA, van Belkum A, Etienne J, Lina G, Ramdani-Bouguessa N, van Wamel WJ. Immunogenicity of toxins during *Staphylococcus aureus* infection. *Clin Infect Dis* 2010;50:61-8.
18. Ziebandt AK, Kusch H, Degner M, Jaglitz S, Sibbald MJ, Arends JP, Chlebowicz MA, Albrecht D, Pantucek R, Doskar J, Ziebuhr W, Broker BM, Hecker M, van Dijl JM, Engelmann S. Proteomics uncovers extreme heterogeneity in the *Staphylococcus aureus* exoproteome due to genomic plasticity and variant gene regulation. *Proteomics* 2010;10:1634-44.
19. Dreisbach A, Hempel K, Buist G, Hecker M, Becher D, van Dijl JM. Profiling the surfacome of *Staphylococcus aureus*. *Proteomics* 2010;10:3082-96.
20. Sibbald MJ, Ziebandt AK, Engelmann S, Hecker M, de Jong A, Harmsen HJ, Raangs GC, Stokroos

I, Arends JP, Dubois JY, van Dijl JM. Mapping the pathways to staphylococcal pathogenesis by comparative secretomics. *Microbiol Mol Biol Rev* 2006;70:755-88.

21. Acton DS, Plat-Sinnige MJ, van Wamel W, de Groot N, van Belkum A. Intestinal carriage of *Staphylococcus aureus*: how does its frequency compare with that of nasal carriage and what is its clinical impact? *Eur J Clin Microbiol Infect Dis* 2009;28:115-27.
22. Mertz D, Frei R, Periat N, Zimmerli M, Battegay M, Fluckiger U, Widmer AF. Exclusive *Staphylococcus aureus* throat carriage: at-risk populations. *Arch Intern Med* 2009;169:172-8.
23. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis* 2005;5:751-62.
24. Holtfreter S, Nguyen TT, Wertheim H, Steil L, Kusch H, Truong QP, Engelmann S, Hecker M, Volker U, van Belkum A, Broker BM. Human immune proteome in experimental colonization with *Staphylococcus aureus*. *Clin Vaccine Immunol* 2009;16:1607-14.
25. Holtfreter S, Roschack K, Eichler P, Eske K, Holtfreter B, Kohler C, Engelmann S, Hecker M, Greinacher A, Broker BM. *Staphylococcus aureus* carriers neutralize superantigens by antibodies specific for their colonizing strain: a potential explanation for their improved prognosis in severe sepsis. *J Infect Dis* 2006;193:1275-8.
26. Verkaik NJ, de Vogel CP, Boelens HA, Grumann D, Hoogenboezem T, Vink C, Hooijkaas H, Foster TJ, Verbrugh HA, van Belkum A, van Wamel WJ. Anti-staphylococcal humoral immune response in persistent nasal carriers and noncarriers of *Staphylococcus aureus*. *J Infect Dis* 2009;199:625-32.
27. Colque-Navarro P, Palma M, Soderquist B, Flock JI, Mollby R. Antibody responses in patients with staphylococcal septicemia against two *Staphylococcus aureus* fibrinogen binding proteins: clumping factor and an extracellular fibrinogen binding protein. *Clin Diagn Lab Immunol* 2000;7:14-20.
28. Colque-Navarro P, Soderquist B, Holmberg H, Blomqvist L, Olcen P, Mollby R. Antibody response in *Staphylococcus aureus* septicaemia--a prospective study. *J Med Microbiol* 1998;47:217-25.
29. Dryla A, Prustomersky S, Gelbmann D, Hanner M, Bettinger E, Kocsis B, Kustos T, Henics T, Meinke A, Nagy E. Comparison of antibody repertoires against *Staphylococcus aureus* in healthy individuals and in acutely infected patients. *Clin Diagn Lab Immunol* 2005;12:387-98.
30. Lorenz U, Ohlsen K, Karch H, Thiede A, Hacker J. Immunodominant proteins in human sepsis caused by methicillin resistant *Staphylococcus aureus*. *Adv Exp Med Biol* 2000;485:273-8.
31. Ryding U, Christensson B, Soderquist B, Wadstrom T. Antibody response to *Staphylococcus aureus* collagen binding protein in patients with *S. aureus* septicaemia and collagen binding properties of corresponding strains. *J Med Microbiol* 1995;43:328-34.
32. Vytvytska O, Nagy E, Bluggel M, Meyer HE, Kurzbauer R, Huber LA, Klade CS. Identification of vaccine candidate antigens of *Staphylococcus aureus* by serological proteome analysis. *Proteomics* 2002;2:580-90.
33. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. *N Engl J Med* 2001;344:11-6.
34. Jungblut PR, Grabher G, Stoffler G. Comprehensive detection of immunorelevant Borrelia garinii antigens by two-dimensional electrophoresis. *Electrophoresis* 1999;20:3611-22.
35. Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, Roosendaal R, Troelstra A, Box AT, Voss A, van der Tweel I, van Belkum A, Verbrugh HA, Vos MC. Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. *N Engl J Med* 2010;362:9-17.
36. Singer J, Willett J. *Applied Longitudinal Data Analysis*. New York: Oxford University Press, Inc.; 2003.
37. Lorenz U, Lorenz B, Schmitter T, Streker K, Erck C, Wehland J, Nickel J, Zimmermann B, Ohlsen K.

Functional antibodies targeting IsaA of *Staphylococcus aureus* augment host immune response and open new perspectives for antibacterial therapy. *Antimicrob Agents Chemother* 2011;55:165-73.

38. Lorenz U, Ohlsen K, Karch H, Hecker M, Thiede A, Hacker J. Human antibody response during sepsis against targets expressed by methicillin resistant *Staphylococcus aureus*. *FEMS immunology and medical microbiology* 2000;29:145-53.
39. Jacobsson G, Colque-Navarro P, Gustafsson E, Andersson R, Mollby R. Antibody responses in patients with invasive *Staphylococcus aureus* infections. *Eur J Clin Microbiol Infect Dis* 2010;29:715-25.
40. Schlievert PM. Use of intravenous immunoglobulin in the treatment of staphylococcal and streptococcal toxic shock syndromes and related illnesses. *J Allergy Clin Immunol* 2001;108:S107-10.
41. Stolz SJ, Davis JP, Vergeront JM, Crass BA, Chesney PJ, Wand PJ, Bergdoll MS. Development of serum antibody to toxic shock toxin among individuals with toxic shock syndrome in Wisconsin. *J Infect Dis* 1985;151:883-9.
42. Clarke SR, Brummell KJ, Horsburgh MJ, McDowell PW, Mohamad SA, Stapleton MR, Acevedo J, Read RC, Day NP, Peacock SJ, Mond JJ, Kokai-Kun JF, Foster SJ. Identification of in vivo-expressed antigens of *Staphylococcus aureus* and their use in vaccinations for protection against nasal carriage. *J Infect Dis* 2006;193:1098-108.
43. Holtfreter S, Kolata J, Broker BM. Towards the immune proteome of *Staphylococcus aureus* - The anti-*S. aureus* antibody response. *Int J Med Microbiol* 2010;300:176-92.
44. Etz H, Minh DB, Henics T, Dryla A, Winkler B, Triska C, Boyd AP, Sollner J, Schmidt W, von Ahsen U, Buschle M, Gill SR, Kolonay J, Khalak H, Fraser CM, von Gabain A, Nagy E, Meinke A. Identification of in vivo expressed vaccine candidate antigens from *Staphylococcus aureus*. *Proceedings of the National Academy of Sciences of the United States of America* 2002;99:6573-8.
45. Weichhart T, Horky M, Sollner J, Gangl S, Henics T, Nagy E, Meinke A, von Gabain A, Fraser CM, Gill SR, Hafner M, von Ahsen U. Functional selection of vaccine candidate peptides from *Staphylococcus aureus* whole-genome expression libraries in vitro. *Infect Immun* 2003;71:4633-41.
46. Kaneko J, Kamio Y. Bacterial two-component and hetero-heptameric pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes. *Biosci Biotechnol Biochem* 2004;68:981-1003.
47. Christensson B, Hedstrom SA, Kronvall G. The clinical significance of serological methods in the diagnosis of staphylococcal septicaemia and endocarditis. *Scand J Infect Dis Suppl* 1983;41:140-3.
48. Bubeck Wardenburg J, Schneewind O. Vaccine protection against *Staphylococcus aureus* pneumonia. *J Exp Med* 2008;205:287-94.
49. Imamura T, Tanase S, Szmyd G, Kozik A, Travis J, Potempa J. Induction of vascular leakage through release of bradykinin and a novel kinin by cysteine proteinases from *Staphylococcus aureus*. *J Exp Med* 2005;201:1669-76.
50. Ohbayashi T, Irie A, Murakami Y, Nowak M, Potempa J, Nishimura Y, Shinohara M, Imamura T. Degradation of fibrinogen and collagen by staphopains, cysteine proteases released from *Staphylococcus aureus*. *Microbiology (Reading, England)* 2011;157:786-92.
51. Verkaik N, Brouwer E, Hooijkaas H, van Belkum A, van Wamel W. Comparison of carboxylated and Penta-His microspheres for semi-quantitative measurement of antibody responses to His-tagged proteins. *J Immunol Methods* 2008;335:121-5.
52. Parker DC. T cell-dependent B cell activation. *Annu Rev Immunol* 1993;11:331-60.

53. Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. *Nat Rev Immunol* 2004;4:478-85.
54. Norrby-Teglund A, Chatellier S, Low DE, McGeer A, Green K, Kotb M. Host variation in cytokine responses to superantigens determine the severity of invasive group A streptococcal infection. *European journal of immunology* 2000;30:3247-55.
55. Norrby-Teglund A, Lustig R, Kotb M. Differential induction of Th1 versus Th2 cytokines by group A streptococcal toxic shock syndrome isolates. *Infect Immun* 1997;65:5209-15.
56. Cho JS, Pietras EM, Garcia NC, Ramos RI, Farzam DM, Monroe HR, Magorien JE, Blauvelt A, Kolls JK, Cheung AL, Cheng G, Modlin RL, Miller LS. IL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice. *J Clin Invest* 2010;120:1762-73.
57. Lin L, Ibrahim AS, Xu X, Farber JM, Avanesian V, Baquir B, Fu Y, French SW, Edwards JE, Jr., Spellberg B. Th1-Th17 cells mediate protective adaptive immunity against *Staphylococcus aureus* and Candida albicans infection in mice. *PLoS Pathog* 2009;5:e1000703.

Chapter 8

Sustained low prevalence of methicillin-resistant Staphylococcus aureus (MRSA) upon admission to hospital in The Netherlands

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Abstract

The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage at hospital admission in The Netherlands was 0.03% in 1999-2000. The aim of the present study was to assess whether the prevalence of MRSA carriage in The Netherlands has changed over the last few years. In five Dutch hospitals, 6496 unique patients were screened for nasal *S. aureus* carriage at hospital admission by microbiological culture between 1 October 2005 and 7 June 2007. In total, 2036 of 6496 (31.3%) patients carried *S. aureus* in their nose, and seven of 6496 (0.11%) patients were nasal carriers of MRSA. Compared with 1999-2000, the prevalence of MRSA carriage in the Dutch population at hospital admission has increased more than threefold; however, this increase was not significant ($P = 0.06$, Fisher's exact test). This prevalence is still among the lowest in the world, probably as a result of the stringent Dutch infection control policy, and the restrictive use of antibiotics in The Netherlands.

Introduction

The Netherlands is one of the few countries in the world that has been able to maintain a low prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA).[1] In 1999-2000, the measured prevalence of MRSA in nearly 10,000 patients at hospital admission was 0.03%.[2]

It seems likely that the Dutch search-and-destroy policy, together with the restrictive use of antibiotics, is responsible for this low prevalence. The search-and-destroy policy is based on screening and pre-emptive isolation of patients with known risk factors for MRSA carriage, followed by decontamination of the environment and decolonization of carriers if necessary, as defined by the guideline of the Dutch Working Party on Infection Prevention (WIP). This guideline classifies patients into different risk categories requiring specific control measures (Table 1). Patients who are proven MRSA carriers (Category I) are strictly isolated from the time

Risk category			
I	Proven MRSA carriage		
II	High risk of MRSA carriage	Patients from a foreign country	• Direct transfer from a foreign hospital or nursing home
			• Admission to a foreign hospital or nursing home >24 h within the last two months
			• Admission to a foreign hospital or nursing home <24 h within the last two months, but undergone surgery, intubation, insertion of a device or catheter
			• Admission to a foreign hospital or nursing home <24 h within the last two months, but presence of skin lesions, abscess, furuncles etc.
			• Guest dialysis patients
			• Adopted children who will be admitted to hospital or will frequently be visiting the outpatient clinic
		Patients from The Netherlands	• From a hospital or nursing home with an uncontrolled MRSA outbreak
			• From a hospital or nursing home where he/she shared a room with an MRSA-positive patient
		Patients in contact with living pigs or meat calves*, irrespective of their reason for contact with animals (occupationally or not), and irrespective of the place where contact occurs	
III	Increased risk of MRSA carriage	Patients from a foreign country	• Admission to a foreign hospital more than two months ago with persistent skin lesions or risk factors such as chronic airway or urinary tract infections
			• Patients who underwent dialysis abroad
		Patients successfully MRSA-eradicated previously, within the first year after eradication	
IV	Not belonging to risk category I, II or III		

Table 1. Risk categories of patients for MRSA carriage as defined by the Dutch Working Party on Infection Prevention (WIP) guideline "MRSA in hospitals", 2007
* Risk category added to the WIP guideline in 2006

of admission until hospital discharge. Patients with risk factors for MRSA carriage but whose MRSA status is unknown are grouped into Categories II and III, depending on the risk factor (Table 1). Patients in Category II are screened at hospital admission and kept isolated until polymerase chain reaction (PCR) or cultures for MRSA are negative. Patients in Category III are screened but are only isolated if the culture results are positive for MRSA. Patients in Category IV are not considered to be at increased risk of MRSA carriage and are not screened.

The European Antimicrobial Resistance Surveillance Network (EARS-Net) collects antimicrobial susceptibility data for different pathogens isolated from clinical cultures in European hospitals. EARS-Net data show that the prevalence of MRSA among blood isolates in The Netherlands was 1.0% in 2009.[3] EARS-Net collects data about invasive isolates, but does not survey MRSA carriage.

The aim of this study was to determine whether MRSA carriage in The Netherlands has increased since 1999-2000, and to assess the prevalence of MRSA nasal carriage in patients at hospital admission between 2005 and 2007.

Methods

Between 1 October 2005 and 7 June 2007, patients expected to be admitted for at least four days to one of the participating wards of five Dutch hospitals (Amphia Hospital Breda, Erasmus University Medical Center Rotterdam, Free University Medical Center Amsterdam, University Medical Center Utrecht, Canisius-Wilhelmina Hospital/St. Maartenskliniek Nijmegen) were screened for nasal carriage of *S. aureus* within the first 24 h of admission. *S. aureus* carriage was one of the eligibility criteria for enrolment in a randomized, placebo-controlled trial for the prevention of healthcare-associated infections with *S. aureus*.[4] Medical Review Board approval and informed consent were obtained.

Nasal swab samples were inoculated on blood agar plates and in a non-selective phenyl mannitol broth. After 48 h, a loop of broth was subcultured on a blood agar plate. Colonies morphologically suspected for *S. aureus* were tested with an agglutination test (Slidex Plus, bioMérieux, Marcy l'Etoile, France). Slidex-positive strains were tested for cefoxitin susceptibility by disk diffusion according to the criteria of the Clinical and Laboratory Standards Institute. All cefoxitin-resistant strains were sent to Erasmus University Medical Center for confirmation by an *S. aureus*-specific DNA hybridization test (AccuProbe, Gen-Probe Inc., San Diego, CA, USA), an *S. aureus*-specific polymerase chain reaction (PCR) assay,[5] a PBP-2'-Latex agglutination test (MRSA-Screen, Denka Seiken Co., Tokyo, Japan) and PCR to identify the mecA gene. Susceptibility testing

was performed by an automated system (Vitek 2, bioMérieux). MRSA strains were genotyped by multi-locus sequence typing (MLST) and spa typing. The presence of the Panton Valentine Leucocidin (PVL) toxin gene was assessed by PCR.

Results

In total, 6771 nasal swabs obtained from 6496 patients were analyzed. Two hundred and forty-five patients were screened on more than one occasion. During the study period, approximately 276,000 patients were admitted to the participating hospitals for at least two days. Two thousand and thirty-six of 6496 (31.3%) patients tested positive for *S. aureus* at least once, and seven of 6496 (0.11%) patients had positive nasal swabs for MRSA.

Table 2 shows the characteristics of the MRSA-positive patients and their strains. The seven MRSA strains were isolated from patients in four different hospitals. They all had different spa types (t003, t008, t011, t044, t052, t445 and t567) and belonged to five clonal complexes as determined by MLST: ST5, ST8 (two strains), ST45, ST80 and ST398 (two strains). One strain was PVL positive.

Discussion

This study found a prevalence of MRSA carriage at hospital admission in The Netherlands of 0.11%. Compared with 1999-2000, the prevalence of MRSA carriage in the Dutch population at hospital admission has increased by a factor of 3.7; however, this increase was not significant (P=0.06, Fisher's exact test).[2] According to the surveillance of clinical samples by EARS-Net, the incidence of MRSA infections in The Netherlands remained fairly constant around 1% from 2002 to 2009 (range 0.65-1.97%; data not yet available for 2010).[3] Therefore, the prevalence found in the present study, measured from 2005 to 2007, is considered to be representative of the current situation. The prevalence of MRSA in The Netherlands is still very low compared with almost all other countries in the world, probably due to the restrictive use of antibiotics, the stringent infection control measures in hospitals, and the emphasis on prevention of MRSA transmission in The Netherlands.[6-8] The Dutch search-and-destroy policy was introduced in 1988, and is based on identification, isolation and decolonization of MRSA carriers.[9] This policy, produced and continuously updated by the Dutch WIP, appears to have been successful in the prevention of MRSA transmission and the resolution of MRSA outbreaks.[10-12] Recently, people in contact with pigs and calves were identified as being at increased risk of MRSA

Patient	*Hospital*	*Gender*	*Age (yrs)*	*Department*	*MRSA risk**	*Admitted previously*	*MRSA characteristics*								
							MLST	***Spa***	**PVL**	**Oxa**	**Cli**	**Sxt**	**Cip**	**Gen**	**Rif**
A	I	Male	84	Gastroenterology	No	Yes	ST8	t008	neg	32	S	S	R	S	S
B	I	Male	48	General surgery	No	1 day	ST80	t044	pos	96	S	S	S	S	S
C	I	Male	59	General surgery	Yes†	Yes	ST5	t003	neg	≥256	R	S	R	S	S
D	II	Male	63	Vascular surgery	No	No	ST398	t567	neg	≥256	S	S	S	S	S
E	II	Male	41	Vascular surgery	No	No	ST45	t445	neg	16	R	S	R	S	S
F	III	Female	53	General surgery	No	Yes	ST8	t052	neg	≥256	R	S	R	R	R
G	IV	Male	70	Orthopaedics	Yes‡	Yes	ST398	t011	neg	48	S	R	I	S	S

Table 2. Characteristics of the MRSA carriers and their nasal strains

* MRSA risk according to national WIP guideline

† This patient was cared for in isolation at the time of screening. He had previously been admitted to a ward where, after his discharge, MRSA was isolated in another p (Table 1), and was screened and isolated at his next admission.

‡ This patient was a pig farmer who was admitted and screened before the addition of this risk category to the WIP guideline

MLST, multi-locus sequence typing; PVL, Panton Valentine Leucocidin; Oxa, oxacillin; Cli, clindamycin; Sxt, trimethoprim/sulfamethoxazole; Cip, ciprofloxacin; Gen, gent Working Party on Infection Prevention, Antimicrobial susceptibility according to the criteria of the Clinical and Laboratory Standards Institute.

carriage.[13-15] As such, this risk group was added to the WIP guideline in 2006.

In total, seven MRSA carriers were found during this study. At the time of screening, two of these patients were at high risk for MRSA carriage according to the current, updated WIP guideline: Patients C and G. Patient C was already in contact isolation (Category II). He had previously been admitted to a ward where, after his discharge, MRSA was isolated in another patient. Immediately upon his next admission, he was isolated pre-emptively and screened. This screening confirmed that he was an MRSA carrier, and isolation was continued. Patient G was screened in February 2006; at that time, he was not known to be at increased risk for MRSA carriage. His medical chart noted that he was a pig farmer. Since July 2006, he would have been considered to be at high risk for MRSA carriage (Category II). Thus, the current WIP guideline appears to be fairly effective for the identification of possible MRSA carriers. However, patients with MRSA of unknown origin who are not recognized as being at increased risk (such as five patients in this study) can be a source of transmission of this pathogen. The authors believe that the early recognition of carriers is an important aspect of the Dutch guideline to help prevent transmission of MRSA in hospitals. It is important to update the guideline continuously by the addition of every newly found source of MRSA. Therefore, studies are currently underway to search for new risk factors associated with MRSA of unknown origin.

Methicillin resistance was an exclusively nosocomial problem until the mid-1990s. Since then, increasing numbers of MRSA cases have been described which are not related to healthcare settings or hospital contact. These cases are described as 'community-onset' or 'community-acquired' MRSA (CA-MRSA). However, when MRSA carriage is detected at hospital admission in patients who have been admitted to hospital previously, it is difficult to discriminate between CA-MRSA and hospital-acquired MRSA. MLST can be used to discriminate between genetic lineages of *S. aureus*. In the USA, community-associated infections are mainly caused by multi-locus sequence type 8 (ST8) strains,[16-18] whereas in Europe, ST80 is the predominant strain associated with CA-MRSA infections.[19-21] Another characteristic of CA-MRSA is the susceptibility to non-β-lactam antibiotics.[22] Furthermore, it has been argued that the PVL toxin gene is typically found in CA-MRSA.[23] In the present study, only one strain harboured these three characteristics possibly associated with CA-MRSA (Table 2). The strain was isolated from a patient who had been admitted to hospital previously and who had not been screened for MRSA carriage in the past. Therefore, it is not known whether this strain was acquired in the community or during a hospital admission.

Two of the seven MRSA strains found were identified as sequence type 398 by MLST (spa types t011 and t567). This sequence type is associated with pigs and pig farming.[24-25] One of the two carriers of these strains was a pig farmer. The profession of the other ST398 (t567) carrier is not known, but as he lived in a rural part of The Netherlands, it is possible that he had

contact with pigs or calves. Also, person-to-person transmission of pig strains of MRSA has been described.[26] Since 2007, all MRSA isolates sent to the Dutch National Institute for Public Health and the Environment have been genotyped by spa typing, and spa types t011 and t108, both belonging to the livestock-associated clonal complex CC398, have been found to represent the largest cluster of MRSA isolates in The Netherlands (30-35%).[27-29] Other frequently isolated spa types are t008, t002 and t064. These spa types are found worldwide and may be related to the epidemic strains USA300/ST8 (t008), EMRSA-3 (t002) or USA500 (t064), but additional data, such as the presence of PVL genes or the type of SCCmec, are not available. However, it is known that epidemic strains have spread in The Netherlands.[30-31] As such, it is unlikely that the low prevalence found in this study was due to the absence of epidemic strains in these hospitals.[32]

The population screened for this study differs slightly from the target population of Wertheim *et al*. They screened non-surgical patients, irrespective of their expected duration of hospital stay. In the present study, both surgical and non-surgical patients with an expected length of stay of at least four days were screened. To the authors' knowledge, there is no difference in risk of MRSA carriage between these two populations, nor does the Dutch WIP guideline make a difference between surgical and non-surgical patients, nor take length of stay into account in assessing risk groups for MRSA. Therefore, it is not thought that the results of this study are subject to sampling bias. Nevertheless, patients were not asked for the reason for their expected duration of stay. These patients may suffer from comorbidities or have a history of hospital stay, and may therefore be at increased risk of MRSA carriage. If the study population was at increased risk for MRSA carriage compared with the average population, the prevalence found in this study could be relatively high. If this is the case, the abovementioned study conducted on MRSA carriage of unknown origin should be able to identify these risk factors.

This study provides a good estimate of the prevalence of MRSA carriage in The Netherlands, as it was carried out among patients on admission to five hospitals in rural and urban areas. It found that the prevalence has remained fairly constant over the past few years, which is probably a result of the Dutch search-and-destroy policy, the restrictive use of antibiotics, and stringent infection control measures in hospitals in The Netherlands. However, the majority of the MRSA isolates found were of unknown origin. Currently, an observational study and case-control study are underway to identify possible new sources and transmission routes of MRSA by reviewing Dutch MRSA carriers and isolates of unknown origin. If new sources are found, they should be incorporated into the existing WIP guideline to prevent the spread of MRSA. Surveillance of MRSA prevalence should be performed regularly to monitor the effects of the guideline.

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References

1. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. Lancet. 2006 Sep 2;368(9538):874-85.
2. Wertheim HF, Vos MC, Boelens HA, Voss A, Vandenbroucke-Grauls CM, Meester MH, et al. Low prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) at hospital admission in the Netherlands: the value of search and destroy and restrictive antibiotic use. J Hosp Infect. 2004 Apr;56(4):321-5.
3. Control ECfDPa. Antimicrobial resistance surveillance in Europe 2009. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC; 2010.
4. Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, Roosendaal R, et al. Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. N Engl J Med. 2010 Jan 7;362(1):9-17.
5. Martineau F, Picard FJ, Roy PH, Ouellette M, Bergeron MG. Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. Journal of clinical microbiology. 1998 Mar;36(3):618-23.
6. Tiemersma EW, Bronzwaer SL, Lyytikainen O, Degener JE, Schrijnemakers P, Bruinsma N, et al. Methicillin-resistant *Staphylococcus aureus* in Europe, 1999-2002. Emerg Infect Dis. 2004 Sep;10(9):1627-34.
7. van de Sande-Bruinsma N, Grundmann H, Verloo D, Tiemersma E, Monen J, Goossens H, et al. Antimicrobial drug use and resistance in Europe. Emerg Infect Dis. 2008 Nov;14(11):1722-30.
8. Humphreys H. National guidelines for the control and prevention of methicillin-resistant *Staphylococcus aureus*-what do they tell us? Clin Microbiol Infect. 2007 Jun 30.
9. Vandenbroucke-Grauls CM. Methicillin-resistant *Staphylococcus aureus* control in hospitals: the Dutch experience. Infect Control Hosp Epidemiol. 1996 Aug;17(8):512-3.
10. Vos MC, Behrendt MD, Melles DC, Mollema FP, de Groot W, Parlevliet G, et al. 5 years of experience implementing a methicillin-resistant *Staphylococcus aureus* search and destroy policy at the largest university medical center in the Netherlands. Infect Control Hosp Epidemiol. 2009 Oct;30(10):977-84.
11. Bocher S, Skov RL, Knudsen MA, Guardabassi L, Molbak K, Schouenborg P, et al. The Search and Destroy Strategy Prevents Spread and Long-term Carriage of MRSA; Results from Follow-up Screening of a Large ST22 (E-MRSA 15) Outbreak in Denmark. Clin Microbiol Infect. 2009 Dec 23.
12. van Trijp MJ, Melles DC, Hendriks WD, Parlevliet GA, Gommans M, Ott A. Successful control of widespread methicillin-resistant *Staphylococcus aureus* colonization and infection in a large teaching hospital in the Netherlands. Infect Control Hosp Epidemiol. 2007 Aug;28(8):970-5.
13. Armand-Lefevre L, Ruimy R, Andremont A. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. Emerg Infect Dis. 2005 May;11(5):711-4.
14. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis. 2005 Dec;11(12):1965-6.
15. Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuvel MG, Heck ME, Pluister GN, et al. Community-acquired MRSA and pig-farming. Ann Clin Microbiol Antimicrob. 2006;5:26.
16. Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, et al. Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. N Engl J Med. 2005 Apr 7;352(14):1445-53.
17. Kazakova SV, Hageman JC, Matava M, Srinivasan A, Phelan L, Garfinkel B, et al. A clone of methicillin-resistant *Staphylococcus aureus* among professional football players. N Engl J Med. 2005 Feb 3;352(5):468-75.

18. Pan ES, Diep BA, Carleton HA, Charlebois ED, Sensabaugh GF, Haller BL, et al. Increasing prevalence of methicillin-resistant *Staphylococcus aureus* infection in California jails. Clin Infect Dis. 2003 Nov 15;37(10):1384-8.
19. Ferry T, Etienne J. Community acquired MRSA in Europe. BMJ (Clinical research ed. 2007 Nov 10;335(7627):947-8.
20. Francois P, Harbarth S, Huyghe A, Renzi G, Bento M, Gervaix A, et al. Methicillin-resistant *Staphylococcus aureus*, Geneva, Switzerland, 1993-2005. Emerg Infect Dis. 2008 Feb;14(2):304-7.
21. Hedin G, Fang H. Epidemiology of methicillin-resistant *Staphylococcus aureus* in Southern Stockholm, 2000-2003. Microbial drug resistance (Larchmont, NY. 2007 Winter;13(4):241-50.
22. Cunha BA. Antimicrobial therapy of multidrug-resistant Streptococcus pneumoniae, vancomycin-resistant enterococci, and methicillin-resistant *Staphylococcus aureus*. The Medical clinics of North America. 2006 Nov;90(6):1165-82.
23. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis. 2003 Aug;9(8):978-84.
24. van Belkum A, Melles DC, Peeters JK, van Leeuwen WB, van Duijkeren E, Huijsdens XW, et al. Methicillin-Resistant and -Susceptible *Staphylococcus aureus* Sequence Type 398 in Pigs and Humans. Emerg Infect Dis. 2008 Mar;14(3):479-83.
25. van Loo I, Huijsdens X, Tiemersma E, de Neeling A, van de Sande-Bruinsma N, Beaujean D, et al. Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. Emerg Infect Dis. 2007 Dec;13(12):1834-9.
26. Wulf MW, Markestein A, van der Linden FT, Voss A, Klaassen C, Verduin CM. First outbreak of methicillin-resistant *Staphylococcus aureus* ST398 in a Dutch hospital, June 2007. Euro Surveill. 2008 Feb 28;13(9).
27. Haenen A, Huijsdens XW, Pluister GN, Van Luit M, Bosch T, Van Santen-Verheuvel MG, et al. Surveillance of MRSA in the Netherlands in 2007: an increasing trend of livestock-related MRSA. Inf Bull. 2009;20:138-45.
28. Haenen APJ, Huijsdens XW, Pluister GN, Van Luit M, Bosch T, Van Santen-Verheuvel MG, et al. Surveillance of MRSA in the Netherlands in 2008. Inf Bull. 2010;21:162-9.
29. Haenen A. Registration of infectious diseases. Inf Bull. 2011;22:39.
30. Stam-Bolink EM, Mithoe D, Baas WH, Arends JP, Moller AV. Spread of a methicillin-resistant *Staphylococcus aureus* ST80 strain in the community of the northern Netherlands. Eur J Clin Microbiol Infect Dis. 2007 Oct;26(10):723-7.
31. Deurenberg RH, Nulens E, Valvatne H, Sebastian S, Driessen C, Craeghs J, et al. Cross-border dissemination of methicillin-resistant *Staphylococcus aureus*, Euregio Meuse-Rhin region. Emerg Infect Dis. 2009 May;15(5):727-34.
32. Vos MC, Ott A, Verbrugh HA. Successful search-and-destroy policy for methicillin-resistant *Staphylococcus aureus* in The Netherlands. J Clin Microbiol 2005 Apr;43(4):2034; author reply.

Chapter 9

Summarizing discussion, conclusions, and future perspectives

Summarizing discussion, conclusions, and future perspectives

The aim of this thesis was to add to the prevention of healthcare associated *S. aureus* infections.

Healthcare associated *S. aureus* infections often start with a breach of skin or mucosa, for example when a catheter is inserted or surgical incision is made.[1] In carriers, the skin and mucosa are colonized with *S. aureus*. These patients are thus at risk of developing endogenous infections, i.e. infections with the strain colonizing the patient before the procedure.

Mupirocin has been shown to effectively eradicate *S. aureus* from the nose and skin of carriers.[2-4] The postulated preventive effect of mupirocin decolonization therapy on healthcare associated infections has been subject of several studies before.[5] Although a reduction in the incidence of healthcare associated infections was reported in several patient groups, the intervention was either compared with historical control groups, or the result was obtained from subgroup analysis.[6, 7] In the study by Perl *et al*., patients were assigned to mupirocin or placebo, irrespective of carrier state. In the intention-to-treat analysis, no difference in the incidence of *S. aureus* infections was observed between the two treatment groups. However, in the subgroup of *S. aureus* carriers, *S. aureus* infections were significantly decreased in the mupirocin treated group.[6] A randomized, double blind, placebo-controlled trial in 1,602 nonsurgical *S. aureus* carriers, who were treated after the results of nasal culture became available, did not show a significant effect of mupirocin against healthcare associated infections.[8] A randomized, double-blind, placebo-controlled trial in orthopedic patients neither showed a significant effect of mupirocin.[9] Failure in these studies may have been the result of timing of intervention, an unsatisfactory effect of mupirocin on decolonization of extra-nasal sites, recolonization after therapy, and/or a relatively low incidence of infections in the targeted study group.[10]

Chapter 2 describes a multicenter randomized placebo-controlled trial, in which design the before mentioned determinants of possible failure were taken along. *S. aureus* carriers, admitted for at least four days, were detected by a rapid test upon admission to hospital. They were subsequently assigned to either mupirocin nasal ointment and chlorhexidine gluconate medicated soap, or placebo ointment and placebo soap. Treatment was repeated every three weeks if still hospitalized. The primary study outcome was the incidence of *S. aureus* healthcare associated infections: these were prevented by almost 60% in the mupirocin/chlorhexidine group compared with the placebo group. Since 90% of the patients enrolled were surgical patients, and the incidence of *S. aureus* infections in the nonsurgical group was low, the benefit of the intervention was most evident in surgical patients. Deep surgical site *S. aureus* infections were prevented by almost 80%.

Since the majority of patients enrolled were patients undergoing surgery, we could not draw any conclusions in the subgroup analyses on the prevention of infections in the non-surgical population. This study may though be seen as a "proof-of-principle". Also in non-surgical patients, the majority of bacteremias are of endogenous origin.[11, 12] Eradication of *S. aureus* from the nose and skin of non-surgical patients may well decrease the incidence of all kinds of *S. aureus* infections. However, the incidence of infections in the non-surgical population is lower than in the surgical population, resulting in a higher number needed to treat to prevent one infection. To estimate the effect size of the intervention in non-surgical patients, a randomized clinical trial targeting this subgroup of patients is needed. The major limitation for conducting such a study is the large number of participants that will be needed to reject the null hypothesis. Therefore, modeling may be an alternative for clinical trials.

The design of our trial was a conventional one: we calculated the number of patients needed to randomize based on the estimated cumulative incidence, desired power, type II error rate and reduction of healthcare associated *S. aureus* infections. We planned to perform the statistical analysis after enrolment of all patients. Since the rate of enrolment was slower than we expected, and some surgeons reported a change in the incidence of serious surgical-site infections, we changed to the design of sequential analysis with permission of the medical ethics board.[13] A major advantage of this design is the ability of stopping the trial when a beneficial effect of the intervention is achieved, but also a beneficial effect of placebo or futility can be a reason to stop the trial. The major disadvantage of this design is the correction that has to be applied for multiple analyses. Nevertheless, this relatively new type of study design has great potential to be used in the future more often. It minimizes the number of patients needed to be randomized, it avoids unnecessary exposure to placebo medication in the case of a larger beneficial effect of the intervention than expected, may avoid harm when the intervention is inferior to placebo, and saves trial budget in the case of futility.

A large, randomized controlled trial may provide answers on the primary study question at a high level of evidence, but may also leave policy makers with many additional questions. Implementation of the screen-and-treat strategy hampers when issues such as the following arise: Which patients benefits most from this strategy in terms of morbidity and mortality, and should be targeted for this strategy? Is the screen-and-treat strategy cost-effective? Will patients, doctors and nurses commit to the screen-and-treat strategy, and what if compliance hampers? When should a patient be screened, to have a reliable screening result at the date of admission? Why not just prescribing mupirocin and chlorhexidine to every patient entering the hospital?

In May 2010, four months after publication of our RCT, Diekema et al conducted a survey among members of the IDSA Emerging Infections Network, a healthcare provider-based network

of infectious disease clinicians who are members of the IDSA (Infectious Diseases Society of America) or the Pediatric Infectious Diseases Society.[14] Of their responders, 13% screened for MSSA carriage and only 8% decolonized MSSA carriers. Apparently, implementing the seemingly simple strategy of screening patients and treating carriers to achieve a substantial reduction of *S. aureus* surgical-site infections, appears not to be that simple. And although The US Centers for Disease Control and Prevention added the *S. aureus* screen-and-treat strategy to their top recommendations for safer health care, up to now, only a small proportion of surgical patients are screened for MSSA carriage and treated if colonized, and recommendations on screening and treatment of carriers have not been implemented in national guidelines yet. This thesis may add to the knowledge that is needed to implement the *S. aureus* screen-and-treat strategy in practice and subsequently prevent healthcare associated *S. aureus* infections.

The selection of patients that may benefit most from a screen-and-treat strategy is addressed in **Chapter 3.** Many different types of patients, both surgical and non-surgical, come to the hospital. As mentioned before, we could not draw conclusions from the non-surgical population. But, for reasons of costs and logistics, the screen-and-treat strategy should neither be applied to every surgical patient entering the hospital. In the randomized controlled trial, we already selected those patients that were supposed to be at highest risk for developing healthcare associated *S. aureus* infections: Patients, admitted for at least four days, to wards with a known high incidence of *S. aureus* infections. However, also on a single ward, many types of patients may be admitted, especially in general hospitals. Therefore, we conducted an observational follow-up study, to compare long-term mortality between patients who received mupirocin/chlorhexidine and placebo. By checking the municipal personal records database for the presence of a mortality date three years after enrolment, we compared mortality rates one and three years after admission in all surgical patients allocated to the intervention, and in different subgroups. Mortality was not reduced when all patients were taken together, but a significant reduction in one-year mortality was shown in patients who had undergone a clean procedure and had received mupirocin/chlorhexidine, as compared to these patients who had received placebo.

The US Centers for Disease Control define a clean (class I) wound as follows: “An uninfected operative wound in which no inflammation is encountered and the respiratory, alimentary, genital, or uninfected urinary tract is not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage. Operative incisional wounds that follow non-penetrating (blunt) trauma should be included in this category if they meet the criteria”.[16] Other types of operative wounds are classified as clean-contaminated (class II), contaminated (class III) or dirty-infected (class IV). Since clean wounds are per definition not in contact with colonized tracts, but usually breach the skin only, clean wound infections are most likely caused by skin flora, particularly *S. aureus*. It is therefore not surprising that the effect of the screen-and-treat

strategy for *S. aureus* carriage on mortality appeared most effective in patients who undergo clean procedures.

An important limitation of this study was the lack of the cause of death, which is not registered in the municipal personal records database. We tried to obtain the cause of death from the hospitals' discharge letters, but these letters were often absent, or did not specifically address the cause of death. No reliable database could thus be used to infer these data from. But although causality cannot not be proven, it is striking that in the placebo group of patients who died within a year, clearly more patients had suffered from a deep surgical site infection than in the mupirocin/chlorhexidine group (24% vs. 0%), and it is likely that the excess of deaths in the placebo group is indeed attributable to the higher incidence of serious *S. aureus* infections. Therefore, the screen-and-treat strategy should primarily focus on surgical patients undergoing clean procedures.

We did not study morbidity or functional impairment after allocation to mupirocin/chlorhexidine or placebo. However, the consequences of *S. aureus* infections can be devastating. Such consequences are not taken into account in studies with mortality as single endpoint. Therefore, excluding patient groups from the screen-and-treat strategy should not be based on mortality studies only. Decisions about the target groups should be made based on effect sizes, infection rates, cost-effectiveness studies, mortality studies, but particularly on common sense.

Healthcare associated infections are extremely expensive. Excess costs of these infections are estimated at € 3,700 - 30,000 per event.[17] Mean length of stay increases with 4.3 to 32.2 days, depending on many factors such as the type of procedure, and severity of the SSI.[18] New strategies, like the screen-and-treat strategy for *S. aureus* carriage, usually require a cost-effectiveness analysis in order to provide policy makers with tools to decide whether they should be implemented in clinical practice. Cost-effectiveness of the preoperative use of mupirocin has been studied before by Young and Winston.[17] The screen-and-treat strategy was cost-saving by preventing healthcare associated *S. aureus* infections. However, a model was used for this study, based on several assumptions. Furthermore, costs for conventional cultures for nasal screening were included, not for rapid screening tests.[17] In **Chapter 4**, we compared hospital costs of cardiothoracic and orthopedic patients who were allocated to mupirocin and chlorhexidine in the RCT, to the costs of those who received placebo treatment. The strength of this study is the use of real data on costs per patient enrolled in the RCT, provided by the financial department of the Amphia Hospital in Breda, The Netherlands.

Total costs of care for patients who had received placebo were on average €1911 higher than the costs for patients who had received mupirocin and chlorhexidine. The subgroup analyses of

orthopedic and cardiothoracic patients revealed a cost reduction of €955 in orthopedic patients, and a reduction of €2841 in cardiothoracic patients treated with mupirocin and chlorhexidine compared with placebo. Thus, the screen-and-treat strategy is beneficial in terms of hospital costs, at least for these two patient groups. Patients with healthcare associated infections that manifest after discharge do not always return to the hospital where they underwent surgery, and these hospitals may lack a stimulus for implementation of the strategy. Therefore, guidelines for implementation and definition of patient groups are urgently needed, and patient safety programs or health insurance companies may play a role in encouraging the implementation of the strategy.

Since mupirocin and chlorhexidine are very cheap, the screen-and-treat strategy is sometimes replaced by a treat-all strategy. Patients are not screened, but all are treated, irrespective of nasal carrier state. This strategy is calculated to be cheaper than the screen-and-treat strategy.[19] However, it is absolutely unadvisable to treat non-carriers with mupirocin, for several reasons. First, although the side-effects and risks of the use of mupirocin are low, one case of toxic epidermal necrolysis after intranasal application of mupirocin has been described.[20, 21] Mupirocin should therefore be used with prudence. In general, it is unethical to prescribe unnecessary medication. In cardiac surgery, it has been assessed that withholding mupirocin to non-carriers does not increase postoperative infectious complications.[22] It is plausible that this does also apply to other types of patients. Moreover, mupirocin also eradicates other gram-positive colonizers of the nose, and acquisition of *S. aureus* in non-carriers after treatment with mupirocin has been reported. This phenomenon may be the result of elimination of bacterial interference, thereby facilitating colonization with *S. aureus*.[23] Thus, non-carriers may become (transient) carriers after a course of mupirocin. Since bloodstream infections in patients who were non-carriers upon admission have a worse outcome than those in patients who were carriers, acquisition of *S. aureus* carriage in hospital should be avoided.[11]

Second, use of mupirocin may lead to development of resistance.[24] Mupirocin resistance is either the result of base changes in the native tRNA synthetase *ileS*, or of the acquisition of a novel plasmid-mediated tRNA synthetase *mupA*.[24, 25] Base changes result in low-level resistance to mupirocin (minimum inhibitory concentrations (MICs) of 8-64 μg/mL). Initially, low-level resistant *S. aureus* isolates are eradicated after treatment with mupirocin, but after stopping treatment, recolonization occurs rapidly.[26] High-level resistance to mupirocin (MICs of ≥512 μg/mL), due to acquisition of *mupA,* leads to treatment failure.[24]

Worldwide, mupirocin resistance is more common in MRSA than in MSSA, and low-level mupirocin resistance is more common than high-level resistance, although high-level resistance rates have been reported to be up to 25% in selected patient groups. [27, 28] The emergence and

spread of resistant pathogens is related to the use of antibiotics.[29] There also seems to be a relationship between the use of mupirocin and the emergence of resistance in *S. aureus*: resistance rarely occurs when it is used intranasally for short periods, for example as perioperative prophylaxis.[24,27,30] However, resistance is more common when it is used for exit-sites in patients on peritoneal dialysis, for treatment of MRSA carriage, or when it is available over-the-counter.[24,31-33]

Emerging mupirocin resistance in coagulase-negative staphylococci (CoNS) has also been reported, and seems to be correlated with the increased use of mupirocin as perioperative prophylaxis.[34-36] In **Chapter 5,** we show that dispension of the amount of mupirocin to the different wards in the Erasmus MC varies greatly, and that high-level mupirocin resistance in CoNS is significantly more frequent on wards where the highest amounts (>200 g ointment 2% per year) of mupirocin are dispensed. Almost 50% of the CoNS isolates from these wards have a mupirocin MIC of ≥512 µg/mL. On three out of these four wards, all in the cardiothoracic center, mupirocin is used as perioperative prophylaxis in all patients admitted for surgery, regardless of carrier state: patients are not screened for *S. aureus* carriage. The fourth ward is the dialysis ward, where mupirocin is used for the prevention of infections in CAPD and hemodialysis patients. On wards where intermediate amounts (50-200 g ointment 2% per year) of mupirocin are delivered, the proportion of mupirocin resistant strains is significantly lower (21%), and when <50 g ointment 2% per year is dispensed, mupirocin resistance in CoNS is approximately 10%.

A limitation of this study is the risk of selection bias: the numbers of CoNS isolates available for analysis depended on the numbers of cultures taken, and the sites where cultures were taken from. In general, antibiotic susceptibility is only determined if CoNS is isolated from blood, from another normally sterile site, or if an infection persists. As a result, from some departments hardly any isolates were available, and mupirocin resistance may have been underreported; while from other departments only the more resistant isolates causing persisting problems were extensively analyzed. A point-prevalence study investigating CoNS isolates from carriage sites may set this problem to right.

Mupirocin resistance in CoNS may have consequences for therapeutic options, since in mupirocin resistant isolates, resistance to fluoroquinolones, erythromycin and clindamycin is also more frequently found.[36] Furthermore, *mupA* is plasmid mediated, which means that it can be transferred vertically to daughter cells as well as horizontally to other species.[37] With the increase in mupirocin resistance in CoNS, the risk of transfer of the plasmid to *S. aureus* increases. One study nicely showed transfer of mupirocin resistance from *S. epidermidis* to *S. aureus* in a clinical situation.[38] Although acquisition of the plasmid carrying *mupA* by *S. aureus* does not seem to occur often, the risk of emerging resistance should be kept as low as possible, to preserve mupirocin as an important topical therapeutic and preventive agent acting against *S.*

aureus.[39] Future studies should focus on the development of alternatives for mupirocin.

In the randomized, controlled trial, we were not able to prevent all healthcare associated infections in the mupirocin/chlorhexidine treated group. Several explanations can be thought of. First, we did not aim to prevent exogenous *S. aureus* infections, i.e. infections caused by strains that were not present on the skin or mucosa before. Of the 17 infections that developed in the mupirocin/chlorhexidine group, at least four were classified as exogenous, according to the pulsed-field genotyping comparison of the nasal and infecting isolates. Second, we did not monitor compliance to treatment. The first dose of mupirocin was usually applied to the nose of the patient by one of the researchers, but the remainder of the course had to be applied by the patient himself or by one of his caretakers. Chlorhexidine bathing or showering was prescribed, but also not monitored. Thus, it is possible that patients did not complete the five days of treatment. Furthermore, we did not take nasal swabs after treatment, to check whether eradication of carriage was successful. None of the infecting strains in the RCT was mupirocin resistant, but chlorhexidine susceptibility was not assessed. Nevertheless, mupirocin alone does not eradicate nasal carriage in 100% of patients, even when isolates are mupirocin susceptible.[30,40,41] Mupirocin and chlorhexidine together eradicated nasal carriage in 100% of nursing home residents, but in patients on long-term hemodialysis in 83% of cases.[42, 43] The dynamics of carriage before and after eradication are not completely clear. Recolonization from other body sites may have occurred, for example from the throat, a site we did not target to decolonize. Therefore, endogenous infections may have developed in unsuccessfully eradicated patients.

With the randomized, placebo-controlled trial, we focused on the prevention of endogenous healthcare associated *S. aureus* infections, thereby passing by the exogenous infections that occur in both carriers and non-carriers. However, exogenous infections make up an important part of the total burden of *S. aureus* infections. The size of that burden is estimated in **Chapter 6.** Although the majority of infections in carriers are of endogenous origin, the majority of *S. aureus* infections in the total hospital population are of exogenous origin. Based on the carriage rate and infection rates established in the study, the proportion of exogenous infections is 1.9-2.2%, while the proportion of endogenous infections is 0.6-0.9% ($P \leq 0.01$). Carriers have a higher risk of infection than non-carriers, but only 20-30% of all patients are *S. aureus* carriers, explaining the higher proportion of exogenous infections. This underlines the importance of preventive measures that do not only target *S. aureus* carriers, but all patients admitted to hospital (the horizontal approach), next to targeted programs for carriers (the vertical approach).[44]

Quite worrying are the small clusters we recognized by typing the isolates with Raman spectroscopy. Although we screened only a fraction of the population admitted to hospital, we

could identify small clusters of identical strains consisting of infecting and nasal isolates. This is highly suspicious for transmission between patients, which occurs most likely via healthcare workers and the inanimate environment. More money and effort should therefore be put in the education of healthcare workers about general infection control practice, in increasing the awareness of possible transmission, and improvement of compliance to hand hygiene.

In the study described in Chapter 6, the proportion of exogenous infections in carriers was relatively high (30-70%) compared to previous studies and the RCT, where approximately 80% of infections were of endogenous origin.[11, 12] Unfortunately, since several isolates were lost, the exact proportion of exogenous infections was not established, but would have been between 45 and 70%. This difference might be explained by the difference in culture techniques between this study and the previous ones. In this study, we enrolled all patients with a nasal swab which grew *S. aureus*. In contrast to the studies by Von Eiff and Wertheim, we used broth enrichment next to direct plating for the detection of *S. aureus*.[11, 12] In the RCT, we used a PCR to detect *S. aureus* carriers. A peak-height cut-off of the *S. aureus* meltingcurve was used to avoid false-positive results. High numbers of *S. aureus* (>100 CFU in the sample) are detected reliably with this kit with a sensitivity of approximately 97%, but low-level carriers may have been missed. By using not only direct culture results, but also culture results from broth enrichment in Chapter 6, carrier detection was more sensitive, and we probably have assigned low-level, intermittent or transient carriers to the *S. aureus* carrier group. This also explains the relatively high carriage rate of 30% in this study. The risk of endogenous infections in low-level carriers is considered to be low.[45, 46] If misclassification of carriers indeed occurred, it did not affect the classification of endogenous and exogenous infections, and did thus not affect the estimated proportion of exogenous infections in the total hospital population of 1.9-2.2%. The classification of endogenous and exogenous infections in carriers may though have been affected by comparing the infecting strain with the nasal strain only, both in the RCT as well as in the study of Chapter 6. More than just one strain colonizes the body in almost 7% of carriers, as was modeled by Cespedes et al.[47] Thus, an infection may have been endogenous, but not of nasal origin; or, more than one strain colonized the nose, but strains were morphologically identical and not recognized as different, and therefore not both were stored. In this way, we may have misclassified endogenous infections as exogenous. Future studies should therefore address the dynamics of carriage by focusing on the numbers of different *S. aureus* strains people carry, not only in the nose, but at other body sites as well. Raman spectroscopy can be helpful to assess the difference between the strains.

Colonization with *S. aureus* is not only a risk factor for the development of *S. aureus* infections, but it also seems to have a protective effect on mortality due to *S. aureus* bacteremia.[11] In **Chapter 7,** we aimed to unravel the underlying immunological basis for this phenomenon, by investigating the humoral immune response to *S. aureus* bacteremia using immune

proteomics. We were able to show that the immune response patterns for endogenous and exogenous infection differed. In carriers, IgG antibodies directed against antigens specific for their colonizing strain were present before onset of bacteremia. In carriers who developed an endogenous infection, binding of these antibodies was enhanced during bacteremia, but the specificity of the antibodies remained the same. On the other hand, in carriers and non-carriers who developed an exogenous bacteremia, novel antibodies were recognized in serum obtained during bacteremia with specificity for antigens of the invasive strains, which were not present before onset of infection.

It has been shown before, that strain-specific antibodies are produced in nasal carriers of *S. aureus*.[48, 49] However, in artificially inoculated subjects, antibody titers against the inoculated strain could not be detected.[50] Apparently, a longer period of carriage is necessary to induce an antibody response. Probably, in persistent carriage subclinical (micro)infections trigger this response. Persistent carriage of *S. aureus* thus seems to confer immunity, albeit only protective for death from bacteremia, not for invasion of the pathogen.

The results of this study show that exogenous *S. aureus* infections are unexpected events, at least for the patient's immune system, which does not have the potential to build up an immune response rapidly enough to prevent severe detoriation and death. The heterogeneity of *S. aureus* strains is enormous, as a result of the wide variety of antigens individual strains possess and express.[51] Up to now, this heterogeneity hampers the development of effective vaccins directed against *S. aureus*.[52] Therefore, this study emphasizes the importance of prevention of exogenous infections caused by *S. aureus*.

In **Chapter 8,** the prevalence of MRSA carriage on admission to hospital is reported for the period 2005-2007. This prevalence is 0.11%, not statistically significant from the prevalence in 1999-2000 when it was 0.03%.[53]

Among the seven MRSA carriers we found in the study, one was a pig farmer. Nowadays, we are aware of the high prevalence of MRSA carriage in pig farmers.[54, 55] However, at the time of screening, this risk category was not yet added to the guidelines of the Dutch Working Party on Infection Prevention (WIP). In 2006, these guidelines were updated, and from that moment included persons in contact with living pigs or veal calves. Recently, the prevalence of MRSA in broilers and in persons working with live broilers was estimated at 6.9% and 13.8%, respectively.[56] In 2012, the guidelines were updated again, and now also include persons in contact with broilers.[57] Identifying possible new sources and transmission routes of MRSA remains important, to incorporate these in the existing guidelines and prevent the spread of MRSA. For this purpose, the MUO (MRSA of Unknown Origin) study is currently carried out, investigating cases of MRSA

that were unexpectedly found, by means of an observational epidemiological study and a case-control design together with genotyping analyses in different risk groups. The results of this study will hopefully elucidate the sources of unexpectedly encountered MRSA. Furthermore, MRSA prevalence in the community should be monitored closely and frequently.

The low prevalence of MRSA in The Netherlands is a result of the Search-and-Destroy policy, and frequently associated with the restrictive use of antibiotics in health care.[53] In stock farming, antibiotics are frequently used, and the high prevalence of MRSA may be explained by this.[56,58] Restriction of antibiotic use in livestock is therefore urgently needed, as was recommended by the Health Council of The Netherlands.[59] The continuous exposure to MRSA in stock farming hampers the "destroy" part of the Dutch Search-and-Destroy policy, since continuous exposure is a contra-indication for eradication therapy.[58] Furthermore, with the addition of more risk groups to the WIP guidelines, the costs for screening and pre-emptive isolation rise. This might endanger the Search-and-Destroy policy, while maintenance of this policy is important to avert an endemic situation. MRSA endemicity finally results in other choices for prophylactic, empirical and therapeutical antibiotics. These antibiotics, like glycopeptides, have more side-effects, need to be administered intravenously, are expensive, and their use might induce resistance in other microorganisms.[60] Besides, MRSA infections are associated with higher mortality rates and costs than MSSA infections.[61, 62] MRSA infections add to the total number of healthcare associated bloodstream infections, without replacing bloodstream infections caused by MSSA.[63] Modeling suggests that without any infection control measure, in-hospital MRSA prevalence will rise from <1% to 15% within approximately 10 years.[64]

Conclusions

- The number of healthcare associated *S. aureus* infections in nasal carriers of *S. aureus* can be reduced with almost 60% by rapid screening and decolonization on admission.
- In the subgroup of surgical patients, deep surgical-site infections can be reduced with almost 80% by the intervention.
- No conclusions can be drawn from the RCT about the effect of decolonization on the incidence of infections in non-surgical *S. aureus* carriers.
- Rapid detection and decolonization of *S. aureus* carriers significantly reduces one-year mortality in surgical patients who undergo clean operations.
- The screen-and-treat strategy should primarily focus on surgical patients undergoing clean procedures.
- In patients undergoing cardiothoracic or orthopaedic surgery, screening for *S. aureus* nasal carriage and treating carriers with mupirocin and chlorhexidine results in a substantial reduction of hospital costs.
- Mupirocin resistance in *S. aureus* is observed only sporadically, but is emerging in CoNS, and is most likely associated with the frequent use of mupirocin as part of the preoperative *S. aureus* decolonization strategy to prevent hospital associated *S. aureus* infections.
- Mupirocin as perioperative prophylaxis should exclusively be used by patients colonized with *S. aureus*, who are at risk for developing healthcare associated *S. aureus* infections.
- Although the majority of infections in carriers are of endogenous origin, the majority of *S. aureus* infections in the total hospital population are exogenous infections.
- The humoral immune response in endogenous bloodstream infections differs from the response in exogenous infections, which may contribute to the better outcome of bacteremia in carriers of *S. aureus*.
- Prevention of *S. aureus* infections should not only focus on carriers, but on the hospital population in general, by preventing endogenous as well as exogenous infections.
- The prevalence of MRSA upon admission to hospital in The Netherlands remains among the lowest in the world, indicating that the Search-and-Destroy policy is still successful and should be maintained.

Future perspectives

Although some issues have been resolved with the results of the studies described in this thesis, questions on nasal *S. aureus* carriage and the prevention of healthcare associated *S. aureus* infections remain. How do we realize commitment to the screen-and-treat strategy, by patients, managers, doctors, and nurses? How do we get the screening results from the lab to the docter as soon as possible? What is an acceptable time lag between the moment of nasal culture, and the start of intervention? And between the start of intervention and the moment of admission? Do we have to use rapid diagnostics upon admission, or can we screen patients upon preadmission visits? What exactly will be the target population for the strategy? Currently, a study addressing these questions on the facilitators and barriers of implementation is carried out in the Erasmus MC. For this study, behavioral science is applied, to achieve maximum compliance of all healthcare workers involved. Nevertheless, the results of this study will only partly be generalizable to other hospitals. Protocols on the implementation of the strategy should therefore be adapted to the local situation of a hospital.

After implementation of the strategy, mupirocin resistance should be monitored closely, preferably with point-prevalence measurements. An important subject for future studies should be the development of alternatives for mupirocin. Promising agents are under investigation yet, but their efficacy has to be evaluated further.[65] Ideally, these agents have a low propensity for development of resistance, and eradicate *S. aureus* even more efficiently than mupirocin already does.

With the RCT, we showed that the screen-and-treat strategy reduces healthcare associated infections with *S. aureus* in surgical patients. For the non-surgical population however, no conclusions on the effectiveness of this strategy can be drawn from our studies, or from previous ones. Since not only surgical-site infections, but also bloodstream infections are more prevalent in carriers than in non-carriers of *S. aureus*, one would expect that eradication of *S. aureus* carriage also reduces the incidence of bloodstream infections with this pathogen. However, due to the relatively low frequency of these infections, a large number of patients need to be enrolled to show this effect in a RCT. Furthermore, additional measures might have to be taken, since insertion of a catheter involves both a breach of the skin and implantation of foreign body material. For instance, chlorhexidine baths, or local application of an antibacterial agent or disinfectant may be needed to sufficiently eradicate *S. aureus,* and to reduce the incidence of catheter-related bloodstream infections.

Additionally, more insight into the dynamics of *S. aureus* carriage is needed. Does only one *S. aureus* strain colonize the body, or more, and on which sites then? What exactly happens

after eradication of carriage with mupirocin and chlorhexidine? Where on the body does the bacterium persist, and in what quantities? How long does it take before recolonization occurs? What happens with other colonizing bacteria on skin and mucosa, and are rates of healthcare associated infections caused by other pathogens altered? A long-term, prospective follow-up study in a large group of volunteers as well as in patients should be carried out to resolve these issues, focusing not only on *S. aureus,* but also on other pathogens. An informative patient group to start with would be a ward where mupirocin is currently used in all patients without assessment of carrier state. Information about the before mentioned issues, but also about the dynamics of mupirocin resistance, can then be obtained.

Based on one-year mortality rates, we recommend targeting patients undergoing clean procedures for the screen-and-treat strategy. However, an exact definition of the targeted population is necessary to save costs, and to restrict to use of mupirocin and chlorhexidine. A significant beneficial effect in terms of morbidity, and not only mortality, could also be a condition on which to decide to implement the strategy. In the ideal world, we would assess effect size of the intervention in terms of prevention of infections, morbidity, mortality, and cost-effectiveness per procedure to decide whether or not to implement the strategy for that particular intervention. However, this is not only prohibitive, but it is also unethical to conduct a study and deny patients a proven preventive strategy. Observational studies with historical control groups will therefore have to be conducted to assess the effect size in different patient populations.

Proteomics, the study of the structures and functions of proteins, was used earlier to provide information on cell physiology and pathogenicity of *S. aureus*.[66] Here, it was applied to unravel the immune response in carriers and non-carriers of *S. aureus*, and to study the difference in the humoral immune response in endogenous and exogenous infections. In the future, it might be used to diagnose *S. aureus* bloodstream infections at an early stage, by identification of the core immune proteome of the bacterium in peripheral blood. Its usefulness for therapy of bloodstream infections seems to be far-fetched, but might once become an option when resistance to antibiotics has become an insuperable problem: If a blood isolate is available, proteomics can be applied to identify the antigens present on the infecting strain, after which specific neutralizing immunoglobulins should be administered.

Exogenous infections are not only more prevalent among the total hospital population, but also have a higher mortality compared to endogenous infections.[11] Therefore, money and effort should be put in the education of healthcare workers, in increasing the awareness of development of healthcare associated infections by transmission, and improvement of compliance to hand hygiene.

References

1. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998; **339**(8): 520-32.
2. Casewell MW, Hill RL. Elimination of nasal carriage of Staphylococcus aureus with mupirocin ('pseudomonic acid')--a controlled trial. J Antimicrob Chemother. 1986; **17**(3): 365-72.
3. Bulanda M, Gruszka M, Heczko B. Effect of mupirocin on nasal carriage of Staphylococcus aureus. J Hosp Infect. 1989; **14**(2): 117-24.
4. Reagan DR, Doebbeling BN, Pfaller MA, Sheetz CT, Houston AK, Hollis RJ, et al. Elimination of coincident Staphylococcus aureus nasal and hand carriage with intranasal application of mupirocin calcium ointment. Ann Intern Med. 1991; **114**(2): 101-6.
5. van Rijen M, Bonten M, Wenzel R, Kluytmans J. Mupirocin ointment for preventing Staphylococcus aureus infections in nasal carriers. Cochrane database of systematic reviews (Online). 2008; (4): CD006216.
6. Perl TM, Cullen JJ, Wenzel RP, Zimmerman MB, Pfaller MA, Sheppard D, et al. Intranasal mupirocin to prevent postoperative *Staphylococcus aureus* infections. N Engl J Med. 2002; **346**(24): 1871-7.
7. Kluytmans JA, Mouton JW, VandenBergh MF, Manders MJ, Maat AP, Wagenvoort JH, et al. Reduction of surgical-site infections in cardiothoracic surgery by elimination of nasal carriage of *Staphylococcus aureus*. Infect Control Hosp Epidemiol. 1996; **17**(12): 780-5.
8. Wertheim HF, Vos MC, Ott A, Voss A, Kluytmans JA, Vandenbroucke-Grauls CM, et al. Mupirocin prophylaxis against nosocomial *Staphylococcus aureus* infections in nonsurgical patients: a randomized study. Ann Intern Med. 2004; **140**(6): 419-25.
9. Kalmeijer MD, Coertjens H, van Nieuwland-Bollen PM, Bogaers-Hofman D, de Baere GA, Stuurman A, et al. Surgical site infections in orthopedic surgery: the effect of mupirocin nasal ointment in a double-blind, randomized, placebo-controlled study. Clin Infect Dis. 2002; **35**(4): 353-8.
10. Chambers HF, 3rd, Winston LG. Mupirocin prophylaxis misses by a nose. Ann Intern Med. 2004; **140**(6): 484-5.
11. Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, et al. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet. 2004; **364**(9435): 703-5.
12. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. N Engl J Med. 2001; **344**(1): 11-6.
13. Whitehead J. The design and analysis of sequential clinical trials. Revised Second Edition ed. Chichester: John Wiley & Sons; 1997.
14. Diekema D, Johannsson B, Herwaldt L, Beekmann S, Jernigan J, Kallen A, et al. Current practice in Staphylococcus aureus screening and decolonization. Infect Control Hosp Epidemiol. 2011; **32**(10): 1042-4.
15. Top CDC Recommendations to Prevent Healthcare-Associated Infections. [cited April 24, 2013]; Available from: http://www.cdc.gov/HAI/prevent/top-cdc-recs-prevent-hai.html
16. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. Infect Control Hosp Epidemiol. 1999; **20**(4): 250-78; quiz 79-80.
17. Young LS, Winston LG. Preoperative use of mupirocin for the prevention of healthcare-associated *Staphylococcus aureus* infections: A cost-effectiveness analysis. Infect Control Hosp Epidemiol. 2006; **27**(12): 1304-12.
18. Boltz MM, Hollenbeak CS, Julian KG, Ortenzi G, Dillon PW. Hospital costs associated with surgical site infections in general and vascular surgery patients. Surgery. 2011; **150**(5): 934-42.
19. Wassenberg MW, de Wit GA, Bonten MJ. Cost-effectiveness of preoperative screening and

eradication of Staphylococcus aureus carriage. PLoS One. 2011; **6**(5): e14815.
20. Praz SM, de Torrente A, Zender H, Schmied E, Schleppy CA, Genne D. Toxic epidermal necrolysis after topical intranasal application of mupirocin. Infect Control Hosp Epidemiol. 2003; **24**(6): 459-60.
21. Hudson IR. The efficacy of intranasal mupirocin in the prevention of staphylococcal infections: a review of recent experience. J Hosp Infect. 1994; **27**(2): 81-98.
22. Shrestha NK, Banbury MK, Weber M, Cwynar RE, Lober C, Procop GW, et al. Safety of targeted perioperative mupirocin treatment for preventing infections after cardiac surgery. The Annals of thoracic surgery. 2006; **81**(6): 2183-8.
23. Wertheim HF, Verveer J, Boelens HA, van Belkum A, Verbrugh HA, Vos MC. Effect of mupirocin treatment on nasal, pharyngeal, and perineal carriage of Staphylococcus aureus in healthy adults. Antimicrob Agents Chemother. 2005; **49**(4): 1465-7.
24. Patel JB, Gorwitz RJ, Jernigan JA. Mupirocin resistance. Clin Infect Dis. 2009; **49**(6): 935-41.
25. Farmer TH, Gilbart J, Elson SW. Biochemical basis of mupirocin resistance in strains of Staphylococcus aureus. J Antimicrob Chemother. 1992; **30**(5): 587-96.
26. Harbarth S, Dharan S, Liassine N, Herrault P, Auckenthaler R, Pittet D. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 1999; **43**(6): 1412-6.
27. Fawley WN, Parnell P, Hall J, Wilcox MH. Surveillance for mupirocin resistance following introduction of routine peri-operative prophylaxis with nasal mupirocin. J Hosp Infect. 2006; **62**(3): 327-32.
28. Abbasi-Montazeri E, Khosravi AD, Feizabadi MM, Goodarzi H, Khoramrooz SS, Mirzaii M, et al. The prevalence of methicillin resistant Staphylococcus aureus (MRSA) isolates with high-level mupirocin resistance from patients and personnel in a burn center. Burns. 2013; **39**(4): 650-4.
29. Struelens MJ, Byl B, Vincent JL. Antibiotic policy: a tool for controlling resistance of hospital pathogens. Clin Microbiol Infect. 1999; **5 Suppl 1**: S19-S24.
30. van Rijen MM, Bonten M, Wenzel RP, Kluytmans JA. Intranasal mupirocin for reduction of *Staphylococcus aureus* infections in surgical patients with nasal carriage: a systematic review. J Antimicrob Chemother. 2008; **61**(2): 254-61.
31. Perez-Fontan M, Rosales M, Rodriguez-Carmona A, Falcon TG, Valdes F. Mupirocin resistance after long-term use for Staphylococcus aureus colonization in patients undergoing chronic peritoneal dialysis. Am J Kidney Dis. 2002; **39**(2): 337-41.
32. Upton A, Lang S, Heffernan H. Mupirocin and Staphylococcus aureus: a recent paradigm of emerging antibiotic resistance. J Antimicrob Chemother. 2003; **51**(3): 613-7.
33. Vivoni AM, Santos KR, de-Oliveira MP, Giambiagi-deMarval M, Ferreira AL, Riley LW, et al. Mupirocin for controlling methicillin-resistant Staphylococcus aureus: lessons from a decade of use at a university hospital. Infect Control Hosp Epidemiol. 2005; **26**(7): 662-7.
34. Desroches M, Potier J, Laurent F, Bourrel AS, Doucet-Populaire F, Decousser JW, et al. Prevalence of mupirocin resistance among invasive coagulase-negative staphylococci and methicillin-resistant Staphylococcus aureus (MRSA) in France: emergence of a mupirocin-resistant MRSA clone harbouring mupA. J Antimicrob Chemother. 2013; **68**(8): 1714-7.
35. Lepainteur M, Royer G, Bourrel AS, Romain O, Duport C, Doucet-Populaire F, et al. Prevalence of resistance to antiseptics and mupirocin among invasive coagulase-negative staphylococci from very preterm neonates in NICU: the creeping threat? J Hosp Infect. 2013; **83**(4): 333-6.
36. Bathoorn E, Hetem DJ, Alphenaar J, Kusters JG, Bonten MJ. Emergence of high level mupirocin resistance in coagulase-negative staphylococci associated with increased short-term mupirocin

use. Journal of clinical microbiology. 2012.
37. Lindsay JA, Holden MT. Understanding the rise of the superbug: investigation of the evolution and genomic variation of Staphylococcus aureus. Functional & integrative genomics. 2006; **6**(3): 186-201.
38. Hurdle JG, O'Neill AJ, Mody L, Chopra I, Bradley SF. In vivo transfer of high-level mupirocin resistance from Staphylococcus epidermidis to methicillin-resistant Staphylococcus aureus associated with failure of mupirocin prophylaxis. J Antimicrob Chemother. 2005; **56**(6): 1166-8.
39. Bastos MC, Mondino PJ, Azevedo ML, Santos KR, Giambiagi-deMarval M. Molecular characterization and transfer among Staphylococcus strains of a plasmid conferring high-level resistance to mupirocin. Eur J Clin Microbiol Infect Dis. 1999; **18**(6): 393-8.
40. Doebbeling BN, Breneman DL, Neu HC, Aly R, Yangco BG, Holley HP, Jr., et al. Elimination of Staphylococcus aureus nasal carriage in health care workers: analysis of six clinical trials with calcium mupirocin ointment. The Mupirocin Collaborative Study Group. Clin Infect Dis. 1993; **17**(3): 466-74.
41. Trautmann M, Stecher J, Hemmer W, Luz K, Panknin HT. Intranasal mupirocin prophylaxis in elective surgery. A review of published studies. Chemotherapy. 2008; **54**(1): 9-16.
42. Watanakunakorn C, Axelson C, Bota B, Stahl C. Mupirocin ointment with and without chlorhexidine baths in the eradication of Staphylococcus aureus nasal carriage in nursing home residents. Am J Infect Control. 1995; **23**(5): 306-9.
43. Watanakunakorn C, Brandt J, Durkin P, Santore S, Bota B, Stahl CJ. The efficacy of mupirocin ointment and chlorhexidine body scrubs in the eradication of nasal carriage of Staphylococcus aureus among patients undergoing long-term hemodialysis. Am J Infect Control. 1992; **20**(3): 138-41.
44. Wenzel RP, Edmond MB. Infection control: the case for horizontal rather than vertical interventional programs. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2010; **14 Suppl 4**: S3-5.
45. Nouwen JL, Fieren MW, Snijders S, Verbrugh HA, van Belkum A. Persistent (not intermittent) nasal carriage of Staphylococcus aureus is the determinant of CPD-related infections. Kidney Int. 2005; **67**(3): 1084-92.
46. White A. Increased infection rates in heavy nasal carriers of coagulase-positive staphylococci. Antimicrobial Agents Chemother (Bethesda). 1963; **161**: 667-70.
47. Cespedes C, Said-Salim B, Miller M, Lo SH, Kreiswirth BN, Gordon RJ, et al. The clonality of Staphylococcus aureus nasal carriage. J Infect Dis. 2005; **191**(3): 444-52.
48. Ritz HL, Kirkland JJ, Bond GG, Warner EK, Petty GP. Association of high levels of serum antibody to staphylococcal toxic shock antigen with nasal carriage of toxic shock antigen-producing strains of Staphylococcus aureus. Infect Immun. 1984; **43**(3): 954-8.
49. Holtfreter S, Roschack K, Eichler P, Eske K, Holtfreter B, Kohler C, et al. Staphylococcus aureus carriers neutralize superantigens by antibodies specific for their colonizing strain: a potential explanation for their improved prognosis in severe sepsis. J Infect Dis. 2006; **193**(9): 1275-8.
50. Holtfreter S, Nguyen TT, Wertheim H, Steil L, Kusch H, Truong QP, et al. Human immune proteome in experimental colonization with Staphylococcus aureus. Clin Vaccine Immunol. 2009; **16**(11): 1607-14.
51. Ziebandt AK, Kusch H, Degner M, Jaglitz S, Sibbald MJ, Arends JP, et al. Proteomics uncovers extreme heterogeneity in the Staphylococcus aureus exoproteome due to genomic plasticity and variant gene regulation. Proteomics. 2010; **10**(8): 1634-44.
52. Holtfreter S, Kolata J, Broker BM. Towards the immune proteome of Staphylococcus aureus - The

anti-S. aureus antibody response. Int J Med Microbiol. 2010; **300**(2-3): 176-92.
53. Wertheim HF, Vos MC, Boelens HA, Voss A, Vandenbroucke-Grauls CM, Meester MH, et al. Low prevalence of methicillin-resistant Staphylococcus aureus (MRSA) at hospital admission in the Netherlands: the value of search and destroy and restrictive antibiotic use. J Hosp Infect. 2004; **56**(4): 321-5.
54. Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuvel MG, Heck ME, Pluister GN, et al. Community-acquired MRSA and pig-farming. Ann Clin Microbiol Antimicrob. 2006; **5**: 26.
55. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerg Infect Dis. 2005; **11**(12): 1965-6.
56. Mulders MN, Haenen AP, Geenen PL, Vesseur PC, Poldervaart ES, Bosch T, et al. Prevalence of livestock-associated MRSA in broiler flocks and risk factors for slaughterhouse personnel in The Netherlands. Epidemiol Infect. 2010; **138**(5): 743-55.
57. Prevention) WDWPoI. Policy for methicillin-resistant *Staphylococcus aureus*. 2012 [cited August 2013]; Available from: www.wip.nl
58. Wulf M, Voss A. MRSA in livestock animals-an epidemic waiting to happen? Clin Microbiol Infect. 2008.
59. Advies gezondheidsraad LA-MRSA. 2012 [cited; Available from: http://www.vwa.nl/onderwerpen/risicobeoordelingen/bestand/2202891/adviesgezondheidsrisico-mrsa
60. Vriens M, Blok H, Fluit A, Troelstra A, Van Der Werken C, Verhoef J. Costs associated with a strict policy to eradicate methicillin-resistant Staphylococcus aureus in a Dutch University Medical Center: a 10-year survey. Eur J Clin Microbiol Infect Dis. 2002; **21**(11): 782-6.
61. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. Clin Infect Dis. 2003; **36**(1): 53-9.
62. Cosgrove SE. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. Clin Infect Dis. 2006; **42 Suppl 2**: S82-9.
63. Ammerlaan HS, Harbarth S, Buiting AG, Crook DW, Fitzpatrick F, Hanberger H, et al. Secular trends in nosocomial bloodstream infections: antibiotic-resistant bacteria increase the total burden of infection. Clin Infect Dis. 2013; **56**(6): 798-805.
64. Bootsma MC, Diekmann O, Bonten MJ. Controlling methicillin-resistant Staphylococcus aureus: quantifying the effects of interventions and rapid diagnostic testing. Proceedings of the National Academy of Sciences of the United States of America. 2006; **103**(14): 5620-5.
65. Saravolatz LD, Pawlak J, Johnson L, Bonilla H, Saravolatz LD, 2nd, Fakih MG, et al. In vitro activities of LTX-109, a synthetic antimicrobial peptide, against methicillin-resistant, vancomycin-intermediate, vancomycin-resistant, daptomycin-nonsusceptible, and linezolid-nonsusceptible Staphylococcus aureus. Antimicrob Agents Chemother. 2012; **56**(8): 4478-82.
66. Hecker M, Engelmann S, Cordwell SJ. Proteomics of Staphylococcus aureus--current state and future challenges. Journal of chromatography. 2003; **787**(1): 179-95.

Chapter 10

Nederlandse samenvatting

Nederlandse samenvatting

Staphylococcus aureus (S. aureus) is een bacterie die op de huid en slijmvliezen van veel gezonde personen aanwezig is, zonder daar problemen te veroorzaken. Deze personen noemen we "dragers". Er zijn persisterend dragers, die de bacterie vrijwel altijd bij zich dragen; intermitterend dragers, die af en toe met *S. aureus* gekoloniseerd zijn, en niet-dragers. De bacterie is bij de meeste dragers in de neus te vinden.

S. aureus is niet alleen een onschuldige bewoner van de huid, maar kan ook vele soorten infecties veroorzaken, variërend van relatief milde huidinfecties zoals krentenbaard (impetigo), tot levensbedreigende bloedbaaninfecties (sepsis). In eerder onderzoek werd aangetoond dat persisterend dragers, die vaak grote hoeveelheden bacteriën bij zich dragen, een groter risico hebben om dergelijke infecties te ontwikkelen dan intermitterend dragers en niet-dragers.

Zorggerelateerde infecties zijn infecties die tijdens of na een ziekenhuisopname ontstaan, en het gevolg zijn van verkregen medische zorg. Voorbeelden zijn bloedbaaninfecties bij patiënten met een intraveneuze lijn, en wondinfecties na een operatie. *S. aureus* is één van de belangrijkste verwekkers van dergelijke infecties, en uit eerder onderzoek is gebleken dat *S. aureus* neusdragers een aanzienlijk groter risico lopen op zorggerelateerde infecties met deze bacterie dan niet-dragers. De infecties die bij dragers ontstaan zijn voor het grootste deel van "endogene" oorsprong, d.w.z. dat de *S. aureus* die de infectie heeft veroorzaakt, identiek is aan de stam waar de drager mee gekoloniseerd was. Dit in tegenstelling tot "exogene" infecties, waarbij de bacterie afkomstig is van de omgeving, veelal via de handen van gezondheidszorgmedewerkers.

Het doel van dit proefschrift was om bij te dragen aan de kennis en de preventie van zorggerelateerde infecties veroorzaakt door *S. aureus*. Hiertoe hebben we een gerandomiseerd, placebo-gecontroleerd onderzoek uitgevoerd in vijf Nederlandse ziekenhuizen **(hoofdstuk 2).** Met het idee dat zorggerelateerde *S. aureus* infecties bij dragers misschien voorkomen kunnen worden door de bacterie te eradiceren van de huid en de neus, werden patiënten bij opname in het ziekenhuis gescreend op dragerschap. Middels een snelle test (PCR) was binnen 24 uur bekend of een patiënt drager was van *S. aureus.* Als dat zo was, kregen patiënten een behandeling van vijf dagen. Circa de helft van de patiënten kreeg mupirocine neuszalf (een antibioticum) en chloorhexidine zeep (desinfecterende zeep); de andere helft kreeg ook neuszalf en zeep, maar zonder werkzame stof (placebo). Dit onderzoek was dubbelblind, d.w.z. dat noch de onderzoekers, noch de behandelaars wisten welke patiënt welke behandeling gekregen had. Uiteraard werd dit wel gecodeerd vastgelegd om later de gegevens te kunnen analyseren. Gedurende de opname en tot zes weken na ontslag werd gekeken of patiënten een infectie met *S. aureus* hadden ontwikkeld. Na beëindiging van de studie en analyse van de resultaten bleek dat in de groep die

mupirocine en chloorhexidine had gekregen, bijna 60% minder infecties met *S. aureus* waren ontstaan dan in de groep die placebo had gekregen. Het grootste deel van de patiënten die aan deze studie deelnam had een operatie ondergaan ("chirurgische patiënten"), en het grootste effect van de behandeling was dan ook aantoonbaar bij postoperatieve wondinfecties. De conclusie van deze studie is dat patiënten die een ingreep ondergaan met een relatief groot risico op infectie, bij opname in het ziekenhuis gescreend moeten worden op *S. aureus* dragerschap, en dat dragers behandeld moeten worden met mupirocine en chloorhexidine.

In de studie waren meerdere soorten chirurgische patiënten geïncludeerd, maar waarschijnlijk hebben niet alle patiënten evenveel baat bij de behandeling met mupirocine en chloorhexidine. Om dit te onderzoeken, hebben we na één jaar en na drie jaar de sterfte vergeleken tussen de groep patiënten die mupirocine en chloorhexidine had gekregen en de groep die placebo had gekregen **(hoofdstuk 3)**. Dit hebben we gedaan voor meerdere subgroepen. Uit deze studie blijkt dat er in de subgroep van patiënten die een schone ingreep ondergingen, na 1 jaar minder patiënten overleden waren in de mupirocine/chloorhexidine groep dan in de placebo groep. Schone ingrepen zijn ingrepen waarbij vrijwel alleen bacteriën in de wond komen die op de huid aanwezig waren, en geen bacteriën uit bijvoorbeeld het spijsverteringskanaal of de luchtwegen. Het screenen van patiënten op dragerschap en het behandelen van dragers zou daarom tenminste moeten worden uitgevoerd bij patiënten die een schone ingreep ondergaan, mits deze ingrepen geassocieerd zijn met een hoog risico op postoperatieve wondinfecties.

Zorggerelateerde infecties brengen extra kosten met zich mee, die kunnen oplopen tot €30.000 per infectie. Het screenen van patiënten en het behandelen van dragerschap is echter ook kostbaar. Zeker gezien de huidige bezuinigingen in de gezondheidszorg is het van belang te weten of een nieuw in te voeren strategie kosteneffectief is. In **hoofdstuk 4** hebben we gekeken naar de kosten die gemaakt zijn voor alle patiënten die in de studie waren geïncludeerd en een orthopedische ingreep of een hartoperatie hadden ondergaan. De totale kosten die per patiënt in de placebo groep waren gemaakt, waren gemiddeld €1911 hoger dan de kosten per patiënt in de mupirocine/chloorhexidine groep. Hieruit blijkt dat de screen-en-behandel strategie voor deze patiëntgroepen kosteneffectief is.

Mupirocine is een antibioticum dat bacteriën zoals staphylococcen (waaronder *S. aureus)* doodt. Er is echter ook resistentie voor mupirocine beschreven. Als bacteriën resistent worden voor een antibioticum, is het niet meer werkzaam. Resistentie onder bacteriën kan toenemen als een antibioticum veel wordt gebruikt. In het Erasmus MC wordt op sommige afdelingen veel mupirocine gebruikt, en op andere afdelingen veel minder of zelfs helemaal niet. In **hoofdstuk 5** hebben we gekeken naar mupirocineresistentie bij staphylococcen ten opzichte van het gebruik van mupirocine. Hieruit blijkt dat resistentie bij *S. aureus* gelukkig slechts sporadisch

voorkomt, maar dat resistentie bij coagulase-negatieve staphylococcen (CoNS; een ander soort staphylococcen) frequent gevonden wordt. Op de afdelingen waar veel mupirocine door de apotheek geleverd wordt, is het aandeel uit bloed gekweekte CoNS stammen die mupirocine resistent zijn zelfs ruim 45%. Omdat mupirocine resistentie vaak gepaard gaat met resistentie voor andere antibiotica, en omdat resistentie van CoNS aan *S. aureus* doorgegeven kan worden, is dit een zeer onwenselijke situatie. Het is daarom noodzaak om mupirocine slechts aan die patiënten voor te schrijven, die er ook daadwerkelijk baat bij hebben. Omdat er geen effect verwacht wordt van mupirocine bij niet-dragers, is het belangrijk om patiënten te screenen op dragerschap en alleen dragers mupirocine voor te schrijven.

Met de studie uit hoofdstuk 2 hebben we ons slechts gericht op het voorkomen van endogene *S. aureus* infecties. Bij zowel dragers als niet-dragers komen echter ook exogene infecties voor. Om de juiste infectiepreventie maatregelen te kunnen nemen, is het van belang te weten welk deel van de infecties in het ziekenhuis van endogene oorsprong is, en welk deel exogeen. Omdat we ruim 2000 patiënten bij binnenkomst in het Erasmus MC op dragerschap gescreend hadden, konden we voor een grote groep patiënten nagaan of ze infecties hadden ontwikkeld, en konden we de neusstammen met de infectiestammen vergelijken **(hoofdstuk 6)**. Uit deze studie blijkt dat dragers weliswaar een groter risico lopen op een *S. aureus* infectie, maar dat er in het ziekenhuis absoluut gezien meer exogene dan endogene infecties zijn. Daarnaast zagen we dat een aantal van de exogene infecties door dezelfde stam veroorzaakt werd, wat erop wijst dat er overdracht heeft plaatsgevonden tussen patiënten. Deze studie laat zien dat preventie van *S. aureus* infecties zich niet alleen moet richten op endogene infecties, maar op preventieve maatregelen in het algemeen. Het is tevens een goede aanleiding om personeel te wijzen op de juiste hygiënemaatregelen, in het bijzonder handhygiëne.

Een eerdere studie wijst uit dat dragerschap van *S. aureus* niet alleen een risicofactor is voor het ontwikkelen van *S. aureus* infecties, maar ook dat dragers minder vaak overlijden aan een bloedbaaninfectie met deze bacterie dan niet-dragers. In **hoofdstuk 7** hebben we gekeken naar de afweerreactie van het lichaam op bloedbaaninfecties bij dragers en niet-dragers. Uit dit onderzoek blijkt dat dragers, die een endogene infectie ontwikkelen, de bacterie eerder herkennen en een betere afweerreactie op gang brengen dan niet-dragers. Een infectie met onbekende stam is voor het afweersysteem een onverwachte gebeurtenis, waardoor het tijd kost een goede afweerreactie te ontwikkelen. Dit zou het verschil in sterfte tussen dragers en niet-dragers kunnen verklaren.

In **hoofdstuk 8** tenslotte, hebben we gekeken naar dragerschap van methicilline-resistente *S.*

aureus (MRSA). Deze bacterie is ongevoelig voor een belangrijke groep antibiotica, en komt wereldwijd veel voor. In Nederland is het aantal MRSA-dragers echter altijd zeer laag geweest. Om verspreiding van deze resistente bacterie te voorkomen, worden patiënten die met deze bacterie gekoloniseerd zijn in isolatie verpleegd. De Werkgroep Infectie Preventie (WIP) heeft richtlijnen opgesteld hoe om te gaan met patiënten die mogelijk gekoloniseerd zouden kunnen zijn met MRSA, zoals patiënten die in het buitenland opgenomen geweest zijn, of patiënten die in aanraking zijn geweest met bekende MRSA-dragers. Met deze studie wilden we meten wat het percentage patiënten is dat opgenomen wordt met MRSA-dragerschap. Uit onze studie blijkt dat het aantal dragers over een periode van zes jaar weliswaar gestegen is, maar niet statistisch significant verschillend is van voorgaande meting. Aangezien het grootste deel van de gevonden MRSA van onbekende oorsprong was, is het belangrijk nieuwe bronnen op te sporen en de richtlijnen aan te passen, zoals dat eerder het geval is geweest bij patiënten afkomstig uit de varkenshouderij. Regelmatige prevalentiemetingen van MRSA-dragerschap kunnen bijdragen aan het vroegtijdig identificeren van zulke nieuwe bronnen.

Curriculum Vitae, PhD portfolio en Dankwoord

Curriculum Vitae

Lonneke Gabriëlle Maria Bode werd op 27 december 1977 geboren te Gouda. Haar eindexamen VWO behaalde zij in 1996 aan het Minkema College te Woerden. Wegens uitloting voor de studie geneeskunde startte zij in 1996 met de studie Biologie aan de Universiteit Utrecht, maar die werd in 1997 gestaakt toen zij voor de studie Geneeskunde inlootte aan diezelfde universiteit. De studie werd in 1999-2000 een jaar onderbroken om deel uit maken van het bestuur van de Medische Studenten Faculteitsvereniging Utrecht “Sams”. Het doctoraalexamen werd behaald in 2002, de artsenbul in 2004. Na haar studie werkte zij een klein jaar als arts-assistent niet in opleiding in het Wilhelmina Kinderziekenhuis. In september 2005 startte zij haar promotieonderzoek naar de preventie van ziekenhuisgerelateerde *Staphylococcus aureus* infecties op de afdeling Medische Microbiologie en Infectieziekten van het Erasmus MC te Rotterdam (begeleiders: prof.dr. M.C. Vos en prof.dr. H.A. Verbrugh). In 2007 begon zij aan de opleiding tot arts-microbioloog in het Erasmus MC en het Reinier de Graaf Gasthuis (opleiders: prof.dr. H.A. Verbrugh en dr. R.W. Vreede). Tevens startte zij in dat jaar met de masteropleiding Clinical Epidemiology aan het Netherlands Institute of Health Sciences Rotterdam (NIHES), welke in 2009 succesvol afgerond werd. In het UMC Utrecht volbracht zij het laatste onderdeel van de opleiding tot arts-microbioloog (opleider: dr. A.M.J. Wensing). Sinds februari 2014 is zij in het UMC Utrecht werkzaam als arts-microbioloog met als aandachtsgebied infectiepreventie. Zij woont samen met Frank de Booij en zij hebben twee kinderen, Meike en Kalle.

PhD Portfolio

Naam PhD student	Lonneke Gabriëlle Maria Bode
Erasmus MC department	Medische Microbiologie & Infectieziekten
Research School	MolMed
PhD period	2005-2014
Promotor	Prof.dr. M.C. Vos

Opleidingen en werk

2005-2007 Erasmus MC Rotterdam, afdeling Microbiologie & Infectieziekten, arts-onderzoeker
2007-2009 NIHES Rotterdam, Master in Clinical Epidemiology. Examen: 28 augustus 2009
2007-2014 Erasmus MC Rotterdam/Reinier de Graaf Gasthuis/UMC Utrecht, specialisatie Medische Microbiologie. Opleiders: H.A. Verbrugh/R.W. Vreede/A.M.J. Wensing
2014-heden UMC Utrecht, afdeling Medische Microbiologie, arts-microbioloog

Aanvullende cursussen

2006 Molmed Postgraduate School, Molecular Medicine
2007 Erasmus MC Rotterdam, Biomedical English writing
2008 DOO Desiderius school Rotterdam, module Medische Ethiek
2009 DOO Desideriusschool Rotterdam, module Gezondheidsrecht
2010 UMCG Groningen, Anaërobe bacteriologie
2010 CBS Utrecht, Medical mycology
2010 LUMC Leiden, Boerhaave, Parasitologische diagnostiek
2010 DOO Desideriusschool Rotterdam, module Ziekenhuismanagement
2011 Erasmus MC Zorgacademie, Inleiding ABCD-E methodiek
2011 Noordwijkerhout, Boerhaave, (Na)scholingscursus infectieziekten
2011 DOO Desideriusschool Rotterdam, module Samenwerking
2011 RIVM, Openbare Gezondheidszorg
2012 Molmed Postgraduate School, Course in Virology
2012 DOO Desideriusschool Rotterdam, module Evidence Based Medicine
2012 Academie voor Medisch Specialisten/NVMM, Management cursus
2013 Amphia Academy Infectious Disease Foundation, Infectiepreventie
2013 Erasmus MC Rotterdam, Antimicrobiële Resistentie

Bijgewoonde symposia en congressen

2006 NVMM Voorjaarsvergadering Papendal
2006 International Symposium on Staphylococci and Staphylococcal Infections, Maastricht
2006 Avondsymposium Infectieziekten
2007 NVMM Voorjaarsvergadering Papendal
2007 ECCMID München
2007 MRSA Symposium: “Van sneldiagnostiek tot behandeling”
2008 NVMM Voorjaarsvergadering Papendal
2008 Symposium “De sterkste schakel. Antibiotische therapie & resistentie: Zijn de sluizen nu definitief open?”
2008 Molmed Postgraduate School, Workshop on Phylogeny & Genetics in microbiology

and virology

2008 International Symposium on Staphylococci and Staphylococcal Infections, Cairns

2008 ICAAC/IDSA Washington

2009 NVAMM Symposium “Infectiepreventie: Paniek onder controle?”

2010 NVMM Voorjaarsvergadering Papendal

2010 International Symposium on Staphylococci and Staphylococcal Infections, Bath

2010 Federation of Infection Societies Scientific Meeting, Edinburgh

2011 NVAMM Symposium “Vaccinologie: Beyond trials and errors”

2011 NVMM Voorjaarsvergadering Papendal

2012 NVAMM Symposium “Medical Microbiology & Globalisation”

2012 Molmed Postgraduate School, “Carbapenemase-producing organisms: the beginning of the end?”

2012 NVMM Voorjaarsvergadering Papendal

2013 NVAMM Symposium “Biofilms on the move: foreign material-related infections”

2013 NVMM Voorjaarsvergadering Papendal

2013 Regionaal Infectiemenu, Rotterdam

2013 UMCU “All you always wanted to know about decontamination and antibiotic resistance in ICU”

2013 BD Diagnostics HAI event, Rosmalen

Posters en presentaties

2006 ISSSI Maastricht: ”The LightCycler Staphylococcus kit: Increasing the positive predictive value for *Staphylococcus aureus* bacterial counts => 10 CFU by using a fluorescence intensity threshold.” Poster presentation

2007 NVMM Papendal: “Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) carriage at hospital admission in the Netherlands: 2005-2006 compared with 1999-2000”. Poster presentation

2007 ECCMID Muenchen: “Evaluation of the 3M Rapid Detect Staph aureus: An in vitro diagnostic device for direct detection of *Staphylococcus aureus* nasal colonisation.” Poster presentation

2008 ISSSI Cairns: “Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) carriage upon hospital admission in the Netherlands remains very low (0.08%)”. Poster presentation

2008 ISSSI Cairns: “A genome-wide association study on *Staphylococcus aureus* nasal carriage”. Poster presentation

2008 Nascholing analisten, SSDZ Delft: “*Staphylococcus aureus* neusdragerschap en preventie van nosocomiale infecties”.

2008 Scientific meeting on GWA, Greifswald: “*Staphylococcus aureus* nasal carriage and genome-wide association: How to interpret the Rotterdam results?” Invited speaker

2008 Symposium ter gelegenheid van het afscheid van Diana Bogaers, Breda: “Opgescheept zitten met *Staphylococcus aureus*.” Invited speaker

2008 ICAAC/IDSA Washington: “Admission screening and decolonization of *Staphylococcus aureus* carriers to prevent nosocomial *S. aureus* infections”. Oral presentation

2009 NVMM Werkgroep West, Rotterdam: “Nasal carriage of *Staphylococcus aureus* and prevention of nosocomial infections”. Invited speaker

2010 Federation of Infection Societies Scientific Meeting, Edinburgh: “Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*”. Invited speaker

2010 Refereeravond Infectieziekten, Groningen: “Preventie van infecties bij *Staphylococcus*

aureus dragers". Invited speaker

2010 Regionale nascholing voor ziekenhuishygiënisten, Erasmus MC: "Preventie van infecties bij *Staphylococcus aureus* neusdragers". Invited speaker

2010 Refereeravond "Infectiepreventie" voor arts-assistenten Spaarneziekenhuis Hoofddorp: "Preventie van nosocomiale *Staphylococcus aureus* infecties bij dragers". Invited speaker

2010 PAOG nascholing "Perioperatieve zorg, een grote zorg" voor verpleegkundigen en OK-assistenten, St. Radboudziekenhuis, Nijmegen, april 2010: "Het komt je neus uit: Het voorkomen van post-operatieve wondinfecties met *Staphylococcus aureus*". Invited speaker

2010 Refereeravond Centrum voor Infectieziekten, LUMC: "Preventie *Staphylococcus aureus* infectie bij dragerschap". Invited speaker

2010 Nascholing analisten MMIZ, Erasmus MC Rotterdam: "*Staphylococcus aureus* infecties".

2011 NVMM Werkgroep West Rotterdam: "Een verraderlijke verwekker van subacute endocarditis" (*S. epidermidis*)

2011 Nascholing analisten, SSDZ Delft: "VZV: Eén virus, meerdere gedaanten"

2011 Nascholing analisten SSDZ Delft: "Een griepje?" (endocarditis)

2011 (Na)scholingscursus Infectieziekten (Boerhaavecursus), Noordwijkerhout: "Postoperatieve wondinfecties, NEJM". Invited speaker

2011 Nascholing analisten SSDZ Delft: "Hoofdbrekens en kopzorgen" (*Taenia solium*)

2011 Webinar APIC (Association for Professionals in Infection Control and Epidemiology): "Towards an efficient strategy to implement evidence based measures to prevent *S. aureus* infections; difficulties to overcome" (met M. Vos). Invited speakers

2012 Nascholing sociaal verpleegkundigen GGD Rotterdam-Rijnmond: "Inleiding in de microbiologie"

2012 Cepheid symposium Impact of Rapid and Easy-to-Use Molecular Diagnostics on Health Care, Antwerpen: "Staphylococcus aureus screening and decolonization: The journey from principle to practice". Invited speaker

2012 Nascholing analisten Havenziekenhuis: "Toxoplasma"

2012 Nascholing analisten MMIZ Erasmus MC Rotterdam: "*Burkholderia pseudomallei*"

2013 London Knee Meeting. "Prevention of *Staphylococcus aureus* infections in carriers". Invited speaker

2013 BD Diagnostics HAI event: "Postoperatieve *Staphylococcus aureus* infecties en de preventie hiervan" (met M. van Rijen). Invited speaker

Grants

2008 Fellows Travel Grant ICAAC/IDSA. Awarded to fellows in training who submitted excellent research.

2008 Travel Fellowship ISSSI. Awarded for "A genome-wide association study on *Staphylococcus aureus* nasal carriage".

2010 Kiemprijs award for first scientific paper in the field of microbiology.

Overige activiteiten

2005 – 2013 Assistent bij vaardigheidsonderwijs Microbiologie & Infectieziekten voor tweedejaars geneeskundestudenten

2010 – 2012 Wetenschapscommissie NVAMM

Organisatie van twee symposia voor arts-assistenten Medische Microbiologie: "Vaccinology" op 17 februari 2011 en "Medical Microbiology and Globalisation" op 9 februari 2012.

2013 Begeleiding ziekenhuishygiënist in opleiding

2013 Begeleiding 2 HLO-studenten

2013 Begeleiding 6 geneeskunde studenten bij Community Project

Publicaties

Reduced costs for *Staphylococcus aureus* carriers treated prophylactically with mupirocin and chlorhexidine in cardiothoracic and orthopaedic surgery. MML van Rijen*, **LGM Bode***, DA Baak, JAJW Kluytmans, MC Vos. *PLoS One* 2012;7(8):e43065

Rapid detection of methicillin-resistant *Staphylococcus aureus* in screening samples by relative quantification between the *mecA* gene and the *SA442* gene. **LGM Bode**, P van Wunnik, N Vaessen, PH Savelkoul, Smeets LC. *J Microbiol Methods* 2012;89:129-32.

Distinctive patterns in the human antibody response to *Staphylococcus aureus* bacteremia in carriers and non-carriers. J Kolata, **LGM Bode**, S Holtfreter, L Steil, H Kusch, B Holtfreter, D Albrecht, M Hecker, S Engelmann, A van Belkum, U Völker, BM Bröker. *Proteomics* 2011;11:3914-27

Sustained low prevalence of meticillin-resistant *Staphylococcus aureus* upon admission to hospital in the Netherlands. **LGM Bode**, HFL Wertheim, JAJW Kluytmans, D Bogaers-Hofman, CMJE Vandenbroucke-Grauls, R Roosendaal, A Troelstra, ATA Box, A Voss, A van Belkum, HA Verbrugh, MC Vos. *J Hosp Infect* 2011;79:198-201

Heterogeneity of the humoral immune response following *Staphylococcus aureus* bacteremia. NJ Verkaik, HA Boelens, CP de Vogel, M Tavakol, **LGM Bode**, HA Verbrugh, A van Belkum, WJ van Wamel. *Eur J Clin Microbiol Infect Dis* 2010;29(5):509-18

Preventing surgical site infections in *Staphylococcus aureus* nasal carriers. **LGM Bode**, JAJW Kluytmans, HFL Wertheim, D Bogaers-Hofman, CMJE Vandenbroucke-Grauls, R Roosendaal, A Troelstra, ATA Box, A Voss, I van der Tweel, A van Belkum, HA Verbrugh, MC Vos. *NEJM* 2010;362(1):9-17

Platelet count, previous infection and FCGR2B genotype predict development of chronic disease in newly diagnosed idiopathic thrombocytopenia in childhood: results of a prospective study. M Bruin, M Bierings, C Uiterwaal, T Révész, **L Bode**, ME Wiesman, T Kuijpers, R Tamminga, M de Haas. *Br J Haematol* 2004;127(5):561-7

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