Determinants, Risks & Dynamics of *Staphylococcus aureus* Nasal Carriage

J. L. Nouwen

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Natural Science:
"When the ebbing tide retreats
Along the rocky shoreline
It leaves a trail of tidal pools
In a short-lived galaxy
Each microcosmic planet
A complete society
A simple kind of mirror
To reflect upon our own
All the busy little creatures
Chasing out their destinies
Living in their pools
They soon forget about the sea . . ."

- Neil Peart -

Aan Angélique

Voor Anouk en Lonneke

In herinnering aan mijn ouders

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1

What Determines *Staphylococcus aureus* Nasal Carriage?

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Introduction

S. aureus is an important pathogen in human disease and the cause of a range of infections from mild, such as skin infections and food poisoning, to life-threatening, such as pneumonia, sepsis, osteomyelitis, and infective endocarditis.[1] Over the last 20 years the incidence of both community-acquired and hospital-acquired *S. aureus* infections has increased, accounting for 13 percent of nosocomial infections in U.S. hospitals between 1979 and 1995.[2-4] Despite antibiotic therapy these infections still have severe consequences, stressing the importance of prevention.[5,6]

S. aureus produces many toxins and is capable of developing resistance to all available antibiotics. In 1961 methicillin resistance was first noted[7] and since the 1970s, methicillin-resistant *S aureus* (MRSA) has become the main cause of nosocomial infections in many countries all over the world.[8-10] Glycopeptides such as vancomycin are the last resort antibiotics in these countries. However, in 1997 a partially vancomycin-resistant *S aureus* (VRSA) strain was isolated.[11-13]

S. aureus nasal carriage is the major risk factor for the development of *S. aureus* infections in various clinical settings, including post-operative wound infections,[5,14] in patients undergoing continuous ambulatory peritoneal dialysis and hemodialysis,[15,16] and in patients infected with the human immunodeficiency virus.[17] A large majority of these infections are of endogenous origin, in that individuals are infected by their own *S. aureus* isolate,[15,16,18] as was recently confirmed by von Eiff et al.[19] Eradication of *S. aureus* nasal carriage by application of topical mupirocine results in a reduction in endogenous infections in various risk populations.[16,20-22] In contrast, the absolute risk of developing a *S. aureus* infection as a nasal carrier is low (less than 5% for nosocomial bacteraemia). The problem of *S. aureus* is still considered an important one, and further research is required to elucidate the host-pathogen interplay and to develop effective strategies in the prevention of *S. aureus* infections.[19,23-27] This article aims to review what is currently known of the host and bacterial factors determining *S. aureus* nasal carriage and to set the stage for this thesis.

S. aureus Nasal Carriage

Humans, as other mammals, are a natural reservoir of *S. aureus* and the primary ecological niches of *S. aureus* strains in humans are the anterior nares, although staphylococci can be isolated from many sites, including throat, axilla and perineum.[28-31] However, the elimination of *S. aureus* from the nose results in the subsequent disappearance from other areas of the body.[28,32-34] While carriage of *S. aureus* in the nose plays a key role in the epidemiology and pathogenesis of infection, and is a major risk factor for the development of both community-acquired and nosocomial infections, the biology of nasal colonization remains incompletely understood.[5,15,19,23,35,36]

Cross-sectional surveys of healthy adult populations have reported *S. aureus* nasal carriage rates between 20 and 55%.[31,37-42] From longitudinal studies it became clear that *S. aureus* nasal carriage patterns differ between individuals, and that 10 to 35% of individuals carry *S. aureus* persistently, 20 to 75% carry *S. aureus* intermittently, and 5 to 50% never carry *S. aureus* in their nose.[37,38,43-47] Persistent carriage is more common in young children than in adults, and many people change their pattern of carriage between the age of 10 and 20 years.[43] The reasons for these differences in colonization patterns remain unknown so far.

The number of *S. aureus* bacteria in the anterior nares is significantly higher in persistent carriers than in intermittent carriers,[48] resulting in an increased risk of *S. aureus* infections.[49-51] Moreover, persistent carriers are often colonized by a selected single strain of *S. aureus* over long time periods, while intermittent carriers carry many different strains over time.[37,38,47,52,53] Persistent carriage seems to have a protective effect on the acquisition of other strains.[54] These data suggest that the basic determinants of persistent and intermittent carriage are different.
Comparison of results between studies is made difficult by the lack of conformity in both the methods of ascertainment and varying criteria for the definition of persistent or intermittent carriage used, as well as the absence of information on antibiotic exposure, an important confounding variable. In clinical studies,

often due to logistic reasons, only one nasal swab culture is performed to ascertain *S. aureus* nasal carriage. The result of this is that the group with one negative culture will in fact consist of a mix of true non-carriers plus intermittent carriers, while the group with one positive culture will in fact consist of a mix of true persistent carriers plus intermittent carriers. When studying determinants of *S. aureus* nasal carriage or when performing an intervention trial in this way, the differences between the positive- and negative-culture groups will be blurred by the presence of intermittent carriers in both groups. The correct separation of the population into persons who are true persistent carriers versus intermittent and non-carriers is a prerequisite to adequately perform studies into the molecular and genetic basis of *S. aureus* nasal carriage, as well as intervention studies.

Determinants of *S. aureus* Nasal Carriage

Bacterial factors

Much research has focused on specific staphylococcal factors like cell wall components (lipoteichoic acid[55,56]), surface proteins (protein A[52], microbial surface components recognizing adhesive matrix molecules (MSCRAMM)[57-59]) and staphylococcal interactions with other host proteins and carbohydrate moieties such as mucin[60,61], or other mucus components.[62,63] Other substances found in the respiratory tract, including secretory immunoglobulin A,[64] glycolipids,[65] gangliosides[66,67] and surfactant protein A,[68] may also constitute receptor sites for staphylococcal adherence. Hydrophobic interactions and surface charge provide forces that are probably also involved in mediating staphylococcal binding to epithelia.[56,60,69]
On the basis of all these results, however, no common genetic or phenotypic characteristics segregating persistent from intermittent colonizing strains have been identified, so far. Recently Day et al. reported the existence of ecologically abundant hypervirulent clones. They suggested that factors promoting the ecological fitness, i.e. the capacity to colonize persons, also increase its virulence and that *S. aureus* is not solely an opportunistic pathogen.[24] Their study was, however, retracted later because in repeated experiments this association could not be corroborated. Future studies will hopefully dissect

the interrelation between colonization capacity and virulence and shed new light on the mechanisms of disease pathogenesis. The just finished '*S. aureus* genome project' would be the logical starting point.[26]

Bacterial interference may be another explanation of the non-carrier state: when an ecological niche is already occupied by other bacteria, e.g. coagulase-negative staphylococci, *Corynebacterium* species or artificially with *S. aureus* 502A, wild type *S. aureus* does not seem to have the means to replace this resident bacterial population.[38,70-72] The exact mechanism for this effect has not been elucidated so far.[73,74] Cross-inhibition of the expression of various virulence factors by the recently identified accessory gene regulator (*agr*) and staphylococcal accessory regulator (*sar*), may be one mechanism by which one strain excludes others from colonizing sites including the anterior nares,[75-80] although a large *S. aureus* population genetics analysis failed to confirm this suggestion.[81] Bacterial interference by active colonization using *S. aureus* 502A has been successful in nurseries during out-breaks of *S. aureus* infections in the 1960s and for treatment of patients with recurrent furunculosis.[82-84] Bacterial interference using *Corynebacterium* species has recently been reported to be successful in eradicating MRSA nasal carriage.[85]

Host factors

The observation that different *S. aureus* nasal carriage patterns (non, intermittent and persistent) can be discerned, suggests a host influence. This view is supported by the fact that persistent carriage rates vary between different ethnic groups,[40,42,86] are higher in males than in females[87] and depend on age (higher in early childhood, lower at old age)[43,54,88-90] and hormonal status.[91] In addition, *S. aureus* seems to have a greater affinity for nasal epithelial cells obtained from carriers than from noncarriers[92] and adheres better to nasal epithelial cells from patients with eczema than to cells from patients without eczema.[93]
Genetic studies have not provided us with a definitive answer, yet. Two twin studies have been performed, the first showing concordant results in monozygotic twins,[94] while the other could not confirm these results.[95] An earlier study that evaluated the relationship between HLA Class II haplotype and nasal carriage demonstrated HLA-DR3 to be associated with carriage, but a large proportion of the patient group suffered from an

autoimmune disease, which was not adjusted for in the analysis.[96]
Environmental factors can also influence the *S. aureus* nasal carrier state. Hospitalization for example, has been shown to be an important risk factor for *S. aureus* nasal carriage.[41,44,97-99]

Many underlying diseases or conditions have been associated with a higher *S. aureus* nasal carriage and subsequent infection rate: insulin-dependent diabetes mellitus,[100,101] hemodialysis and continuous ambulatory peritoneal dialysis,[15,16,102,103] intravenous drug abuse,[104,105] repeated injections for allergies,[106] *S. aureus* skin infections and other skin diseases,[107,108] river-rafting,[109] liver cirrhosis,[110,111] liver transplantation,[112] human immunodeficiency virus (HIV) infection or AIDS,[17,18] qualitative or quantitative defects in leukocyte function,[113] Wegener's granulomatosis,[114] nasal abnormalities,[115] and rhinosinusitis.[116]
One common factor in these seems to be the repeated violation of the skin or mucosa as anatomical barriers. However, local or systemic immune deficiencies probably also play an important role. Cole et al. reported that nasal secretions obtained from *S. aureus* nasal carriers lacked antimicrobial activity against *S. aureus* in vitro, while nasal fluid from non-carriers was bactericidal.[117] Defensins (antimicrobial peptides) as part of the innate immune system, and/or the local immune IgA response could well be involved.[117-119]

Scope of this Thesis

Much is known about *S. aureus, S. aureus* infections and the host and bacterial factors involved in *S. aureus* nasal carriage. However, many questions remain unanswered. To fully exploit *S. aureus* nasal carriage as the key to preventing *S. aureus* infections, we will first have to unlock the fundamental mechanisms underlying it. It was the general aim of the work presented in this thesis to contribute to the further elucidation and understanding of the determinants and consequences of *S. aureus* nasal carriage in humans. The aim of the research presented in this thesis was addressed in three separate lines of work:

Section 1

Defining *S. aureus* nasal carriage

Currently no internationally accepted definitions of the different *S. aureus* nasal carrier states exist. To compare findings from different studies and to be able to extrapolate results from studies to 'real life' situations, consensus on how to define the *S. aureus* carrier states is essential.

- In chapter 2 we set out to derive and validate a 'culture rule' for accurate determination of the individual carrier state, to be used in large scale epidemiologic studies to follow.

Section 2

Determinants of *S. aureus* nasal carriage

In trying to explain carriage, research has focused primarily on bacterial factors. Often in vitro data could not be corroborated in vivo, not to mention real life. Furthermore, most studies on host factors associated with carriage have been performed in the hospital setting. We, therefore, decided to study the **host** as the main source of determinants of *S. aureus* nasal carriage in the **community setting**. To achieve this a total of five studies were undertaken:

- The first study (chapter 3) describes the development of a 'human model' as a tool to study if the human host was indeed an important determinant of *S. aureus* nasal carriage. After artificially inoculating long-term persistent carrier and long-term non-carrier volunteers with a mixture of *S. aureus* strains, these persons were followed-up to investigate whether nasal survival of *S. aureus* was different between these two groups.
- Using the 'culture rule' presented in chapter 2, in a large population-based study in the city of Rotterdam, we intended to find (new) host factors associated with persistent *S. aureus* nasal carriage in the community (chapter 4).
- The third study (chapter 5) aimed to investigate the hypothesis that environmental factors (defined as living within the same household) and/or familial factors (defined as being first degree relatives) are important determinants of *S. aureus* nasal carriage.
- Finally, based on the results from the first three studies and biased to the opinion that the human host is indeed a major determinant of *S. aureus* nasal carriage, we

performed two genetic association studies. First (chapter 6) we investigated the association of polymorphisms in the vitamin D receptor gene and secondly (chapter 7) the association of polymorphisms in the glucocorticoid receptor gene with persistent *S. aureus* nasal carriage.

Section 3

Risks of *S. aureus* nasal carriage

Many studies have delineated the risks associated with *S. aureus* nasal carriage. Most of these studies have been performed in the hospital setting and limited data are available on the health effects of *S. aureus* nasal carriage in the community setting. Moreover, studies in populations at risk, such as patients on continuous peritoneal dialysis (CPD), have been hampered by lack of accurate definition of the different *S. aureus* nasal carrier states, thereby impeding the efficient targeting of prophylactic measures. Accurate determination of the true *S. aureus* carrier state would, theoretically, enable us to improve the prevention of *S. aureus* infections in CPD patients. In this section three studies were performed:

- In the first study (chapter 8) we investigated the overall health effects of persistent *S. aureus* nasal carriage in the community.
- In the second (chapter 9) and third (chapter 10) study we set out to determine whether subgroups of *S. aureus* nasal carriers could be distinguished among CDP patients in a university hospital setting (chapter 9) and in a tertiary care hospital (chapter 10), respectively, and whether these subgroups had different risks for *S. aureus* infections.

Section 4

Dynamics of & Interference with *S. aureus* nasal carriage

In the era of emerging antibiotic resistance in *S. aureus* (including nosocomial as well as the recent emergence of community-acquired methicillin-resistant *S. aureus* (MRSA) strains, but also resistance to many commonly used antibiotics, including the 'last resort' of glycopeptides), prudent use of antibiotics in the prevention and therapy is highly needed. Also, alternative strategies to combat *S. aureus* are necessary. Bacterial interference has been hypothesized to be a major

determinant of the *S. aureus* carrier state.[70,72-74] The concept of bacterial interference has been successfully taken into clinical testing, employing artificial nasal inoculation with *S. aureus* 502A (SA-502A) or *Corynebacterium* species.[82-85,108,120] However, the practice of artificial nasal inoculation with SA-502A was abandoned after alleged complications,[121-123] and the advent of newer anti-staphylococcal antibiotics.

- The last section consists of a study (chapter 11) into the colonization dynamics of wild type *S. aureus* and *S. aureus* 502A after bacterial interference with the latter strain. We set out to investigate whether interference with *S. aureus* 502A in well defined persistent carriers was feasible, safe and effective in eliminating the wild type strain.

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2

Predicting the *Staphylococcus aureus* Nasal Carrier State: Derivation & Validation of a 'Culture Rule'

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Abstract

Context To study determinants and risks of *Staphylococcus aureus* nasal carriage adequate differentiation between the different *S. aureus* carrier states is obligatory.

Objective We set out to develop a 'culture rule' capable of differentiating between persistent and intermittent or non-carriers using a minimum of nasal swab cultures.

Design Prospective diagnostic study.

Setting 51 healthy volunteers (derivation-cohort) & 106 participants of an ongoing study in 3882 elderly persons (validation cohort).

Participants In 51 healthy volunteers (derivation-cohort) 12 quantitative nasal cultures were performed to establish their *S. aureus* nasal carrier states. Persons with 11 or 12 cultures positive with S. aureus were classified as persistent carriers, those with all cultures negative as non-carriers. All other persons were classified as intermittent carriers. Using logistic regression and receiver operating characteristic (ROC) curves a 'culture rule' was derived. This 'culture rule' was subsequently validated in 106 participants of an ongoing study in 3882 elderly persons, again using 12 quantitative nasal cultures.

Outcome Measures Areas under the ROC curves (AUC).

Results In both cohorts, the positive predictive value of two consecutive positive cultures for persistent carriage was 79%. The model best differentiating between persistent and intermittent or non-carriers used the number of positive cultures combined with the amount of *S. aureus* in these cultures. Using the outcome of two cultures, the areas under the ROC curves (AUC) were 0.981 (95% CI 0.949-1.0) for the derivation- and 0.936 (95% CI 0.881-0.990) for the validation-cohort.

Conclusions Combining qualitative and quantitative results of two nasal swab cultures accurately predicted the persistent *S. aureus* carrier state with a reliability of 93.6%. This 'culture rule' can thus be used in studies on determinants and risks of *S. aureus* nasal carriage.

Introduction

Staphylococcus aureus nasal carriage is a major risk factor for both community-acquired and nosocomial infections,[1-7] and the anterior nares are the primary reservoir of *S. aureus* in humans.[8-10] Three *S. aureus* nasal carriage patterns can be discerned: persistent-, intermittent- and non-carriage.[11-22] However, no consensus has been reached on how to exactly identify these different states, but most studies use 10-12 weekly nasal swab cultures.[23]

The number of colony forming units (CFU) of *S. aureus* isolated from the anterior nares are higher in persistent than in intermittent carriers,[24,25] resulting in more extensive dispersal of staphylococci in the environment[25] and in an increased risk of *S. aureus* infections.[26-28] Bacterial variability (i.e. the number of *S. aureus* genotypes isolated in repeated cultures from one individual) is lower for persistent than for intermittent carriers,[15,22,29] indicating that the underlying mechanisms determining persistent and intermittent carriage differs. Adequate differentiation between persistent and intermittent carriage is thus relevant for epidemiological studies.

At present a large survey on *S. aureus* nasal carriage in a population aged 60 and over is being conducted at our institution. The main objectives are to study determinants and risks of *S. aureus* nasal carriage. This is part of the Rotterdam Study, a population-based prospective study on chronic diseases in the elderly. The Rotterdam Study started in 1990 in 7983 persons, and has just finished its third phase, in which more than 4000 persons have been included. In this large survey an efficient and reliable way to assess *S. aureus* nasal carriage was obligatory. It would be impossible to perform 10-12 weekly nasal swab cultures in all participants. Thus, we developed a 'culture rule' to discriminate reliably between persistent-carriage and non-or-intermittent carriage, with a minimum of nasal swab cultures.

Our main questions were 1) how many quantitative nasal swab cultures are needed to accurately predict persistent carriage in a cohort of healthy adult volunteers, and 2) does the derived 'culture rule' correctly predict persistent carriage in the elderly cohort of the ongoing Rotterdam study.

Material & Methods

Patient cohorts and microbiological investigations

Derivation cohort

In 1988 a cohort of healthy volunteers (staff members of the departments of Medical Microbiology & Infectious Diseases and Virology of the Erasmus MC) was formed to investigate bacterial and human factors associated with *S. aureus* nasal carriage.[23] Between September 1995 and March 1996 51 volunteers were invited and agreed to participate in this study. Nasal swab cultures were performed weekly for 12 weeks. All nasal swab cultures were taken by one study physician (MFQK-V), according to protocol (see below).

Validation cohort

Based on the results of the derivation cohort, two quantitative nasal swab cultures with one-week interval were performed in 3882 participants of the Rotterdam Study. While this study was ongoing, 106 participants entering the study between October 1997 and April 1998 were invited and agreed to be included in the validation cohort. Persons with 2 positive or 2 negative nasal swab cultures were oversampled to estimate the predictive value of these cultures for persistent-carriage and non-or-intermittent carriage. One trained research assistant visited the participants at home and performed 10 additional nasal swab cultures at one-week intervals, according to protocol.

The study was approved by the Medical Ethics Review Committee of the Erasmus MC, University Medical Center Rotterdam. Informed consent was obtained of all participants.

Definitions

S. aureus nasal carriage state was assessed using the results of nasal swab culture number 3 to 12, as follows:

persistent carrier 9 or 10 out of 10 cultures positive with *S. aureus*

non-carrier no positive cultures

intermittent carrier all intermediate numbers of positive cultures

Microbiological procedures

Nasal swab cultures were performed according to a standard operating procedure as described earlier.[23] Nasal swabs specimens were obtained using sterile cotton-wool swabs (Transwab, Medical Wire & Equipment Co. Ltd., Corsham,

United Kingdom). Both the left and right anterior nares were swabbed by rubbing the swab four times around the inside of each nostril while applying an even pressure and rotating the swab without interruption. The swabs were immediately placed in Stuart's transport medium and kept at 4 ^{0}C until inoculation (within 24 hours).
Swabs were then cultured quantitatively on phenol-red mannitol salt agar (PHMA) and in phenol red mannitol salt broth (PHMB). The flasks with transport media containing the nasal swab were vortexed for 15 seconds. The swab was then pressed firmly against the wall of the flask using a sterile pincet and cultured in 8 ml of PHMB. Subsequently, 500μL of the remaining bacterial suspension was inoculated evenly unto a large PHMA culture plate (∅ 14 cm). Another PHMA culture plate (∅ 8.5 cm) was divided into three sectors, which were inoculated with 10μL of the original bacterial suspension, 10μL of a 1:10 diluted bacterial suspension, and 1μL of the 1:10 diluted bacterial suspension, respectively. The PHMB was incubated at 37 ^{0}C for 7 days; the PHMA culture plates were incubated at 37 ^{0}C for 48 hours and at room temperature for 5 days. Both were interpreted after 7 days of incubation. If after 7 days no *S. aureus* had grown on the PHMA, but the PHMB demonstrated a yellow color, a PHMA culture plate (∅ 8.5 cm) was inoculated with 10μL PHMB and incubated as before. Culture results were recorded as 0 (no *S. aureus*), 1 (*S. aureus* only from PHMB), 2 (2-9 CFU), 3 (10-99 CFU), 4 (100-999 CFU), or 5 (≥ 1000 CFU) (CFU= colony forming units).
Identification of *S. aureus* was based upon colony morphology on the PHMA. Suspected colonies were cultured overnight on Columbia blood agar plates (Becton-Dickinson B.V., Etten-Leur, The Netherlands). A catalase test and a latex agglutination test (Staphaurex Plus^{R}, Murex, Dartford, UK) were then performed. All *S. aureus* isolates were stored at -70 ^{0}C in glycerol containing liquid media.

Statistical analysis

Percentages and continuous data were compared by Fisher's exact test and Mann-Whitney's test, respectively. Logistic regression was performed and receiver operating characteristic (ROC) curves were constructed for different tests and combinations of tests (number of positive cultures, ^{10}Log-transformed CFUs (^{10}Log[CFU+1]) and the geometric mean CFUs of two or more cultures to study their ability to

discriminate between persistent carriage and non-carriage or intermittent carriage.[30] Culture results of the derivation cohort were added as independent covariates to a logistic regression model with our "gold standard" diagnosis of persistent carriage or not (derived from 10 consecutive cultures) as binary outcome variate. The right side of the regression equation was: [ß0 + ß1*number of positive cultures + ß2*geometric mean of CFUs]. Fitting the model gave us ß0 to ß2. Then, we calculated the odds of persistent carriage for all persons of the validation cohort by adding their respective culture outcomes in the formula: odds = [e(ß0 + ß1*number of positive cultures + ß2*geometric mean of CFUs)]. Subsequently, the probability of persistent carriage was obtained by: odds / (1+odds). We choose the midpoint between 0 and 1 as cut-point. Areas under the ROC curves (AUC) and the corresponding standard errors (SE) were estimated using a non-parametric method (two sample Wilcoxon test).[31,32] Differences between AUCs of the different test combinations were compared using the method of Hanley & McNeil.[33]

Results

Fifty-one persons were included in the derivation cohort: 19 (37%) males and 32 (63%) females, mean age 29 years (range 20 - 52). Twenty (39%) participants were classified as non-, 16 (31%) as intermittent and 15 (29%) as persistent carriers (Table 1A). Positive predictive values for persistent carriage, derived from regression models that included the results of cultures 1 and 2, ranged from 0.79 in a model containing the qualitative outcome only, to 0.88 in a model including both qualitative and quantitative results (Figure 1A). Using the results of only one culture (either 1 or 2) produced a positive predictive value of only 0.69.

The validation cohort consisted of a subset of 106 participants of the Rotterdam Study cohort: 44 (42%) males, 62 (58%) females, mean age 73 years (range 62 - 89). For the present study, persons with one positive and one negative culture were less informative. Two positive cultures could either indicate persistent or intermittent carriage. Possibly, the number of CFUs *S. aureus* cultured could differentiate between persistent and intermittent carriage. Persons with two negative cultures could

Table 1 *Classification of the S. aureus nasal carrier state based on results from the first two cultures as compared with cultures 3 to 12 for the derivation cohort (A) and the validation cohort (B) respectively. For the validation cohort persons with both culture 1 and 2 positive or negative were oversampled (see methods). Therefore the distribution of the different carrier states does not represent the population prevalence.*

Table 1A : Derivation Cohort

		Results of cultures 1 and 2			Total
		Both Negative	1 Positive, 1 Negative	Both Positive	
S.aureus carrier state based on cultures 3-12	non	19	1		20
	intermittent	7	5	4	16
	persistent			15	15
Total		26	6	19	51

Table 1B: Validation Cohort

		Results of cultures 1 and 2			Total
		Both Negative	1 Positive, 1 Negative	Both Positive	
S.aureus carrier state based on cultures 3-12	non	53	4		57
	intermittent	7	2	8	17
	persistent	1		31	32
Total		61	6	39	106

help to assess the predictive value for true non-carriage. Therefore, after initial random inclusion of participants, we decided to oversample persons with two positive or two negative screening cultures. Fifty-seven (54%) participants were classified as non-, 17 (16%) as intermittent and 32 (30%) as persistent carriers (Table 1B). In one participant both screening cultures were negative, while cultures 3 to 12 were all positive. The most probable explanation for this would be either sample handling mistakes or a laboratory error. Since exclusion of this person did not significantly alter the data and mistakes happen in real life, it was decided not to exclude this person. The positive predictive value derived from regression models that included the results of cultures 1 and 2 was 0.79 in a model containing the qualitative outcome only as well as in a model including also the quantitative results (Figure 1B). Using the results of only one culture (either 1 or 2) produced a positive predictive value of 0.74.

The numbers of CFUs of *S. aureus* were significantly higher in the validation than in the derivation cohort (Figure 2). The median geometric mean in intermittent and persistent carriers were 1.4 (range 0.3–3.3) and 3.6 (1.9-3.9) in the validation versus 1.0 (0.3–2.0) and 1.8 (0.9–3.2) in the derivation cohort ($p=0.001$ and $p<0.001$), respectively. Persistent carriers had significantly higher CFUs of *S. aureus* in their positive nasal swab cultures than intermittent carriers (Figure 2): 1.8 (0.9–3.2) versus 0.98 (0.30–2.0;$p=0.001$) in the derivation cohort and 3.6 (1.9–3.9) versus 1.4 (0.30–3.3;$p<0.001$) in the validation cohort (Figure 2).

In the derivation cohort logistic regression showed that the model best differentiating between persistent and non-or-intermittent carriage used qualitative culture results in combination with quantitative data (Figure 1A). The model using the results of 2 cultures performed significantly better than a model using the results of only one culture. Adding the results of a third or fourth culture did not significantly improve the model. Results from the ROC analysis showed that all tests used had good performance (all areas under the ROC curves were above 0.9), with the combined model being slightly, but not significantly better than the qualitative result of two nasal swab cultures (Figure 1A).

In the validation cohort, two qualitative culture results (positive or negative) discriminated similarly between persistent and non-or-intermittent carriage as the combined qualitative and

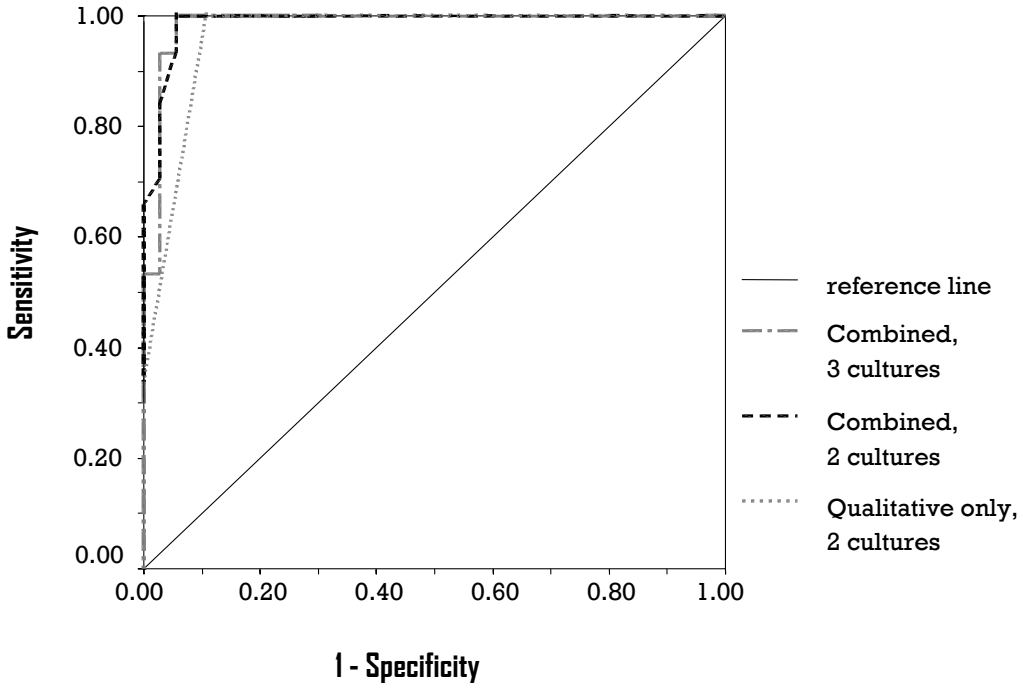
quantitative results (Figure 1B). All logistic regression models were significantly improved by adding data on a third culture. However, in the ROC analysis the differences between the models were small. Adding data on a third (but not a fourth culture) only significantly improved the model using both qualitative and quantitative culture results (Figure 1B).
The areas under the ROC curves (AUC) using the combination of qualitative culture results and the geometric mean CFUs of 2 cultures were 0.981 [95% CI: 0.949-1] for the derivation cohort and 0.936 [95% CI: 0.881-0.990] for the validation cohort, respectively (Figure 1). The logistic regression equation, using the combination of qualitative culture results and the geometric mean of CFUs from 2 cultures, could be written as:

Probability of persistent *S. aureus* nasal carriage =

$$\frac{e^{(\beta 0 + \beta 1 * \text{number of positive cultures} + \beta 2 * \text{geometric mean of CFUs})}}{1 + e^{(\beta 0 + \beta 1 * \text{number of positive cultures} + \beta 2 * \text{geometric mean of CFUs})}}$$

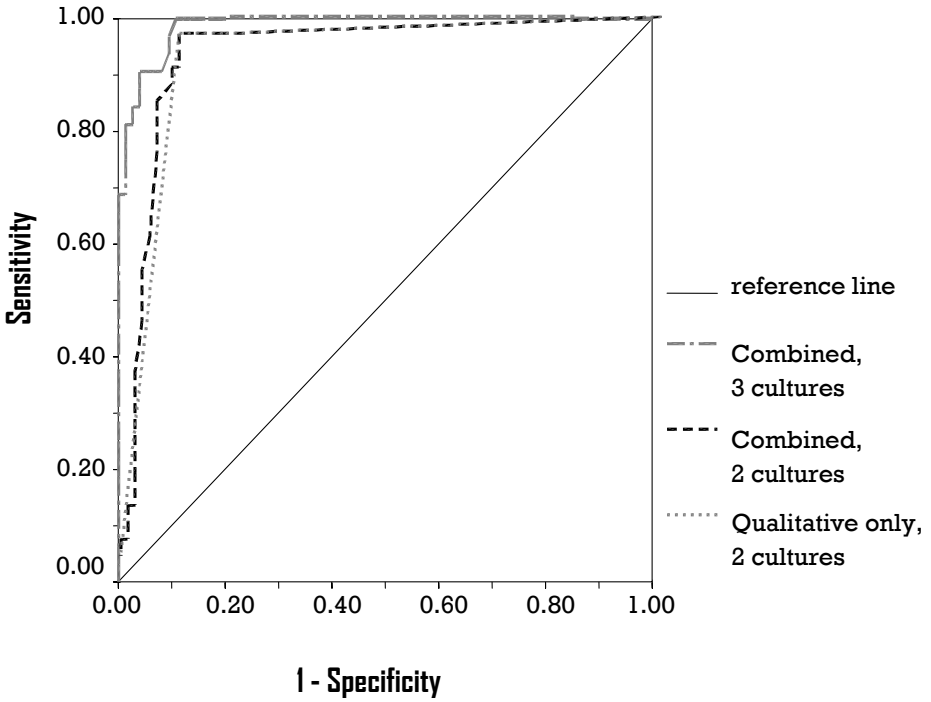
In the derivation cohort the respective values of ß0, ß1 and ß2 were -20.171, 9.341 and 1.661. In the validation cohort these values were -4.572, 2.563 and 0.274, respectively.
Using a cutoff of 0.50, above which probability persons were classified as persistent carriers, it followed from the logistic regression equation from the derivation cohort that a person was a persistent carrier only if both cultures were positive with a geometric mean of at least 0.9 (≈ 8 CFUs per culture). This 'culture rule', when applied to the validation cohort, had a positive predictive value of 0.78, a negative predictive value of 0.96 and an AUC of the corresponding ROC curve of 0.936 (0.881-.990).

Figure IA *Receiver Operating Characteristic (ROC) curve illustrating the predictive value of different tests for the persistent Staphylococcus aureus nasal carrier state in the derivation cohort.*
** AUC-ROC of 2 versus 1 culture p<0.05*



Test	nr. of cultures	Predictive Values		ROC analysis	
		PV+	PV-	AUC	95% CI
Qualitative culture results (nr. of cultures positive)	1	0.69	0.95	0.903*	0.810-0.968
	2	***0.79***	***1.0***	***0.944***	***0.881-1***
	3	0.88	1.0	0.972	0.927-1
Quantitative culture results (Geometric mean of CFUs)	2	0.85	0.89	0.973	0.936-1
	3	0.92	0.92	0.983	0.954-1
Combined	***2***	***0.88***	***1.0***	***0.981***	***0.949-1***
	3	***0.88***	***1.0***	***0.985***	***0.957-1***

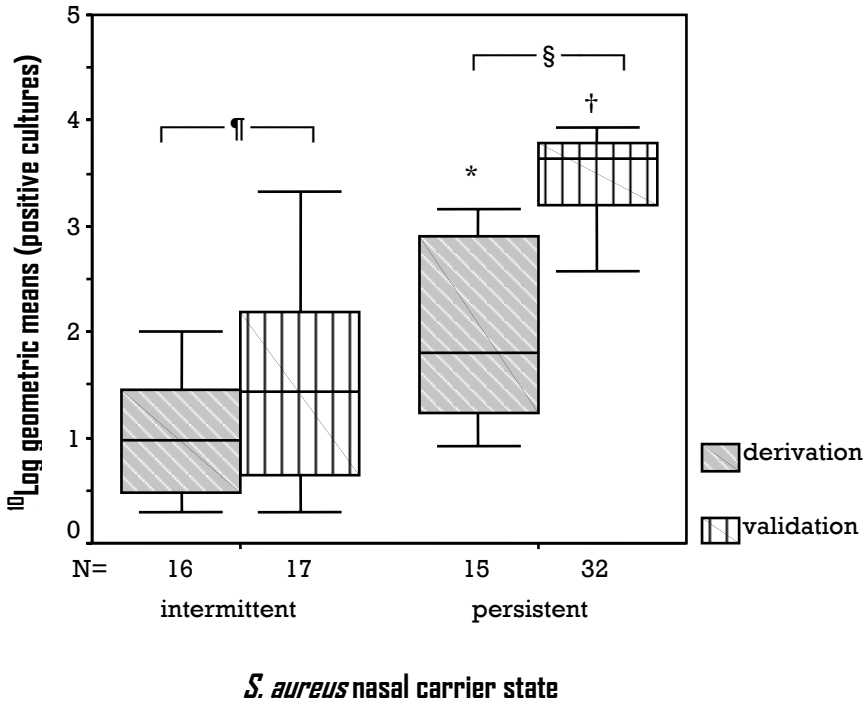
Figure 1B *Receiver Operating Characteristic (ROC) curve illustrating the predictive value of different tests for the persistent Staphylococcus aureus nasal carrier state in the validation cohort.*
** AUC-ROC of 3 versus 2 cultures in the combined test p<0.05*



Test	nr. of cultures	Predictive Values		ROC analysis	
		PV+	PV-	AUC	95% CI
Qualitative culture results (nr. of cultures positive)	1	0.74	0.95	0.901	0.840-0.960
	2	***0.79***	***0.99***	***0.929***	***0.873-0.985***
	3	0.88	0.96	0.967	0.934-1
Quantitative culture results (Geometric mean of CFUs)	2	0.83	0.91	0.936	0.881-0.990
	3	0.84	0.93	0.976	0.953-0.999
Combined	***2***	***0.79***	***0.99***	***0.936***	***0.881-0.990***
	3	***0.85***	***0.96***	***0.986****	***0.971-1***

Figure 2 *Geometric means (^{10}Log) CFUs of S. aureus in positive cultures in intermittent versus persistent carriers from both cohorts. Boxes represent median, quartile and extreme values.*

** persistent vs. intermittent carriers in derivation cohort p=0.001*
† persistent vs. intermittent carriers in validation cohort p<0.001
¶ intermittent carriers: derivation vs. validation cohort p=0.001
§ persistent carriers: derivation vs. validation cohort p<0.001



Discussion

We examined the diagnostic value of two weekly quantitative nasal swab cultures to predict the *S. aureus* nasal carriage state and developed a culture rule to enable adequate differentiation between persistent carriage and intermittent carriage among those individuals with two positive screening cultures.
Using logistic regression and ROC analysis a 'culture rule' was derived under 'ideal' laboratory circumstances in a cohort of healthy adult volunteers. Strictly speaking, the derivation cohort actually was more of an exploratory dataset to help select the variables in the model, but not the actual predictions. The 'culture rule' was subsequently validated under 'real life' conditions in a subset of elderly participants of the Rotterdam Study.
In the derivation cohort, the best test combined qualitative culture results (number of positive cultures) with quantitative data (geometric mean CFUs of *S. aureus* in nasal swab cultures). In the validation cohort, however, the simple qualitative culture result when using data on two cultures performed as good as the more complicated 'culture rule'. The 'culture rule' performed slightly less good in the validation cohort (ROC-AUC 0.981 in the derivation and 0.936 in the validation cohort, respectively). In the ideal laboratory situation, one trained physician performed all nasal swab cultures in a cohort of healthy individuals. In the real life situation of large-scale epidemiologic surveys, misclassification of the carrier state could have occurred for a variety of logistic reasons: differing nasal culturing techniques of study physicians, sample handling mistakes, laboratory errors, etc. In theory, many of these 'errors' are preventable, but can never be totally eradicated. The fact that in the validation cohort the first two cultures were taken at the Rotterdam Study research center by various study physicians, while cultures 3-12 in the validation cohort were performed by one trained person, certainly affected culture results: using cultures 3 and 4 of the validation cohort, instead of culture 1 and 2, the ROC-AUC was increased from 0.936 to 0.996.
Misclassification of the carrier state could also have occurred because of factors associated with individual participants of the Rotterdam study. Culture results will potentially have been influenced by the use of medication (recent courses of

antibiotics), institutionalization (recent hospital admissions) and underlying diseases, as well as other unknown determinants.

We confirm earlier data, showing that the number of CFUs of *S. aureus* in the anterior nares was higher in persistent than in intermittent carriers.[24,25] We also found a striking difference in the amount of *S. aureus* in the nose of persistent carriers between young healthy volunteers and healthy elderly participants. No prior data are available regarding age and the number of CFUs in the noses of persistent carriers. From the Rotterdam study (3851 persons) the high numbers of CFUs (median geometric mean 2.8) in elderly persistent carriers are confirmed (data not shown), but the underlying mechanisms of this finding remain to be elucidated. The differences in number of CFUs in persistent carriers in both cohorts will have affected the performance of the derived 'culture rule' in the validation cohort. When applying this 'culture rule' to other patient populations, it will need to be validated in the specific population first, when possible.

Combining qualitative results with quantitative data is, in our opinion, conceptionally the best choice. Incorporating quantitative data makes it possible to refine associations between potential determinants and *S. aureus* nasal carriage, since not only carriers are compared with non-carriers, but also carriers with low CFUs can be compared with carriers with high CFUs in their anterior nares. Incorporating quantitative data will also make it possible to refine associations between carriage state and morbidity and mortality. However, in large-scale epidemiologic studies, simplicity will often prevail, because of logistic reasons and resources. It is therefore reassuring that in the validation cohort the simple qualitative culture results performed as good as the more complicated 'culture rule'. Thus, two nasal swab cultures taken at a one-week interval can provide sufficient information to indeed adequately predict the *S. aureus* nasal carriage state. Using only one nasal swab culture to predict the carriage state, as is often done, cannot be recommended on the basis of our data since it will lead to misclassification of the carriage state. On the other hand, the addition of a 3rd or 4th quantitative nasal swab culture only minimally improved test performance. Importantly, no persons with the first 2 cultures positive were found to be non-carriers. The finding of two negative screening cultures in one person

with subsequent positive cultures is difficult to explain but may be attributable to sample handling mistakes or laboratory error. These results were included in the evaluation, though. One negative 'screening' culture virtually excludes persistent carriage. Predicting the non-carrier state from 2 nasal swab cultures is more difficult since at least 7 nasal swab cultures would be needed to distinguish intermittent from non-carriers.

At present data on determinants of persistent *S. aureus* nasal carriage in the elderly in the Rotterdam study are being analyzed using this 'culture rule'. This is the first study to validate the potential of a limited number of nasal swab cultures in predicting the *S. aureus* carrier state. Since the incidence of *S. aureus* infections has increased substantially and because of the dramatic worldwide increase in antibiotic resistance (methicillin- and recently even vancomycin resistance) in *S. aureus*, prevention is now more important than ever. Apart from its role in the Rotterdam study, we hope that the presented 'culture rule' will prove to be a helpful tool in identifying determinants of *S. aureus* nasal carriage and infections, as well as in identifying high-risk patient populations and the implementation of new methods in the prevention of *S. aureus* infections.

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3

The Human Factor in *Staphylococcus aureus* Nasal Carriage

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Abstract

Persistent *Staphylococcus aureus* nasal carriers and non-carriers were inoculated with a mixture of different *S. aureus* strains. The majority of non-carriers and nearly all persistent carriers returned to their original carrier state after artificial inoculation. Furthermore, the majority of persistent carriers selected again for their historic resident strain. Using a human nasal inoculation model, we here demonstrate that the human factor is an important determinant of *S. aureus* nasal carriage.

Introduction

Staphylococcus aureus nasal carriage is a major risk factor for *Staphylococcus aureus* infections.[1,2] Recently, cell wall lipoteichoic acid was described as the core factor for *S. aureus* nasal colonization.[3] However, in earlier studies no single staphylococcal factor essential for nasal colonization could be identified.[4-7] Furthermore, host factors,[1,8,9] as well as environmental factors are recognized determinants of the *S. aureus* nasal carrier state.[10,11]

Three human nasal *Staphylococcus aureus* carriage patterns can be distinguished: persistent-, intermittent- and non-carriage.[12] *S. aureus* density in the anterior nares is higher in persistent carriers,[13] which may partly explain their increased risk of *S. aureus* infections.[14] Variation among colonizing strains is higher for intermittent carriers,[15] suggesting that the basic determinants of persistent and intermittent carriage are different. The biology of *S. aureus* nasal carriage remains incompletely understood, although the importance of various host factors has been demonstrated.[8,16-18] In seeking further clarification, we performed a study in which persistent carriers and non-carriers were inoculated intranasally with a mixture of *S. aureus* strains.

Material & Methods

In 1988 a cohort of healthy volunteers (staff members of the departments of Medical Microbiology & Infectious Diseases and Virology of the Erasmus MC) was formed to investigate bacterial and human factors associated with *S. aureus* nasal carriage.[15] The composition of this volunteer cohort was flexible, in that outgoing personnel were considered lost to follow-up and replaced by incoming personnel. All volunteers were screened initially with 12 quantitative nasal swab cultures with one-week intervals. After this initial establishment of *S. aureus* nasal carriage status, volunteers were re-screened regularly with 4 quantitative nasal swab cultures with one-week intervals. For the present study only volunteers in follow-up for at least 2 years and with at least 16 nasal swab cultures done, were included. Long term persistent carriers were defined as all preceding cultures positive and long term non-carriers as all preceding cultures negative. Participants were excluded if they suffered from diabetes mellitus, skin diseases, chronic obstructive pulmonary disease, and cardiac valve abnormalities or were taking immunosuppressive agents. Eleven persistent carriers and 8 non-carriers agreed to participate in the present study. All participants gave written informed consent and the study was approved by the Medical Ethics Review Committee (METC Erasmus MC, nr. 156.137/1996/186).

For the non-carriers, a mixture of 4 different *S. aureus* strains was prepared consisting of *S. aureus* 502A (a strain used in intervention studies in the 1960s and 1970s[19]), *S. aureus* DU 5819 (a protein A deficient Dublin strain, courtesy of Dr. T. Foster), *S. aureus* 274 (a strain from a persistent carrier) and *S. aureus* 1036 (a strain from an intermittent carrier). Strains were selected from different carriage classes to analyze whether they have different colonization capacities.[15] The strains did not produce super-antigens and did not show different *in vitro* growth characteristics (data not shown). For the persistent carriers the same mixture of 4 *S. aureus* strains was used, while adding their own resident strain.

Nasal swabs were obtained using cotton-wool swabs (Transwab, Corsham, United Kingdom).[15] The left and right anterior nares were swabbed four times around. The swabs were immediately placed in Stuart's transport medium (Transwab) and kept at 4^0C

until quantitative culture on phenol-red mannitol salt agar (PHMA) and in phenol red mannitol salt broth (PHMB). The PHMB was incubated at 37^0C (7 days); the PHMA culture plates were incubated at 37^0C (48 hrs) and at room temperature (5 days). Identification of *S. aureus* was based upon colony morphology and a catalase- and latex-agglutination test (Staphaurex Plus^R, Murex, Dartford, UK). The geometric mean of colony forming units (CFUs) in the 26 post-inoculation cultures was calculated as ($10\text{Log}[CFU_1+1] + 10\text{Log}[CFU_2+1] + \ldots + 10\text{Log}[CFU_{26}+1])/26$. Per culture, 16 *S. aureus* colonies (maximum amount allowing for efficient molecular characterization), including all *S. aureus* morphotypes, were stored at -70^0C. To obtain bacterial DNA, *S. aureus* isolates were grown overnight at 37^0C on Brucella blood agar and processed according to Boom et al.[20] DNA was stored at -20^0C. Restriction fragment length polymorphisms (RFLP) of the coagulase and protein A genes were determined for strain identification purposes in the four *S. aureus* strains and all resident *S. aureus* strains from persistent carriers before inoculation.[21] Furthermore, all *S. aureus* strains isolated two and thirteen weeks after inoculation and/or from the last positive culture were genotyped using this method. Pulsed-field gel electrophoresis (PFGE) was performed to confirm the results.[22] All persistent carriers were treated with mupirocin nasal ointment (Bactroban®, GlaxoSmith Kline, Zeist, The Netherlands) 2 times daily for 5 days. The non-carriers did not receive mupirocin treatment. Ten weeks later, with nasal swab cultures negative, all participants were inoculated. Inoculation was performed using cotton-wool swabs drenched in PHMB containing 10^9 CFUs/mL of each strain. Per nostril, one swab was firmly applied against the inner side of the anterior nares and turned around 4 times. In this way, strains were inoculated in a total amount of 10^9 CFUs. At inoculation, blood was drawn for the determination of the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), leukocyte count and differentiation and anti-staphylococcal antibodies (ASTA). These tests were repeated when required. Nasal cultures were performed weekly during the study period. All participants with positive cultures at the end of the study were offered mupirocin nasal ointment (Bactroban®).

The primary endpoint was survival of *S. aureus* in the nose after artificial colonization. Survival ended when at least two consecutive nasal swab cultures were negative. Kaplan-Meier

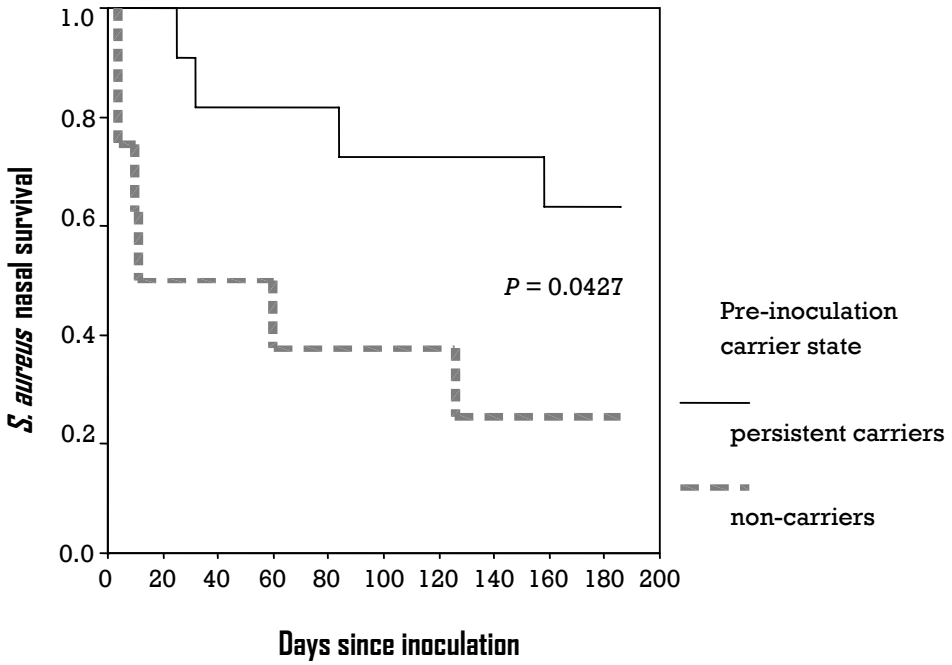
curves and the log-rank test were used to compare *S. aureus* survival curves. Participant's still carrying *S. aureus* in their nose at the end of the study were considered as censored in the analysis. The secondary endpoint was the geometric mean of CFUs over 26 weeks. Percentages and continuous data were compared by Fisher's exact test and Mann-Whitney's test, respectively.

Results

After artificial inoculation with a mixture of *S. aureus* strains, median nasal survival of *S. aureus* was 186 days in persistent carriers versus 35.5 days in non-carriers ($P = 0.0427$; Figure 1). Also, the 26 weeks Log geometric mean of CFUs was higher in persistent carriers (median 2.9 [range 0.6-3.7] versus 0.7 [0-3.6]), nearly reaching statistical significance ($P = 0.069$).
Six out of eight non-carriers became non-carriers again: 4 within 2 weeks after inoculation (non-carriers 224, 317, 244 and 207), and 2 after 19 (non-carrier 302) and 23 weeks (non-carrier 311; inoculation strain *S. aureus* 502A), respectively (Figure 2). Non-carrier 302 remained persistently positive with inoculation *S. aureus* strain 274 until week 11, intermittently between weeks 12-18 and reverted to the non-carrier state again at week 19 (Figure 2). Two non-carriers still had positive nasal cultures at the end of the study. Non-carrier 233 had positive cultures up to week 8, negative cultures between week 9-20 and then cultured positive again until the end of the study. This person first selected for *S. aureus* DU 5819, while ultimately a foreign *S. aureus* strain not included in the inoculation mixture was retained. Non-carrier 249 remained persistently positive after inoculation until the end study with inoculation *S. aureus* strain 274, and thus had become a persistent *S. aureus* nasal carrier (Figure 2). Two of the non-carrier volunteers developed minor self-limiting skin lesions: non-carrier 311 colonized with *S. aureus* 502A and non-carrier 249 colonized with *S. aureus* strain 274. No antibiotic treatment was necessary and all laboratory parameters remained completely normal. No side-effects were not noted in the persistent carrier group (P=0.1637).
In the persistent carrier group, 7 persons became persistent

Figure 1 *S. aureus survival after artificial nasal inoculation in long-term persistent and non S. aureus nasal carriers.*

Kaplan-Meier curves of S. aureus nasal survival in persistent carriers depicted by the black line and in non-carriers depicted by the dotted grey line. Survival ended when at least two consecutive nasal swab cultures were negative. After artificial inoculation with a mixture of S. aureus strains, median nasal survival of S. aureus was 186 days in persistent carriers versus 35.5 days in non-carriers (P = 0.0427; log-rank test).



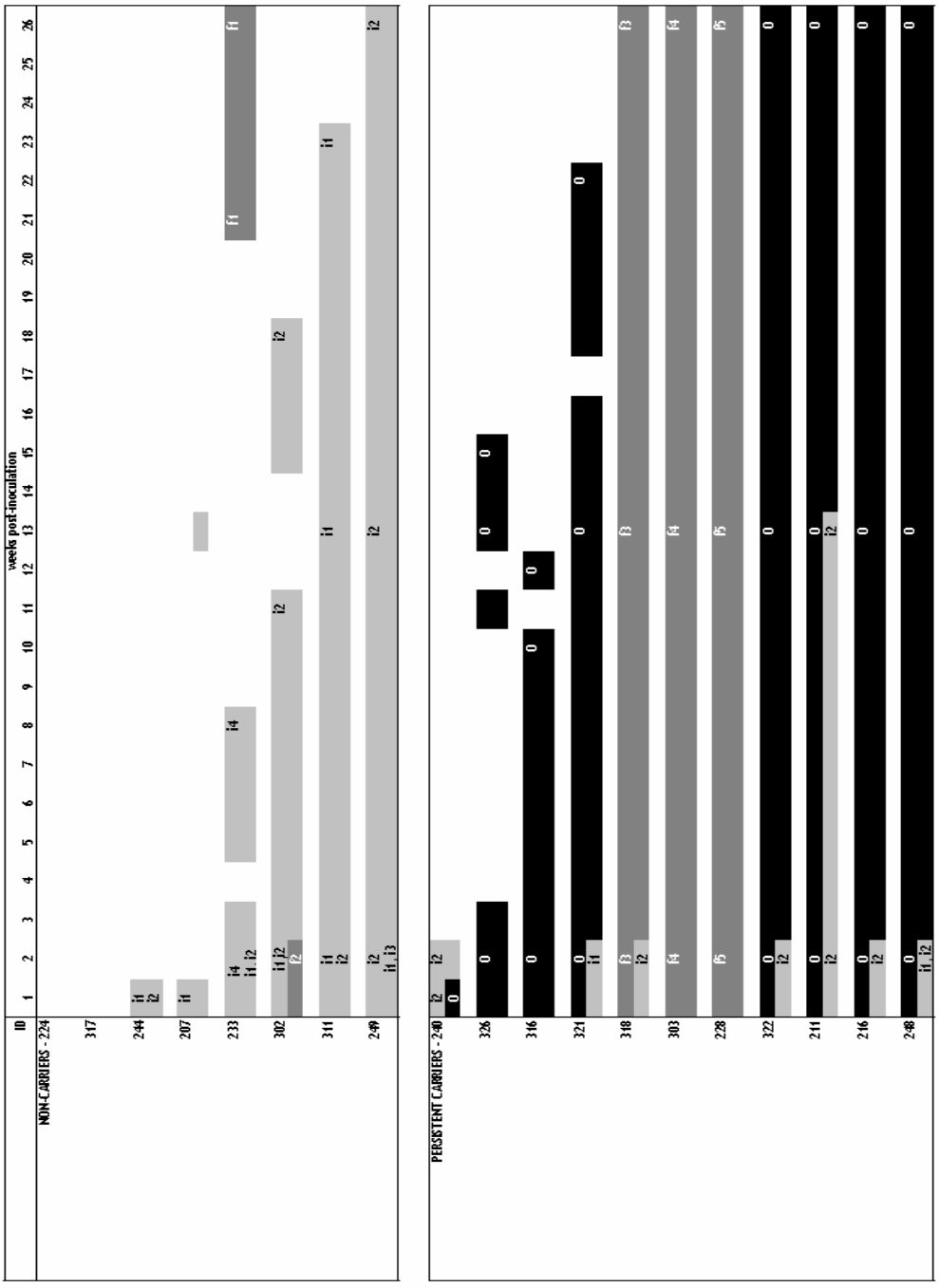
carriers again after artificial nasal inoculation: 4 carrying their own resident strain (persistent carriers 322, 211, 216 and 248) and 3 (persistent carriers 318, 303 and 228) carrying unique foreign strains not included in the mixture. These new strains were all genetically different and did not represent a laboratory contamination. Three persistent carriers became intermittent carriers with their own resident strain (persistent carriers 326, 316 and 321) and one person (persistent carrier 240) reverted to the non carrier state (Figure 2).

Discussion

The present results identify the importance of host factors in determining the *S. aureus* nasal carrier state in healthy adults. Half of non-carriers became non-carriers again within 2 weeks after inoculation. Only one non-carrier became a persistent carrier, coinciding with minor self-limiting skin lesions. These data suggest that most non-carriers are inherently resistant to colonization, but when *S. aureus* carriage is imposed, minor skin lesions can develop. Bacterial interference may be an explanation of the non-carrier state: when an ecological niche is already occupied by other bacteria *S. aureus* does not seem to have the means to establish a local population.[23] Recent data indicate that when the non-carriers were treated with mupirocin prior to inoculation elimination was as efficient: only 1 out of 16 volunteers was found to be still colonized after 16 weeks (data not shown). This suggests that non-carriers are not protected by a mupirocin-susceptible resident population of bacteria. Among the 11 persistent carriers, 7 became a persistent carrier again: 4 with their own resident strain and 3 with genetically unique foreign strains not included in the mixture. Three persons became intermittent carriers, all with their own resident strain. Only one person reverted to the non carrier state. Given the opportunity, persistent carriers will select an optimally fitting *S. aureus* strain, either from the inoculation mixture or from their environment.[15] When the intermittent carriers are included 7 out of 11 volunteers did select their original resident strain again. So far, no single common genetic or phenotypic characteristics

Figure 2 *Post-artificial nasal inoculation culture results from 11 long-term persistent and 8 non S. aureus nasal carriers during 26 weeks of follow-up.*

***ID** stands for the identification number of the individual participants. At time points 2 and 13 post-inoculation and of the last positive culture, all S. aureus strains cultured were genotyped. The various genotypically distinct S. aureus strains cultured are identified by colors and codes.*
*The historic resident strains of the persistent carriers are colored black and coded with an **O** as of 'own'.*
*The 4 S. aureus strains used in the inoculation mixture are colored light grey and coded **i1** (inoculation S. aureus strain 502A), **i2** (inoculation S. aureus strain 274 [=persistent carrier strain]), **i3** (inoculation S. aureus strain 1036 [=intermittent carrier strain]) and **i4** (inoculation S. aureus strain DU 5819 [=protein A deficient strain]). Five unique foreign S. aureus strains, which were neither resident strains from persistent carriers, nor inoculation strains from the inoculation mixture, were cultured in 5 participants. These foreign S. aureus strains are colored dark grey and coded **f1**, **f2**, **f3**, **f4** and **f5**. Multiple genotypically distinct S. aureus strains can thus be cultured at each point in time during follow-up.*
No coloring designates that cultures were negative at that point in time.


ID
weeks post-inoculation
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
NON-CARRIERS - 224
317
244 i1 i2
207 i1
233 i4 i1, i2
302 i1 ,i2 i2
311 i1 i2 i1
249 i2 i1, i3 i2
PERSISTENT CARRIERS - 240
i2 i2 0
326 0 0 0
316 0 0 0
321 0 i1 0
318 f3 i2 f3
303 f4 f4
228 f5 f5
322 0 i2 0
211 0 i2 0
216 0 i2 0
248 0 i1, i2 0

segregating successful from less- or non-successful colonizing *S. aureus* strains have been identified.[4,6-9] However, lipoteichoic acid has been implicated as an essential bacterial factor for *S. aureus* nasal colonization in a rat model recently.[3]
Here we conclude that in addition host characteristics significantly co-determine the *S. aureus* carrier state and that optimal fit between host and bacteria seems to be important. Further research should focus on identifying the specific host and bacterial factors involved. New strategies for the prevention of *S. aureus* nasal carriage and endogenous *S. aureus* infection could then be developed.

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4

Smoking Pattern & Fasting Glucose Levels Determine *Staphylococcus aureus* Nasal Carriage

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Abstract

Context *Staphylococcus aureus* nasal carriage is a known risk factor for *S. aureus* infections. Most studies on determinants of *S. aureus* nasal carriage have been performed among hospitalized persons and not in the general community.

Objective To identify determinants of *S. aureus* nasal carriage in a large community-based population.

Design Cross-sectional population-based study; April 1st 1997 and December 31st 1999.

Setting The Rotterdam Study.

Participants Of 4,797 participants in the second follow-up of the Rotterdam Study, 3,882 persons were able to visit the study center and included in the study. Two quantitative nasal swab cultures with one-week intervals were performed. According to an earlier derived 'culture rule' two carriage patterns were distinguished: non-or-intermittent and persistent *S. aureus* nasal carriage. In 31 persons an incomplete set cultures was obtained, leaving 3,851 persons (median age 71 years [range 61 to 101]) with complete bacteriological results, 2,224 women and 1,627 men.

Outcome Measures Determinants of persistent *S. aureus* nasal carriage.

Results Male sex (odds ratio [OR], 1.49; 95% confidence interval [CI], 1.24-1.80), diabetes mellitus (OR, 1.43; 95% CI, 1.11-1.83) and a history of eczema (OR, 1.92; 95% CI, 1.49-2.46) were associated with persistent *S. aureus* nasal carriage. The proportion of persistent carriers increased with fasting glucose level: one mmol/L increase in glucose level was associated with a 1.09 higher risk (95% CI, 1.04-1.15). Higher age (OR, 0.89 per 10 years; 95% CI, 0.78-0.99) and current smoking (OR, 0.64; 95% CI, 0.49-0.84) were negatively associated with persistent carriage.

Conclusions We confirm that age, sex and skin diseases are determinants of *S. aureus* nasal carriage. Smoking status and fasting blood glucose are newly recognized determinants of persistent *S. aureus* nasal carriage. Toxic or inflammatory effects of cigarette smoke and glycation of cellular structures or mucosal glucose level may promote *S. aureus* nasal colonization.

Introduction

S. aureus is an important agent of human disease and the cause of a variety of infections ranging from mild to life-threatening.[1] Over the last 20 years the incidence of both community-acquired and hospital-acquired *S. aureus* infections has increased,[2] while antibiotic treatment is increasingly hampered by the spread of *S. aureus* strains resistant to multiple antibiotics including methicillin and, more recently, vancomycin.[3]
The nose (i.e. the anterior nares) has been shown to be the primary ecological reservoir of *S. aureus* in humans.[4] *S. aureus* nasal carriage is a major risk factor for the development of *S. aureus* infections in various clinical settings[5,6] The majority of staphylococcal infections is of endogenous origin.[7]
Three *S. aureus* nasal carriage patterns can be distinguished: persistent, intermittent and non-carriage.[5] The biology of nasal colonization remains incompletely understood. Several studies have demonstrated the importance of host factors,[5,6,8] but environmental factors also contribute to *S. aureus* nasal carriage.[9-11]
Most studies on the determinants of *S. aureus* nasal carriage have been performed among hospitalized persons or specific patient groups. Large scale population-based studies are scarce[12] and most studies have been performed in restricted populations.[10,11,13] Our main objective was to find determinants of *S. aureus* nasal carriage in a large population of persons aged 55 years or over.

Material & Methods

Population and measurements

This study was conducted as part of the Rotterdam Study, a prospective, population-based cohort study on the occurrence and determinants of disease and disability in elderly persons.[14] In 1990, all inhabitants of Ommoord, a suburb of Rotterdam in the Netherlands, who were 55 years of age or older and had

lived in the district for at least one year were invited to participate in the study. Of the 10,275 eligible persons, 7983 (78%) participated. At baseline, between 1990 and 1993, trained interviewers administered an extensive questionnaire covering socioeconomic background and medical history, among other topics, during a home interview. During subsequent visits to the study center, additional interviewing, laboratory assessments, and clinical examinations were performed. Information on vital status is obtained at regular time intervals from the municipal authorities in Rotterdam. The second follow-up of the Rotterdam Study was performed between April 1st 1997 and December 31st 1999 among 4,797 remaining participants. Of these, 3,882 persons were able to visit the study center and included in the current study. In 31 persons an incomplete set of nasal cultures was obtained, leaving 3,851 persons with complete bacteriological results, 2,224 women and 1,627 men. The study was approved by the Medical Ethics Committee of the Erasmus MC, University Medical Center Rotterdam. Informed consent was obtained of all participants.

On visiting the study center, participating persons were interviewed using standardized questionnaires.[15-18] The main chronic medical disorders assessed were cardiovascular, neurologic, ophthalmologic and endocrine diseases. Persons were asked if they lived independently or institutionalized (home for the elderly or nursing home) and if they were living alone or together with a partner. Smoking habits were assessed by interview. We asked for cigarette, cigar and pipe smoking. Participants were asked if they were currently smoking, if they had smoked in the past or never smoked. Current and past smokers were asked when they had started smoking, when they had stopped smoking and how many cigarettes, cigars and/or pipes they had been or were smoking a day. Furthermore, they were asked if their spouse (when present) (had) smoked. Participants and their spouses were categorized in mutually exclusive categories of current, past and never smokers. A distinction was made between 'all smoking' (including pipe, cigar and cigarette smoking) and cigarette smoking only. Medication was assessed by interview and completed with data supplied by local pharmacies. Fasting glucose level was measured in all participants. Persons were categorized as having diabetes mellitus when either diabetes mellitus had been diagnosed earlier and they were using a diet or antidiabetic medication (tablets orally or insulin subcutaneously), or when

their fasting glucose level was 7.0 mmol/L (126 mg/dl) or greater. Persons were categorized as not having diabetes mellitus when their fasting glucose level was below 6.1 mmol/L and they were not using antidiabetic medication. Persons were categorized as having borderline impaired glucose metabolism when their fasting glucose level was equal to or higher than 6.1 mmol/L (110mg/dl) but lower than 7.0 mmol/L (126 mg/dl).[19]

Microbiological procedures

Nasal swabs were cultured as described earlier.[20] In brief, nasal samples were obtained using sterile cotton-wool swabs (Transwab, Medical Wire & Equipment Co. Ltd., Corsham, United Kingdom). Both the left and right anterior nares were swabbed and swabs were immediately placed in Stuart's transport medium and kept at 4 ^{0}C until further processing (within 24 hours). Swabs were cultured quantitatively on phenol-red mannitol salt agar (PHMA) and phenol red mannitol salt broth (PHMB).
Identification of *S. aureus* was based upon colony morphology on the PHMA. Suspected colonies were cultured overnight on Columbia blood agar plates (Becton-Dickinson B.V., Etten-Leur, The Netherlands). A catalase and latex agglutination test (Staphaurex Plus^R, Murex, Dartford, UK) both had to be positive. All *S. aureus* isolates were stored at -70 ^{0}C in glycerol containing liquid media.

Defining the *S. aureus* nasal carrier state

S. aureus nasal carriage was defined according to an earlier validated culture rule.[21] In brief, this culture rule uses qualitative (number of positive nasal swab cultures) and quantitative results (number of colony forming units (CFUs) in those nasal swab cultures). Persons were classified as persistent carriers when both nasal swab cultures were positive with *S. aureus.* Persons were classified as intermittent carriers when only one of the two nasal swab cultures was positive with *S. aureus*. Persons were classified as non-carriers when both nasal swab cultures were negative. We combined the non-or-intermittent carrier groups to contrast with the persistent carrier group.

Statistical analysis

Potential determinants of persistent *S. aureus* nasal carriage were tested univariately first. We used the Chi-square test (or Fisher's exact test in case of 2x2 tables) to compare proportions.

For comparison of continuous data Student's T-test, Mann-Whitney's test (in case of non-normal distributions), or Kruskal-Wallis' test (for k independent samples) were used where appropriate. Thereafter, variables associated with persistent carriage with a *P* value of less than 0.20 in the univariate analysis, were further tested using logistic regression analysis. Sex and age were entered in all regression models to correct for potential confounding. Some variables had missing data, which never exceeded 2.5% of participants. To include these individuals in the analysis, we created a missing value indicator when applicable. Results are reported as odds ratios with 95 percent confidence intervals. Two-sided *P* values of less than 0.05 were considered to indicate statistical significance.

Results

The majority of persons enrolled in the study was living independently, together with a partner. More than 80% did not currently smoke, while 34% had never smoked. The prevalence of diabetes mellitus was 11.8% (446/3,768). Only 2.5% was using sex hormones (mainly estrogen suppletion in 67 women) and only one person was on intermittent hemodialysis for end stage renal failure. Nearly half (1,627/3,785) had experienced one or more skin diseases prior to this study (Table 1).
The proportion of persistent carriers was higher in men than in women: 0.21 versus 0.16 (odds ratio [OR], 1.49; 95% confidence interval [CI], 1.24-1.80; Table 2). The Log geometric mean of the number of colony forming units (CFU) in the two nasal swab cultures was also significantly higher in men than in women (0.69 versus 0.49; difference 0.20; 95% CI, 0.12-0.27). However, male and female persistent carriers had similar Log geometric mean CFUs (2.86 versus 2.73; difference 0.13; 95% CI, -0.04-0.30).
The proportion of persistent carriers decreased slightly with age from 0.19 in the youngest age group (61 to 65 years) to 0.15 in age group 85 to 90 years (*P* for trend = 0.10). Logistic regression analysis demonstrated each 10 year increase in age to be associated with a 11% lower risk of persistent *S. aureus* nasal carriage (OR, 0.89 per 10 years; 95% CI, 0.78-0.99; Figure 1). Persons living in a nursing home (median age 89, range 74 to

Table 1 *Main Characteristics of the study population.*

Characteristic	**Men (N=1,627)**	**Women (N=2,224)**
Age - yr		
Median	71	72
Range	61-97	61-101
Living with partner - no. (%)		
Yes	1,391 (85.5)	1,233 (55.4)
No	229 (14.1)	978 (44.0)
Missing	7 (0.4)	13 (0.6)
Institutionalized - no. (%)		
Yes	130 (8.0)	308 (13.8)
No	1,490 (91.6)	1,903 (85.6)
Missing	7 (0.4)	13 (0.6)
Diabetes mellitus - no. (%)		
Diabetes mellitus	208 (12.8)	238 (10.7)
Impaired fasting glucose	251 (15.4)	331 (14.9)
Normal fasting glucose	1,140 (70.1)	1,600 (71.9)
Missing	28 (1.7)	55 (2.5)
Smoking - no. (%)		
Current	350 (12.5)	320 (14.4)
Past	1,082 (66.5)	783 (35.2)
Never	186 (11.4)	1,106 (49.7)
Missing	9 (0.6)	15 (0.7)
Skin diseases		
Skin infections past 3 months - no. (%)		
Yes	133 (8.2)	138 (6.2)
No	1,484 (91.2)	2,069 (93.0)
Missing	10 (0.6)	17 (0.8)
Boils ever - no. (%)		
Yes	744 (45.7)	526 (23.7)
No	868 (53.4)	1,677 (75.4)
Missing	15 (0.9)	21 (0.9)
Eczema past 12 months - no. (%)		
Yes	156 (9.6)	208 (9.4)
No	1,449 (89.1)	1,989 (89.4)
Missing	22 (1.3)	27 (1.2)

Table 2 *Associations of sex, living conditions, diabetes mellitus, smoking pattern and skin diseases with persistent S. aureus nasal carriage.*

Determinant		***S. aureus* Nasal Carrier state**			**Crude Odds Ratio** (95% CI)	**Adjusted Odds Ratio§** (95% CI)
		Persistent	*Non or Intermittent*	*Total*		
Sex	Men	333 (20.5)	1,294 (79.5)	1,627	1.40	1.49
- no. (%)	Women	345 (15.5)	1,879 (84.5)	2,224	(1.19-1.66)	(1.24-1.80)
Living with a partner	Yes	481 (18.3)	2,143 (81.7)	2,624	1.18	1.04
– no. (%)	No	193 (16.0)	1,014 (84.0)	1,207	(0.98-1.41)	(0.60-1.81)
Institutionalized	Yes	64 (14.6)	374 (85.4)	438	0.78	0.86
- no. (%)	No	610 (18.0)	2,783 (82.0)	3,393	(0.59-1.03)	(0.64-1.16)
Diabetes mellitus	Yes	99 (22.2)	347 (77.8)	446	1.38*	1.43
- no. (%)	Impaired	110 (18.9)	472 (81.1)	582	(1.09-1.76)	(1.11-1.83)
	No	459 (16.8)	2,281 (83.2)	2,740		
Active smoking	Current	96 (14.3)	574 (85.7)	670	0.75¶	0.64
- no. (%)	Past	348 (18.7)	1,517 (81.3)	1,865	(0.59-0.94)	(0.49-0.84)
	Never	230 (17.8)	1,062 (82.2)	1,292		
Passive smoking	Yes	74 (19.2)	311 (80.8)	385	1.08	1.15
- no. (%)	No	414 (18.1)	1,876 (81.9)	2,290	(0.82-1.42)	(0.86-1.53)
(smoking spouse)						
Skin infections in the	Yes	62 (22.9)	209 (77.1)	271	1.43	1.30
past 3 months	No	611 (17.2)	2,942 (82.8)	3,553	(1.06-1.92)	(0.96-1.75)
- no. (%)						
Boils ever	Yes	254 (20.0)	1,016 (80.0)	1,270	1.27	1.17
- no. (%)	No	419 (16.5)	2,126 (83.5)	2,545	(1.07-1.51)	(0.98-1.40)
Eczema in the past 12	Yes	100 (27.5)	264 (72.5)	364	1.92	1.92
months - no. (%)	No	567 (16.5)	2,871 (83.5)	3,438	(1.50-2.46)	(1.49-2.46)

Figure 1 *Proportion of persistent S. aureus nasal carriage according to age and sex.*

Error bars represent means with 95 percent confidence intervals. Lines depict linear regression line (black) with 95 percent mean prediction interval (grey) according to:
*Probability of persistent S. aureus nasal carriage = 0.24 – 0.001 [95% CI, -0.002-0.000] * age.*
Numbers at the bottom of the graph represent numbers of partcipants in each category.

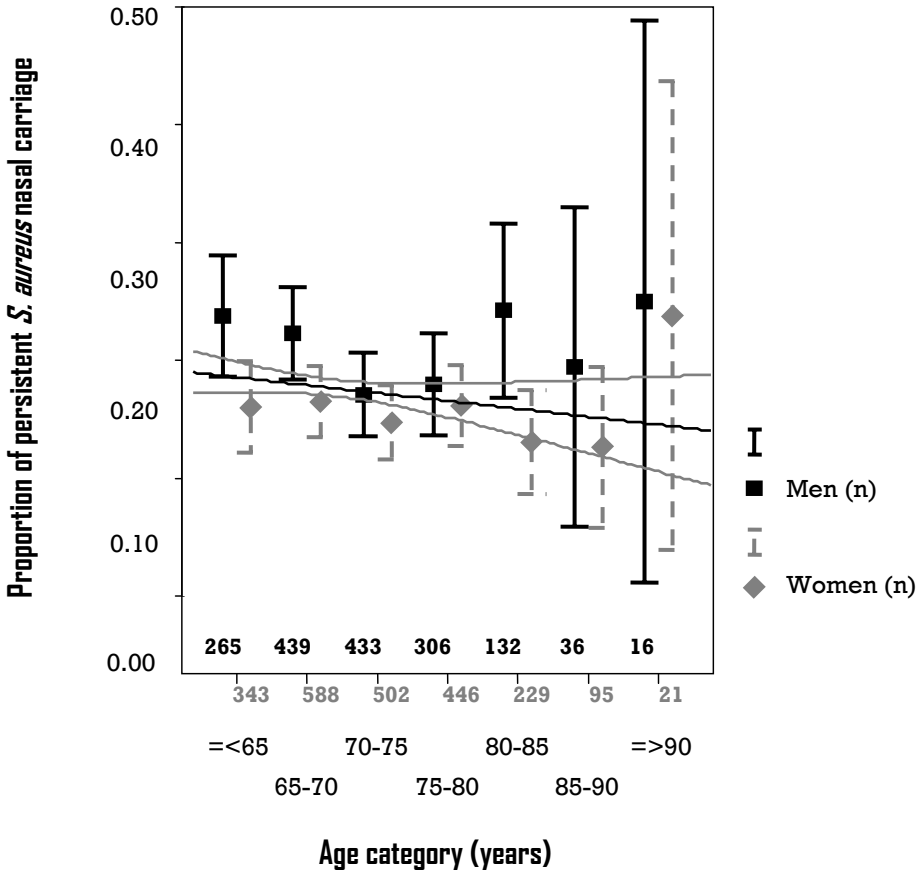


Figure 2A *Proportion of persistent S. aureus nasal carriage according to diabetes mellitus category.*

Error bars represent means with 95 percent confidence intervals. Numbers at the bottom of the graph represent numbers of participants in each category.

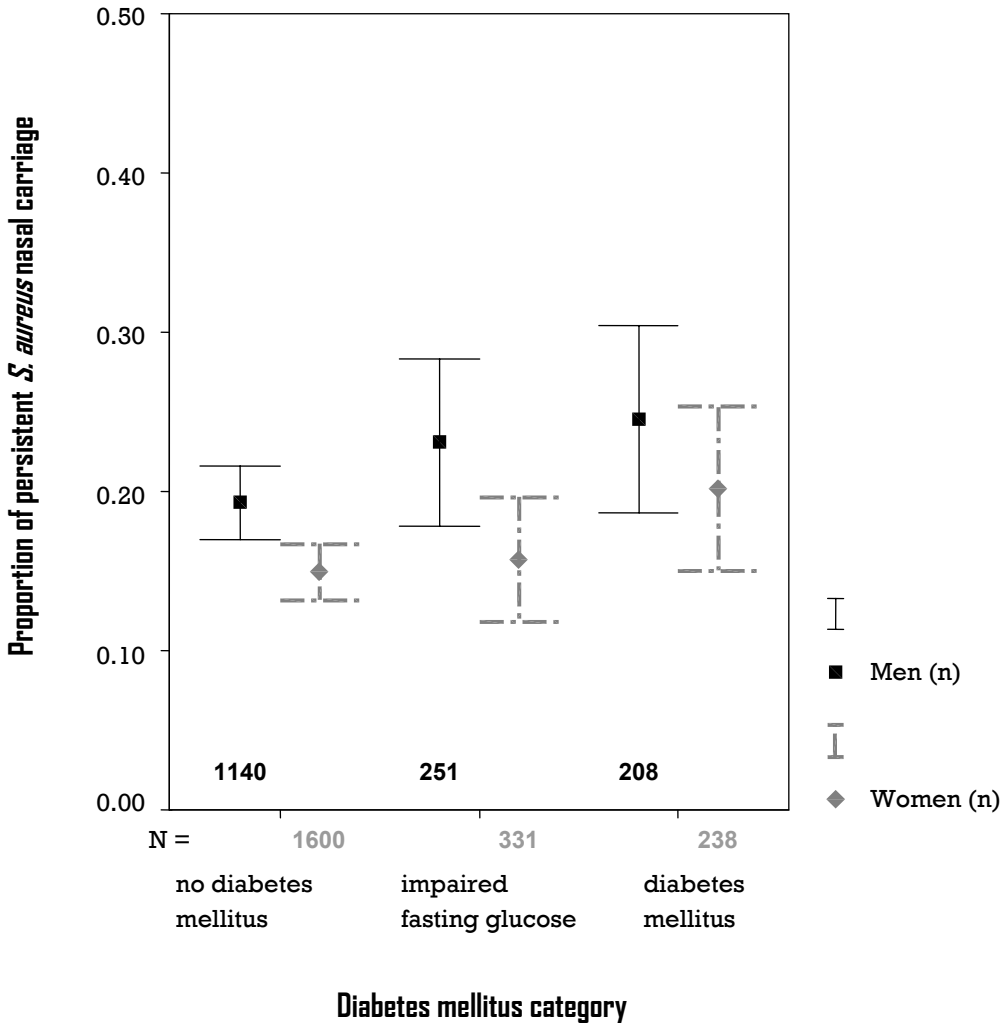
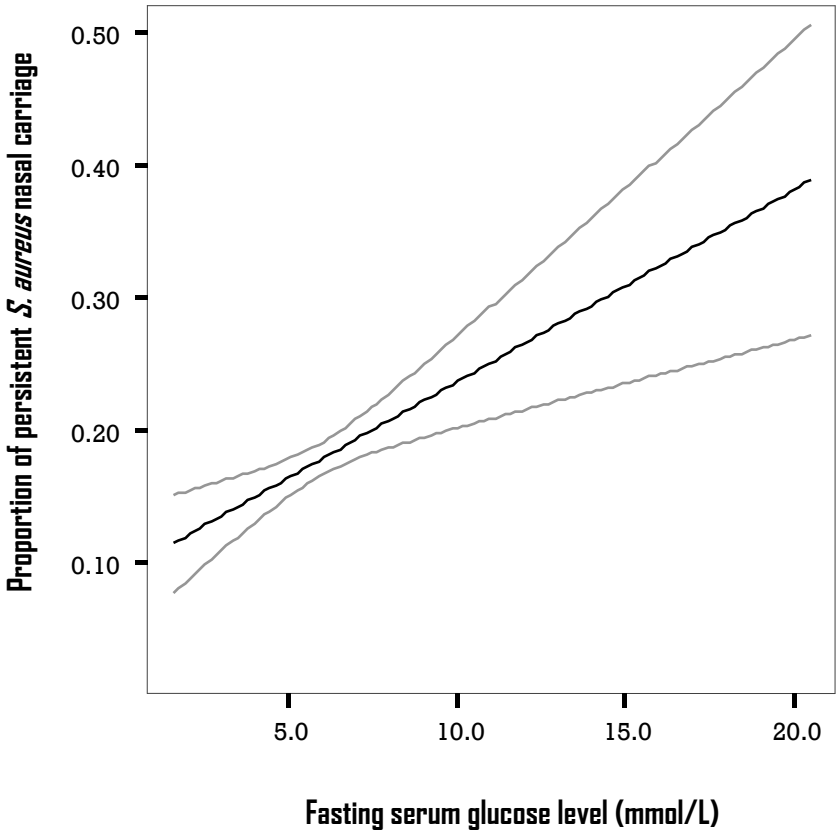


Figure 2B *Proportion of persistent S. aureus nasal carriage according to fasting glucose level in mmol/L.*

Lines depict linear regression line (black) with 95 percent mean prediction interval (grey) according to:
Probability of persistent S. aureus nasal carriage =
*0.09 + 0.012 [95% CI, 0.06-0.18] * fasting glucose level (mmol/L).*



101) and living in a home for the elderly (median age 78, range 62 to 97) were older than persons living independently (median age 71, range 61 to 93; *P* <0.001). Living in an institution was not associated with persistent *S. aureus* nasal carriage (OR, 0.86; 95% CI, 0.64-1.16) after adjustment for, among others, age and sex (Table 2).
The proportion of men living with a partner was significantly higher as compared to women (0.86 versus 0.56; OR, 4.82; 95% CI, 4.10-5.68; Table 1). The presence of a household partner was, however, not an independent determinant of persistent carriage (OR, 1.04; 95% CI, 0.60-1.81; Table 2).

Glucose

More persistent carriers were found among persons suffering from diabetes mellitus: 99/446 (0.22) in the diabetic group, 110/582 (0.19) in the impaired fasting glucose group and 459/2740 (0.17) in the non-diabetic group (*P* for trend = 0.004; Table 2 and Figure 2A). The diabetic group had a 1.43 higher risk (95% CI, 1.11-1.83) of being a persistent carrier than the non-diabetic group (Table 2). The proportion of persistent carriers increased with the fasting glucose level, even in the normal range: one mmol/L increase in glucose level was associated with a 1.09 (95% CI, 1.04-1.15) higher risk of being a persistent carrier (Table 2 and Figure 2B).

Smoking

Current smoking was associated with a 36% lower risk of persistent *S. aureus* nasal carriage when compared to the never smoking group (OR, 0.64; 95% CI, 0.49-0.84). The effect was similar when only cigarette smoking was considered (OR, 0.63; 95% CI, 0.48-0.84). Past smoking (OR, 0.85; 95% CI, 0.70-1.05) and passive smoking (i.e. smoking by a spouse) (OR, 1.15; 95% CI, 0.86-1.53) were not associated with persistent carriage; Table 2).
The negative association with smoking was present in both sexes, but more pronounced in men. In men the proportion of persistent carriers dropped from 0.24 (44/186) in the never, to 0.22 (235/1082) in the past and to 0.15 (53/350) in the current smoking group (*P* for trend = 0.007). In women these figures were 0.17 (186/1106), 0.14 (113/783) and 0.13 (43/320), respectively (*P* for trend = 0.08; Figure 3A). No difference was observed in the number of cigarettes smoked: both currently smoking men and women smoked a mean of 15 cigarettes a day (difference 0.57; 95% CI, -1.72-2.85).

Within the group of persistent carriers, smoking also reduced the mean number of CFUs in the two nasal swab cultures (Figure 3B). The Log geometric mean in never or past smoking persistent carriers was 2.87 versus 2.29 in current smoking persistent carriers (difference 0.58; 95% CI, 0.34-0.863). In the latter group, the daily number of cigarettes smoked tended to be negatively associated with the number of CFUs cultured (one cigarette higher daily intake correlated with 0.007 (≈ 1 CFU) lower Log geometric mean (95% CI, -0.021-0.006). In past smokers, time since quitting was not associated with being a persistent carrier (OR, 0.99 per year; 95% CI, 0.98-1.01).

Skin diseases

Skin diseases were positively associated with persistent carriage, but only a history of eczema (OR, 1.92; 95% CI, 1.49-2.46) was shown to be an independent determinant in the logistic regression analysis (Table 2).

Discussion

The main findings in this population-based study are the positive association of fasting blood glucose levels with persistent *S. aureus* nasal carriage, and the negative association or 'protective' effect of current smoking on the rate and density of *S. aureus* nasal carriage.

Three different patterns of *S. aureus* nasal carriage exist in humans. This suggests that human factors rather than bacterial traits play a major role in determining the carrier state. Indeed, rates of persistent carriage vary with age, sex and ethnic group.[5,10,11] However, the host environment can also influence the carrier state. Hospitalization, for example, has been shown to be an important risk factor for *S. aureus* nasal carriage,[5] while in the community household partners demonstrate highly concordant nasal carriage patterns.[10]

Genetic background,[22-24] local immune status[8] and underlying diseases[25-28] have all been associated with persistent carriage and *S. aureus* infections. A supposed common factor would be repeated or persistent breaches in the integrity of the skin or mucosal surfaces, thought to provide opportunities for staphylococcal adherence and subsequent colonization and infection.

Figure 3A *Proportion of persistent S. aureus nasal carriage according to smoking pattern and sex.*

Error bars represent means with 95 percent confidence intervals. Numbers at the bottom of the graph represent numbers of participants in each category.

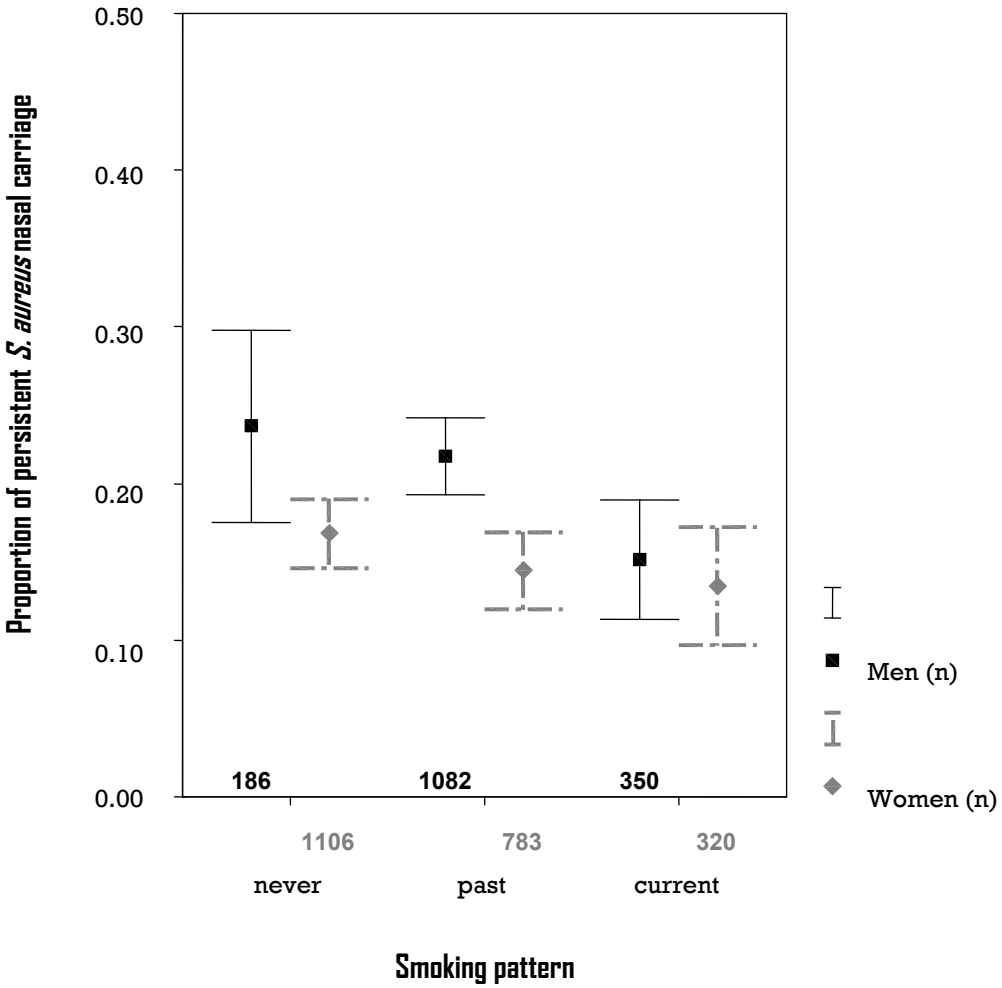
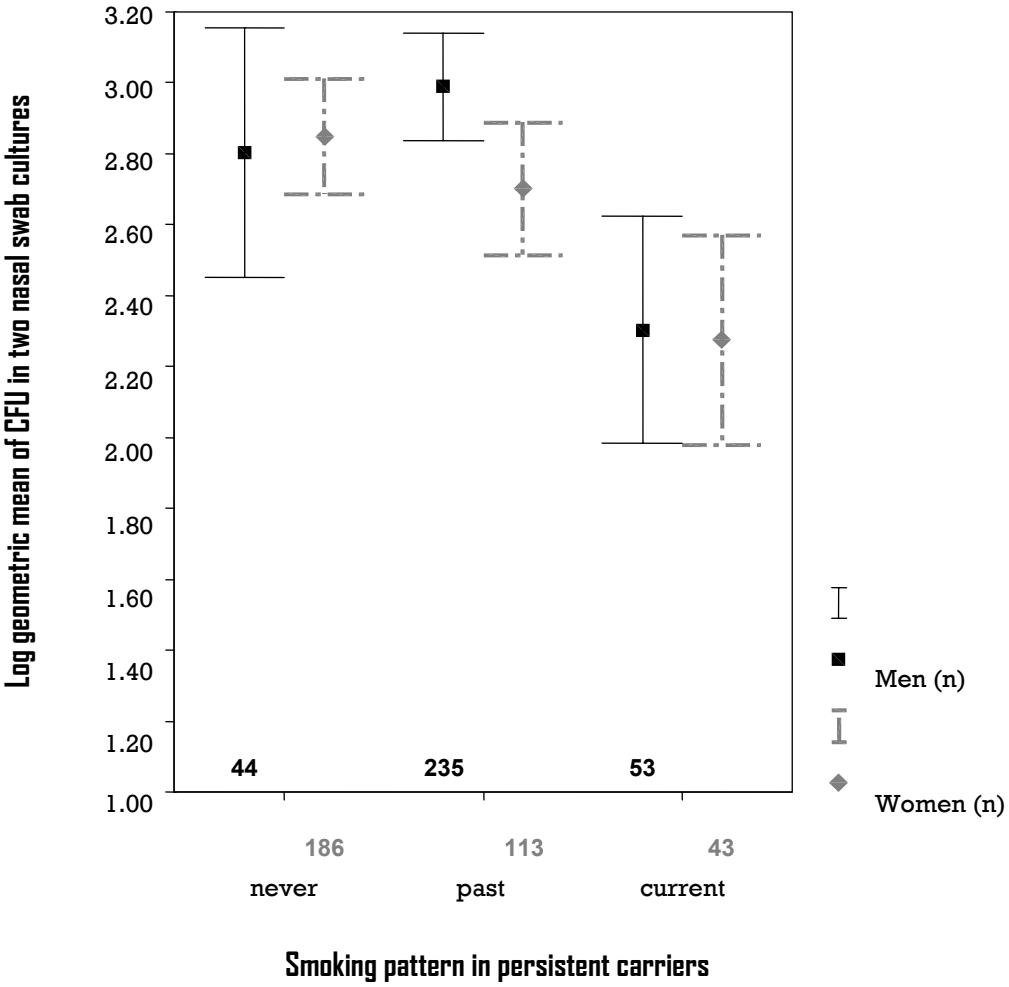


Figure 3B *Log geometric mean of number of CFUs in two nasal swab cultures in persistent S. aureus nasal carriers according to smoking pattern and sex.*

Error bars represent means with 95 percent confidence intervals. Numbers at the bottom of the graph represent numbers of participants in each category.



The present study confirms that age, sex and a history of skin disease are independent determinants of *S. aureus* nasal carriage. As for diabetes mellitus and blood glucose levels, in earlier studies only insulin dependent diabetics were found to have a higher prevalence of persistent carriage.[27,29] We now found that, apart from the diabetic state and its treatment, fasting blood glucose levels, even within the normal range, are associated with persistent carriage. The pathophysiological basis of this association remains to be discovered. Blood or mucosal glucose levels may influence the expression or modulation of bacterial adherence and colonization, perhaps by fostering non-enzymatic glycation of cellular structures in the nasal epithelia[30,31] or by promoting staphylococcal growth or biofilm formation.[32,33] On the other hand, hyperglycaemia is associated with reduced phagocytic activation of neutrophils and macrophages, as well as impaired killing of intracellular micro-organisms (including *S. aureus*).[34]

The second finding is the negative association of smoking and persistent carriage. In a population-based cohort of children and adolescents we recently also found evidence that cigarette smoking lowers the prevalence of *S. aureus* nasopharyngeal carriage.[11] Past smokers have the same prevalence of persistent carriage as those who have never smoked. Therefore, the "protection against carriage" conferred by smoking seems rapidly inducible and transient in nature. The fact that within the cohort of persistent carriers, current smoking was associated with a significantly lower density of *S. aureus* CFUs in the nares also points toward a direct effect of (cigarette) smoke on carriage. In contrast to the study of Bogaert et al., no effect of passive smoking was demonstrated in our study.[11]

The number of studies on the effect of cigarette and/or tobacco smoke on growth and adherence of *S. aureus* are limited and often contradictory. In one study smoking increased nasal colonization with MRSA, while reducing colonization with methicillin sensitive *S. aureus* (MSSA).[35] Ex vivo studies using buccal cells or lower respiratory tract derived cells exposed to cigarette smoke demonstrated increased adherence of *S. aureus*.[36,37] In contrast, one other in vitro study reported that cigarette smoke inhibited the growth of *S. aureus* and other Gram-positive cocci to a greater degree than that of Gram-negative rods. This inhibition was directly related to the concentration of smoke constituents in the experimental

dilutions used, suggesting a direct toxic effect.[38]
Cigarette smoking is known to induce airway inflammation.[39,40] Cole *et al*. reported that nasal secretions obtained from *S. aureus* carriers contained higher levels of inflammatory proteins including Defensins, but lacked antimicrobial activity in vitro, while nasal fluid from non-carriers was bactericidal.[8] However, they did not control for the smoking status of the subjects studied, nor did they properly define the type of nasal carriage. From these and our data, we hypothesize that *S. aureus* colonization of the anterior nares is less likely to occur in smoking persons because of a toxic effect of smoke on *S. aureus* combined with smoke induced airway mucosa infiltration of inflammatory cells.

In conclusion, based on a large population based study we confirm that age, sex, diabetes mellitus and skin diseases are independent determinants of *S. aureus* nasal carriage. In addition, our data suggest that active smoking may preclude persistent *S. aureus* nasal carriage, whereas a positive association exists between fasting glucose levels and the risk of persistent *S. aureus* nasal carriage. Considering the clinical impact of *S. aureus* these newly recognized determinants should prompt novel efforts to further elucidate the biology of *S. aureus* nasal colonization.

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manuscript.
Jan Nouwen, Alewijn Ott, Alex van Belkum, Albert Hofman and Henri Verbrugh had full access to all the data and take responsibility for the integrity of the data and the accuracy of the data analysis.

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5

Staphylococcus aureus Nasal Carriage in Household Contacts & First Degree Family Members

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Abstract

Context *Staphylococcus aureus* nasal carriage is a known risk factor for *S. aureus* infections. However, the precise etiology of carriage is largely unexplained.

Objective To estimate the impact of environmental and genetic factors in determining the *S. aureus* nasal carrier state.

Design Cross-sectional population-based study; April 1st 1997 and March 31st 2001.

Setting The Rotterdam Study and first degree family members of healthy volunteers.

Participants In all participants two quantitative nasal swab cultures with one-week intervals were performed. Two carriage patterns were distinguished: non-or-intermittent and persistent *S. aureus* nasal carriage. To study the impact of environmental factors 1,001 household pairs (2,002 persons) out of 4,797 participants in the Rotterdam Study were identified. To study genetic factors 82 first degree relatives and 23 partners of 16 long-term persistent carriers and 8 long-term non-carriers were cultured.

Outcome Measures The main outcome measure was the risk of sharing identical *S. aureus* nasal carrier states in household partners and first degree family members.

Results The proportion of persistent *S. aureus* nasal carriers was 24% (473 out of 2,002) in the elderly cohort and 27% (28 out of 105) in the family cohort. The risk of sharing identical *S. aureus* nasal carrier states was 2.39 [95% CI: 1.73-3.31] among 1,001 elderly household pairs (n=2,002). Among first degree relatives and partners of 16 persistent- and 8 non-carriers of *S. aureus,* the risk of sharing identical carrier states was 4.18 [95% CI 1.02-17.07] for members of the same household (n=53) versus 1.81 [95% CI: 0.58-5.60] for first degree relatives (n=82). Up to 65% of persons living in one household shared genetically identical strains.

Conclusions Apparently, the repeated (non-) exposure to *S. aureus* in the household environment is a more important determinant of *S. aureus* nasal carriage than the genetic background of first degree family members.

Introduction

Staphylococcus aureus is an important human pathogen and the cause of infections ranging from mild to life-threatening. *S. aureus* nasal carriage is the major risk factor for *S. aureus* (auto-) infections in various clinical settings.[1] Three *S. aureus* nasal carriage patterns can be distinguished: persistent, intermittent and non-carriage.[1] *S. aureus* density in the anterior nares is higher in persistent carriers,[2] which may partly explain their increased risk of *S. aureus* infections.[3] Variation among colonizing strains is higher for intermittent carriers,[4] suggesting that the basic determinants of persistent and intermittent carriage are different.
S. aureus nasal carriage has been associated with host factors such as ethnicity, gender, age, hormonal status in women, local immunity and genetic make-up, as well as concurrent diseases such as eczema and diabetes mellitus and environmental factors, including hospitalization.[1,5] However, the precise etiology of *S. aureus* nasal carriage is largely unexplained.
The aim of the present study was to estimate the relative impact of 'environmental' (defined as living together in the same household with a partner, who is not a family member) versus 'genetic' (defined as being a first degree family member of either a long-term persistent *S. aureus* nasal carrier or a long-term non-carrier) factors in determining the *S. aureus* nasal carrier state.

Material & Methods

This study consisted of two parts. In the first part, two quantitative nasal swab cultures with one-week intervals were taken in 3,851 (on a total number of 4,797) participants aged 55 years and over, as part of the second follow-up of the Rotterdam Study, a population-based prospective cohort study on chronic diseases in the elderly.[6] The second part consisted of a family study in which two quantitative nasal swab cultures with one-week intervals were taken in partners and first degree relatives of 16 long-term persistent carriers and 8 long-term non-carriers

(index persons). According to an earlier validated 'culture rule', two different carrier states were defined: non- or intermittent and persistent *S. aureus* nasal carriage.[2] Genotyping, using pulsed field gel electrophoresis, was done on all *S. aureus* isolates of the family cohort and on a random selection of 100 household pairs of the elderly cohort. Counts and percentages were compared by Fisher's exact test. The study was approved by the Medical Ethics Committee of the Erasmus MC, University Medical Center Rotterdam. Informed consent was obtained of all participants.

Results

In the elderly cohort, 1,001 household pairs (2,002 persons) were identified, of which 473 persons (24%) were persistent carriers (Table 1). In total 1,456 *S. aureus* strains were isolated in 983 persons (510 with only 1 culture positive and 473 with both cultures positive). *S. aureus* nasal carrier states were concordant between household partners, with a risk of 2.39 [95% CI: 1.73-3.31] of sharing identical *S. aureus* nasal carrier states (meaning to say that both partners were either persistent or non-or-intermittent carriers). When only the male-female pairs (983) were included, results were identical. In the random set of 100 household pairs 161 *S. aureus* strains were isolated in 104 persons (47 with only 1 culture positive and 57 with both cultures positive). In 37 household pairs at least one culture was positive in both partners. In 2 pairs both had one culture positive, in 15 pairs one partner had one and the other both cultures positive and in the remaining 20 pairs both partners had both cultures positive. Genotyping demonstrated that in 24 (65%) of these 37 household pairs both persons shared genotypically identical *S. aureus* strains.

In the family cohort, a total of 105 persons (23 partners and 82 first degree relatives of the 24 index persons) were screened, of which 28 persons (27%) were persistent carriers. Fifty-three (50%) persons were part of the index persons' households. In total, 75 *S. aureus* strains were isolated in 46 persons (17 with only 1 culture positive and 29 with both cultures positive), exclusive of the 16 persistent carrier index strains. *S. aureus*

Tabel 1 *S. aureus nasal carrier states of household partners in the Rotterdam Study.*

		S. aureus nasal carrier state partner 1		
		Non or Intermittent	Persistent	Total
S. aureus nasal carrier state partner 2	Non or Intermittent	613	117	730
	Persistent	186	85	271
	Total	799	202	1001

Tabel 2 *S. aureus nasal carrier states of household contacts and first degree family members in the family study.*

		S. aureus nasal carrier state index person		
		Non or Intermittent	Persistent	Total
S. aureus nasal carrier state of household contact	Non or Intermittent	14	19	33
	Persistent	3	17	20
	Total	17	36	53
S. aureus nasal carrier state of first degree family member	Non or Intermittent	22	39	61
	Persistent	5	16	21
	Total	27	55	82

nasal carrier states were concordant between persons living within the same household with a risk of 4.18 [95% CI 1.02-17.07] of sharing the same SNC state (Table 2). No concordance in *S. aureus* nasal carrier states between the index person and first degree family members could be demonstrated (risk 1.81 [95% CI: 0.58-5.60]; Table 2). Overall, genotypically identical *S. aureus* strains were shared in 9 (18%) out of 46 persons with 1 or 2 positive nasal swab cultures. Within households identical strains were shared in 8 (28%) out of 29 persons versus 1 (5.9%) out of 17 non household first degree family members (risk 6.10 [95% CI: 0.69-53.85]). Within the group of household contacts of the persistent carrier index persons, identical strains were shared in 8 (36%) out of 22 persons versus 1 (8%) out of 12 non household first degree family members (risk 6.29 [95% CI: 0.68-58.13]).

Discussion

Recently, Peacock et al. found concordant carrier states between mothers and their children.[7] Furthermore, Bogaert et al. found large households (≥ 5 members) to be positively and colonization with *Streptococcus pneumoniae* to be negatively associated with persistent *S. aureus* nasal carriage.[8] The large study among an elderly population presented here, demonstrates that not only persistent, but also non-or-intermittent *S. aureus* nasal carrier states are shared among household members. Living together with a persistent carrier increases your risk of being a persistent carrier at least twofold, while living together with a non-or-intermittent carrier 'reduces' your chance of being a persistent carrier twofold. Up to 65% of persons with positive cultures living within one household shared genotypically identical strains. These findings suggest that persistent carriers as well as non-carriers can 'impose' their respective carrier state upon their (intermittent carrier) household members.

From the family study it is clear that a simple Mendelian trait does not underlie the different *S. aureus* nasal carrier states. The repeated (non) exposure to *S. aureus* in the household environment is, thus, considered to be a more important

determinant of *S. aureus* nasal carriage than the genetic background of family members.

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6

Staphylococcus aureus Nasal Carriage is Not Associated with Polymorphism in the Vitamin D Receptor Gene

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Abstract

The vitamin D endocrine system has been shown to influence immune response and polymorphisms in the vitamin D receptor (VDR) gene have been associated with susceptibility to infectious diseases. We determined if the Cdx2, FokI and BsmI-ApaI-TaqI polymorphisms in the VDR gene were associated with nasal carriage of *Staphylococcal aureus*. We analyzed over 2,000 subjects from the Rotterdam Study. The prevalence of persistent *S. aureus* nasal carriage was 18%, which was, however, not different by VDR genotype. Our study is one of the largest in the field and suggests that VDR gene variation is not associated with *S. aureus* carriage.

Introduction

S. aureus is an important agent of human disease and the cause of a variety of infections ranging from mild to life-threatening. Over the last 20 years the incidence of both community-acquired and hospital-acquired *S. aureus* infections has increased, while antibiotic treatment is increasingly hampered by the spread of *S. aureus* strains resistant to multiple antibiotics including methicillin and, more recently, vancomycin.

The nose (i.e. the anterior nares) has been shown to be the primary ecological reservoir of *Staphylococcus aureus (S. aureus)* in humans and *S. aureus* nasal carriage is a major risk factor for *S. aureus* infections in various clinical settings.[1] Three nasal carriage patterns can be distinguished: persistent, intermittent and non carriage. The biology of nasal colonization with *S. aureus* remains incompletely understood, but host factors are thought to play a pivotal role.[1] Age, sex, fasting glucose levels, diabetes mellitus and smoking were recently demonstrated to be independent determinants of *S. aureus* nasal carriage .[2]

The vitamin D endocrine system has pleiotropic effects on many different organ systems including calcium absorption, bone biology, cell growth and differentiation, but also the immune system. This is exemplified by associations of polymorphisms in

the Vitamin D receptor (VDR) gene with a number of diseases such as osteoporosis, cancer, diabetes mellitus, but also infections.[3] VDR polymorphisms have been associated with susceptibility to tuberculosis,[4,5] *Mycobacterium malmoense* pulmonary disease,[6] persistent Hepatitis B infection,[4] leprosy[7] and HIV disease progression.[8] VDR gene polymorphisms may determine the likelihood of persistent infection or colonization with intracellular micro-organisms (including *S. aureus)*, since Vitamin D metabolites have been shown to play a role in macrophage activation and differentiation.[9] Furthermore, recent evidence indicates that neutrophils express functional VDR at a level similar to that of macrophages,[10] while neutrophils have been demonstrated to play an important role in the innate immunity against *S. aureus*. Cole et al. for example found that persistent *S. aureus* nasal carriage is accompanied by the release of epithelial and neutrophil-derived host defense peptides into nasal secretions.[11]
Hence, in search for a single host gene marker for *S. aureus* nasal carriage, we aimed to determine whether certain polymorphisms in the Vitamin D receptor gene were associated with persistent carriage.

Material & Methods

This study was conducted as part of the Rotterdam Study, a prospective, population-based cohort study on the occurrence and determinants of disease and disability in elderly persons.[12] In 1990, all inhabitants of Ommoord, a suburb of Rotterdam in the Netherlands, who were 55 years of age or older and had lived in the district for at least one year were invited to participate in the study. Of the 10,275 eligible persons, 7,983 (78%) participated. Participants gave informed consent and permission to retrieve information from medical records. At baseline, between 1990 and 1993, trained interviewers administered an extensive questionnaire covering socioeconomics background and medical history, among other topics, during a home interview. During subsequent visits to the study center, additional interviewing, laboratory assessments, and clinical examinations were performed. Information on vital status is obtained at regular time intervals from the municipal

authorities in Rotterdam. The study was approved by the Medical Ethics Committee of the Erasmus MC, University Medical Center Rotterdam. Informed consent and permission to retrieve information from treating physicians was obtained of all participants.
The second follow-up was performed between April 1st 1997 and December 31st 1999 among 4,797 remaining participants. Of these, 3,882 persons were able to visit the study center and eligible for the present study. In 31 persons an incomplete set of nasal swab cultures was obtained, leaving 3,851 persons with complete bacteriological results, 2,224 females and 1,627 males. Two quantitative nasal swab cultures with one-week intervals were performed. Subjects were classified as persistent carriers if both cultures were positive with at least 8 colony-forming units of *S.* aureus per culture. Those with both cultures negative were classified as non carriers. Intermittent carriers were those with intermediate results.[13]
The intermittent carrier group was excluded from our analyses to increase contrast between the groups and to reduce the risk of dilution of a possible association due to misclassification. We thus gave preference to precision at the cost of power.

The VDR polymorphisms studied were the G to A polymorphism in a Cdx2 binding site in the 1e/1a promoter region (results available for 3,349 subjects),[14] the N-terminal 3 amino acids deletion (also known as the start codon polymorphism; SCP) detected as a FokI RFLP (results available for 2,589 subjects)[15] and the 3' polymorphisms detected as BsmI-ApaI-TaqI RFLPs (results available for 3,502 subjects). Briefly, the Cdx2 and FokI polymorphisms are bi-allelic and, thus, three genotype combinations were distinguished, while three common Bsm-Apa-Taq haplotype-alleles could be distinguished, as described before.[16]

Associations between the different genotypes or haplotypes and *S. aureus* nasal carrier status were first analyzed univariately using the Chi-square test. Thereafter, multivariate logistic regression analysis was employed to evaluate the association of the individual gene polymorphisms on carrier status, while correcting for age, gender, smoking (coded as never, past, and current) and fasting serum glucose levels as potential confounders. In the final sample, data on *S. aureus* nasal carrier state and all possible confounders were available for 2,056

subjects for the Cdx2 genotype, 1,574 subjects for the FokI genotype, and 2,025 subjects for the BsmI-ApaI-TaqI haplotypes (1-3).

Results

In the group of 3,851 persons in whom the *S. aureus* nasal carrier state was determined, median age was 71 years (range 61-101), 58% were women, 34% were current and 50% were past smokers and mean fasting glucose was 5.9 mmol/L (range 1.6-20.5). These baseline characteristics were not different from the complete Rotterdam Study cohort or the final sample in which the analyses were performed (Table 1).
The distribution of the Cdx2 and FokI genotypes and the Bsm-Apa-Taq haplotype genotypes were in compliance with the Hardy-Weinberg equilibrium ($p>0.05$; Table 2). Univariate analysis (analysis I) did not show any significant association between a polymorphism and *S. aureus* nasal carrier state (Table 2). After correction for possible confounders by logistic regression analyses (analysis II), no association could be demonstrated either. There was a slight trend for the Cdx2 A-allele to be increased in frequency in the persistent carrier group but this was not significant. Similarly, the T-allele of the FokI RFLP was slightly increased in frequency in the persistent carrier group, but again this was not significant.

Discussion

Our study demonstrated none of the polymorphisms (Cdx2 or FokI genotype and Bsm-Apa-Taq haplotypes) in the Vitamin D receptor gene to be significantly associated with *S. aureus* nasal carriage status. We assume that selection bias was of no importance in our study, since allele combinations in any genotype or haplotype and the distribution of possible confounders was similar in the complete cohort and the subsets that were analyzed.

Other evidence has pointed to the influence of host genetic variations on the *S. aureus* nasal carriage status.[17-19] However, no earlier study examined specific gene polymorphisms as genetic determinants of *S. aureus* nasal carriage. From the results obtained in this study we conclude that these polymorphisms in the VDR gene do not relate to *S. aureus* nasal carriage. However, future studies of the VDR gene examining additional polymorphisms, or analysis of polymorphisms in other candidate genes might reveal genetic host differences determining S. *aureus* nasal carriage status.

Table 1 *Baseline characteristics of the study population.*

	Rotterdam Study second follow-up study population	***S. aureus* nasal carriage status determined**	**Final sample with all variables available**
Sample size – no.	4,797	3,851	1,547
Median age (range)	73 (61-106)	71 (61-101)	73 (62-96)
Female – no. (%)	2,875 (60)	2,224 (58)	836 (54)
Males – no. (%)	1,922 (40)	1,627 (42)	711 (46)
Smoking			
Never – no. (%)	1,649 (35)[a]	1314 (34)[c]	490 (32)
Past – no. (%)	2,264 (48)	1921 (50)	809 (52)
Current – no. (%)	768 (16)	592 (16)	248 (16)
Fasting serum glucose (mmol/l) (sd)	5.9 (1.35)[b]	5.9 (1.32)[d]	5.8 (1.25)

[a]116 missings
[b]1002 missings
[c]24 missings
[d]245 missings

Table 2 *The distribution of individual VDR polymorphisms and haplotypes and S. aureus nasal carriage status.*

VDR genotype / haplotype	**HWE [a] P-value**	**N-SNC no. (%)**	**P-SNC no. (%)**	**Total no.**	**Analysis I (Crude) P Value [b]**	**Analyses II (Adjusted) P Value [c]**
Cdx2	0.53	1,678 (81.6)	378 (18.4)	2,056		
GG		1,116 (82.1)	244 (17.9)	1,360	0.61	0.40
GA		502 (81.1)	117 (18.9)	619		
AA		60 (77.9)	17 (22.1)	77		
		A=18.5%	*A=20.0%*			
FokI	0.46	1,275 (81.0)	299 (19.0)	1,574		
CC		526 (81.7)	118 (18.3)	644	0.84	0.81
CT		575 (80.4)	140 (19.6)	715		
TT		174 (81.0)	41 (19.1)	215		
		T=36.2%	*T=37.1%*			
Bsm-Apa-Taq	0.24					
Haplo 1 (baT)		1,653 (81.6)	372 (18.4)	2,025		
0 copy		454 (80.5)	110 (19.5)	564	0.44	0.90
1 copy		803 (82.8)	167 (17.2)	970		
2 copies		396 (80.7)	95 (19.3)	491		
		1=48.3%	*1=48.0%*			
Haplo 2 (BAt)		1,653 (81.6)	372 (18.4)	2,025		
0 copy		612 (82.8)	127 (17.2)	739	0.58	0.34
1 copy		749 (80.9)	177 (19.1)	926		
2 copies		292 (81.8)	68 (18.9)	360		
		2=40.3%	*2=42.1%*			
Haplo 3 (bAT)		1,653 (81.6)	372 (18.4)	2,025		
0 copy		1,320 (81.1)	307 (18.9)	1,627	0.49	0.18
1 copy		310 (83.6)	61 (16.4)	371		
2 copies		23 (85.2)	4 (14.8)	27		
		3=10.8%	*3=9.3%*			

[a] Hardy Weinburg equilibrium
[b] Univariate: Pearson Chi-Square test,
[c] Multivariate after correction for age, gender, cigarette smoking behavior and serum glucose

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7

Functional Glucocorticoid Receptor Gene Polymorphisms are the First Genetic Markers for *Staphylococcus aureus* Nasal Carriage

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Abstract

No single bacterial or host factor essential for nasal colonization with *Staphylococcus aureus* in humans has been identified yet.[1-5] Earlier studies into the presumed genetic background of *S. aureus* nasal carriage in humans have generated conflicting results.[6-8] We determined whether four polymorphisms in the glucocorticoid receptor gene were associated with persistent nasal carriage of *S. aureus* in nearly 3,000 participants of a population-based cohort study. We demonstrate that GG-homozygotes of the exon 9beta polymorphism have a 68% reduced risk of persistent *S. aureus* nasal carriage (odds ratio 0.32; 95% confidence interval, 0.13-0.82), while carriers of the haplotype containing the 9beta G-allele and the codon 23 Lys-allele have an 80% increased risk (odds ratio 1.80; 95% confidence interval, 1.08-3.00). This study identifies the first human genetic variants as determinants of individual *S. aureus* nasal carriage.

Introduction

The anterior nares are the primary ecological reservoir of *Staphylococcus aureus* in humans and *S. aureus* nasal carriage is a major risk factor for a variety of infections.[9,10] Three human nasal *S. aureus* carriage patterns can be distinguished: persistent-, intermittent- and non-carriage.[11] *S. aureus* density in the anterior nares is highest in persistent carriers,[12] which may explain their increased risk of developing *S. aureus* infections.[13] Variation among colonizing strains is higher for intermittent carriers,[14] suggesting that the basic host determinants of persistent and intermittent carriage are different. The biology of nasal colonization with *S. aureus* remains incompletely understood. Environmental factors,[15-17]as well as host factors are thought to play a pivotal role in determining the *S. aureus* nasal carrier state.[4,5,18,19]

The hypothalamic-pituitary-adrenal (HPA) axis is known to influence glucose levels by regulating insulin sensitivity, but it

also plays a role in susceptibility to infectious disease.[20] Glucocorticoids repress a large number of pro-inflammatory cytokines and key inflammatory mediators such as TNF, IL-6, IL-12, and prostaglandin E2, while activating a number of anti-inflammatory genes (IL-10, IL-4, and TGFbeta) as well.[21-23] In addition, up-regulation of cell adhesion molecules such as ICAM-1 occurs [21], while down-regulation of neutrophil adhesion molecules has also been documented.[24] Thus, glucocorticoids exert repressive effects on the innate immune system with downstream fallout for adaptive immunity and inflammation. Several glucocorticoid receptor (GR) gene polymorphisms have been described and found associated with variation in glucocorticoid sensitivity, variation in insulin sensitivity, changes in body fat distribution and with autoimmune diseases such as rheumatoid arthritis.[25-30]
Polymorphisms in the glucocorticoid receptor gene can predispose to a more or less active immune system through modulation of the systemic or local pro/anti-inflammatory cytokine balance.[21,22,25-30] Alternatively, concurrent changes in insulin sensitivity may influence the availability of glucose in circulation as well as at the tissue level. Blood or mucosal glucose levels may influence the expression or modulation of bacterial adherence and colonization, by fostering non-enzymatic glycation of cellular structures in the nasal epithelia[31,32] or by promoting staphylococcal growth or biofilm formation.[33,34] Hyperglycaemia and hyperinsulinaemia have also been associated with reduced phagocytic activation of neutrophils and macrophages, as well as with impaired killing of intracellular micro-organisms (including *S. aureus*).[35] The ability of *S. aureus* to evade the inflammatory response of the host by surviving inside neutrophils has been shown to be a virulence factor earlier.[36] Impaired phagocytic activity is probably also a central factor in determining *S. aureus* nasal carriage, as was shown by Pos et al. in patients infected with the human immunodeficiency virus (HIV).[37] Interestingly, in HIV patients serum cortisol levels were shown to be elevated at all stages of HIV infection, while a linear negative association between CD4 cell counts and cortisol level was found.[38] Consequently, changes in glucocorticoid sensitivity may predispose to or protect from microbial colonization or infection on the one hand, or to auto-immune disease on the other.

Hence, in search of host genetic markers for *S. aureus* nasal

carriage, we aimed to determine whether functional polymorphisms in the glucocorticoid receptor gene are associated with persistent *S. aureus* nasal carriage.

Material & Methods

Study population

This study was conducted as part of the Rotterdam Study, a prospective, population-based cohort study on the occurrence and determinants of disease and disability in elderly persons started in Rotterdam, The Netherlands in 1990 among in 7983 participants.[19,39] The second follow-up of this study was performed between April 1st 1997 and December 31st 1999 among 4,797 remaining participants. Persons able to visit the study center were eligible for the present study. We obtained complete bacteriological results in 3,851 persons, 2,224 females and 1,627 males. The study was approved by the Medical Ethics Committee of the Erasmus MC, University Medical Center Rotterdam. Informed consent and permission to retrieve information from treating physicians was obtained from all participants. Two quantitative *S. aureus* specific nasal swab cultures with one-week intervals were performed. Subjects were classified as persistent carriers (n = 678) if both cultures were positive. Those with both cultures negative were classified as non carriers (n = 2804). The remaining subjects were intermittent carriers (n = 369).[12] The intermittent carrier group was excluded from our analyses to increase contrast between the groups and to reduce the risk of dilution of a possible association due to misclassification.

Genotyping

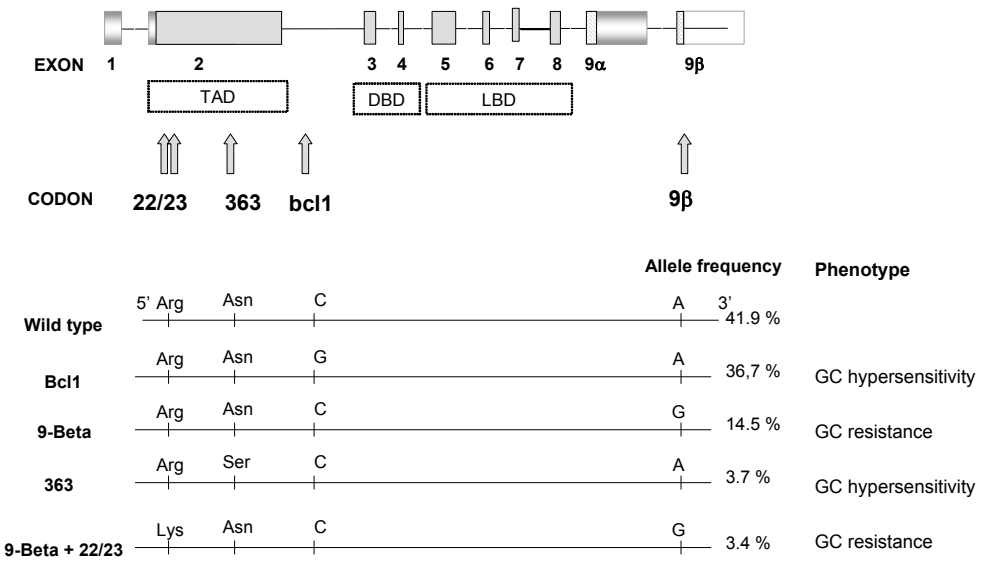
Participants were genotyped for four glucocorticoid receptor gene polymorphisms. Figure 1 schematically depicts the glucocorticoid receptor gene, the location of the four polymorphisms studied in the glucocorticoid receptor gene, their specific nucleotide variations and the allele frequencies. The glucocorticoid receptor gene polymorphisms studied were Bcl-1, an intronic C-to-G nucleotide substitution detected as a restriction fragment length polymorphism (RFLP) of 646 nucleotides downstream from exon 2 which results in fragments

of 2.2 kb and 3.9 kb.[27] We described the Arg23Lys and Asn363Ser polymorphisms in relation to a specific human GR cDNA sequence (accession number NM_ 000176), in which position 198/200 of the protein coding sequence is the first nucleotide of the start of the next closest exon to the polymorphisms studied (exon 2). The polymorphisms have also been described at http://www.ncbi.nlm.nih.gov/SNP under identification numbers rs 6189 (E22E), rs 6190 (R23K), rs.6195 (N363S.). The Asn363Ser polymorphism is an asparagine to serine polymorphism in codon 363 of exon 2, located in the transactivation domain.[25] The Arg23Lys polymorphism is a combination of two linked single-nucleotide variations in codons 22 and 23 leading to an arginine-to-lysine change in codon 23 in the transactivation domain.[29] The 9beta polymorphism is an A-to-G nucleotide substitution located in the 3' end of exon 9β, which encodes for the 3' UTR of the mRNA of the hGRβ isoform. The A to G nucleotide substitution is located in the 'ATTTA' motif (to GTTTA). This 'ATTTA' motif is known to destabilize mRNA.[30,40]

For the analysis DNA was isolated from peripheral blood leucocytes of subjects using standard techniques. DNA was extracted using proteinase K and sodium-dodecyl-sulphate digestion at 37^0C overnight and purified using phenol-chloroform or high-salt extractions. The extracted DNA was then precipitated using 4 mol/L NaCl and two volumes of cold absolute ethanol. DNA was solubilized in double-distilled water and stored at -20^0C until used for DNA amplification. PCR amplification and genotyping were performed using 5 ng genomic DNA for the Taqman allelic discrimination assay. Primer and probe sequences were optimized using the SNP assay-by-design service of Applied Biosystems. For details see http://store.appliedbiosystems.com. Reactions were performed on the Taqman Prism7900HT (Applied Biosystems, Foster City, CA, USA) in 384 wells format.
To calculate the possibilities of these four polymorphisms occurring together on the same allele, we used statistical methods for haplotype reconstruction: the Phase Reconstruction Method.[41] All four polymorphisms are mutually exclusive, except for the codon 23 Lys-allele, which is always present in combination with the 9beta G-allele. Per haplotype allele three combinations were distinguished, carrying 0, 1 or 2 copies of the haplotype allele. For studying the different genotypes all 15

possible haplotype allele combinations were analyzed seperately.

Figure 1 *Schematic overview of the glucocorticoid receptor (GR) gene polymorphisms and haplotypes.*



Statistical analysis

To compare baseline characteristics between the complete Rotterdam Study cohort, participants with complete bacteriologic results (the *S. aureus* cohort) and participants with a complete dataset on *S. aureus* nasal carrier state, confounding variables and all glucocorticoid receptor gene polymorphisms (the final cohort), Pearson's Chi-square test was used for dichotomous variables and Mann-Whitney's test or Kruskal-Wallis' test were used for continuous variables where appropriate.

Associations between the GR haplotypes and *S. aureus* nasal carrier status were first analyzed univariately using Pearson's Chi-square test. Multivariate logistic regression analysis was then employed to correct for the covariates age, gender, smoking (coded as never, past, and current), eczema (coded yes or no) and fasting serum glucose levels. First analysis for dominant, recessive or allele dose effects was performed by stratification for allele copy number 0, 1 and 2 for all haplotype alleles, while correcting for covariates. Thereafter, the 15 possible genotypes were entered into the logistic regression model, again correcting for covariates. Results are reported as odds ratios with 95 percent confidence intervals. Two-sided *P* values of less than 0.05 were considered to indicate statistical significance.

Results

For 2,929 participants (final cohort) a complete dataset on *S. aureus* nasal carrier state, confounding variables (age, gender, smoking, fasting glucose level and eczema) and all glucocorticoid receptor gene polymorphisms were available. Median age was 72 years, 58% were women, 18% were current and 48% were past smokers and mean fasting glucose was 5.9 mmol/L (Table 1). These baseline characteristics were not significantly different from those for the complete Rotterdam Study cohort or the 3,851 participants with complete bacteriologic results (*S. aureus* cohort). Prevalence of persistent *S. aureus* nasal carriage in the final cohort was 19.2% (563/2929), which was somewhat higher than in the *S. aureus* cohort (17.6%;

678/3851) due to selection of only non- and persistent carriers in the present study.

In non *S. aureus* carriers, the distribution of genotypes for all individual glucocorticoid receptor gene polymorphisms were in compliance with the Hardy-Weinberg equilibrium ($p>0.05$; Table 2). Frequencies of the haplotype alleles are presented in Figure 1.

First, the associations of the haplotype alleles of the different polymorphisms and *S. aureus* nasal carrier state were analyzed separately. Univariate analysis demonstrated the G-allele of the exon 9beta polymorphism to be significantly associated with a lower prevalence of *S. aureus* nasal carriage with evidence for a recessive effect (Table 2). After correction for age, gender, fasting glucose, eczema and smoking habit by logistic regression analyses, this association remained essentially unchanged (Table 2). GG homozygotes of the exon 9beta polymorphism had a 68% lower risk of persistent *S. aureus* nasal carriage compared to the AA homozygotes (odds ratio 0.32; 95% confidence interval (0.13-0.82).
The G-allele of the Bcl-1 polymorphism was also associated with a lower prevalence of *S. aureus* nasal carriage. GG homozygotes of the Bcl-1 polymorphism had a 20% lower risk of persistent *S. aureus* nasal carriage compared to CC homozygotes. The association was, however, not statistically significant (odds ratio 0.80; 95% confidence interval (0.59-1.08).
The haplotype containing the G-allele of the exon 9beta polymorphism combined with the Lys-allele of the codon 23 polymorphism was associated with a higher prevalence of *S. aureus* nasal carriage. Heterozygote 23Lys-carriers were shown to have a 38% higher risk of persistent *S. aureus* nasal carriage compared to their 23Arg-carrying counterparts. This association was just not statistically significant (odds ratio 1.38; 95% confidence interval (0.97-1.97).
No direct relation was found between fasting blood glucose levels and any of the polymorphisms investigated.

Hereafter, all potential haplotype allele combinations (i.e. genotypes) were analyzed using logistic regression analysis. Next to the association of the GG homozygotes of the exon 9beta polymorphism, only the combination of the haplotype containing the G-allele of the exon 9beta polymorphism combined with the

Table 1 *Baseline characteristics of the study population.*

	Rotterdam Study second follow-up study population	***S. aureus* nasal carriage status determined**	**Final sample population with all variables available**
Sample size - *n*	4,797	3,851	2,929
Median age (range)	73 (61-106)	71 (61-101)	72 (61-101)
Gender			
Women - *n* (%)	2,875 (60)	2,224 (58)	1,689 (58)
Men - *n* (%)	1,922 (40)	1,627 (42)	1,240 (42)
Smoking			
Never - *n* (%)	1,649 (35)	1,314 (34)	1,003 (34)
Past - *n* (%)	2,264 (48)	1,921 (50)	1,414 (48)
Current – *n* (%)	768 (16)	592 (16)	512 (18)
Eczema			
No - *n* (%)	3,438 (89)	3,438 (89)	2,648 (90)
Yes - *n* (%)	364 (11)	364 (11)	281 (10)
Fasting serum glucose (mmol/l) (sd)	5.9 (1.35)	5.9 (1.32)	5.9 (1.46)

Table 2 *The distribution of the glucocorticoid receptor gene alleles and S. aureus nasal carriage status.*

Total study group by GR genotype	HWE[a] P-value	N-SNC[b] *n* (%)	P-SNC[c] *n* (%)	Total *n*	Multivariate analysis[e] OR (95% CI)
Wild type allele (41.9%)	0.2	2,366 (80.8)	563 (19.2)	2,929	
2 copies		423 (79.7%)	108 (20.3%)	531	1 (reference)
1 copies		1,117 (80.1%)	278 (19.9%)	1,395	0.85 (0.68-1.04)
0 copies		826 (82.4%)	177 (17.6%)	1,003	0.85 (0.65-1.11)
Bcl-1 G-allele (36.7%)	0.5	2,366 (80.8)	563 (19.2)	2,929	
0 = CC		945 (80.2)	233 (19.8)	1,178	1 (reference)
1 = CG		1,099(80.6)	265 (19.4)	1,364	0.97 (0.80-1.18)
2 = GG		322 (83.2)	65 (16.8)	387	0.80 (0.59-1.08)
Exon 9beta G-allele (14.5%)	0.94	2,366 (80.8)	563 (19.2)	2,929	
0 = AA		1,725 (80.5)	418 (19.5)	2,143	1 (reference)
1 = AG		581 (80.6)	140 (19.4)	721	1.01 0.81-1.25)
2 = GG		60 (92.3)	5 (7.7)	65	0.32 (0.13-0.82)
Codon 363 Ser-allele (3.7%)	0.81	2,366 (80.8)	563 (19.2)	2,929	
AA		2,197 (80.9)	520 (19.1)	2,717	1 (reference)
AG		166 (79.4)	43 (20.6)	209	1.13 (0.80-1.61)
GG		3 (100)	0 (0)	3	
Codon 23 Lys-allele + 9beta G-allele haplotype (3.4%)	0.16	2,366 (80.8)	563 (19.2)	2,929	
GG		2,218 (81.0)	519 (19.0)	2,737	1 (reference)
GA		143 (76.5)	44 (23.5)	187	1.38 (0.97-1.97)
AA		5 (100)	0 (0)	5	
Genotype combination of a codon 23 Lys-allele + 9beta G-allele & a wild type allele	0.26	2,366 (80.8)	563 (19.2)	2,929	1.80 (1.08-3.00)
no		2,310 (81.0%)	541 (19.0%)	2,851	
yes		56 (71.8%)	22 (28.2%)	78	

[a] Hardy Weinburg equilibrium
[b] non *S. aureus* nasal carrier
[c] persistent *S. aureus* nasal carrier
[e] Adjusted for age, gender, smoking, eczema and fasting serum glucose

Lys-allele of the codon 23 polymorphism and a wild type haplotype allele was significantly associated with *S. aureus* nasal carriage (Table 2). This latter genotype was shown to increase the risk of persistent *S. aureus* nasal carriage by 80% (odds ratio 1.80; 95% confidence interval, 1.08-3.00).

Discussion

Earlier evidence has pointed to the possible influence of host genetic variation on the *S. aureus* nasal carriage status [4,6-8]. However, no previous study identified specific gene polymorphisms as genetic determinants of *S. aureus* nasal carriage. The current availability of high-tech, high-throughput genomics tools in combination with a large prospective population-based epidemiologic survey, has provided a unique opportunity to study genetic determinants of *S. aureus* nasal carriage. Our study demonstrates that polymorphisms in the glucocorticoid receptor gene are significantly associated with *S. aureus* nasal carriage status.

We assume that selection bias was of little importance in our study, since allele frequencies and genotypes were similar to those observed in other Caucasian study populations [26,28]. Allele frequencies and genotypes and the distribution of possible confounders were also similar in the total Rotterdam study cohort and in the final cohort that was analyzed.

The four glucocorticoid receptor gene polymorphisms have all been previously associated with variation in glucocorticoid sensitivity [28]. We found that homozygous presence of the G-allele of the exon 9beta polymorphism conferred a 68% lower risk of persistent *S.aureus* nasal carriage. The G-allele of the exon 9beta polymorphism has previously been found to be over-represented in a group of patients with rheumatoid arthritis. The mechanism for this is unknown, but stabilization of the GRbeta mRNA by the presence of this G-allele – as indeed observed in vitro [30,40] – may play a role. The G-allele destroys an mRNA destabilizing ATTTA motif, resulting in a higher stability for the GRbeta mRNA. This stabilization may induce relative

glucocorticoid insensitivity through accumulation of the GRbeta protein which has been reported to have a dominant negative influence on GRalpha action [30,40]. This in turn may lead to a slightly more active immune system, predisposing to chronic inflammatory disease such as rheumatoid arthritis on one hand, while protecting from *S.aureus* colonization on the other hand.
The Ser-allele of the Asn363Ser polymorphism has been shown to increase glucocorticoid sensitivity and to increase the insulin response to dexamethasone [25,26]. It was, however, not associated with *S. aureus* carriage.
The G-allele of the Bcl-1 polymorphism was previously found to be associated with increased glucocorticoid sensitivity and increased abdominal obesity, while at higher age a lower body mass index (BMI) accompanied by a tendency towards lower lean body mass was documented [27]. It showed a slight but statistically non-significant, protective effect with regard to *S.aureus* carrier status.
The Lys-allele of the Arg23Lys polymorphism has been associated with increased resistance to glucocorticoids, higher cortisol levels after dexamethasone suppression testing, lower fasting insulin concentrations and better insulin sensitivity [2942]. From our haplotype analysis we determined that this allele is always present on an allele that also carries the G-variant of the exon 9beta polymorphism. Remarkably, we found that heterozygous carriers of this haplotype allele were at an increased risk of persistent *S. aureus* carriage, which just failed to reach significance (OR 1.38; 95% CI, 0.97-1.97). However, persons with the genotypic combination of a wild type allele together with this haplotype allele had an 80% higher risk of persistent *S. aureus* carriage versus all other genotypes. It is difficult to reconcile these observations: two polymorphisms, both associated with reduced glucocorticoid sensitivity have opposite effects. First, it should be noted, however, that the protective effect of the G-allele of the exon 9beta polymorphism was only present in homozygotes, while the genotypic combination of a wild type allele together with this haplotype allele includes only 9beta heterozygotes. This suggests a recessive effect of the 9beta G-allele. Second, all five 23Lys homozygotes were non-carriers of *S. aureus*, which, might be due to the fact that they are also 9beta G-allele homozygous. Due to their low number, this was not amendable to statistical analysis

Our data show that genetic variation in the glucocorticoid receptor gene influences the risk of persistent *S. aureus* nasal carriage. The 9beta GG-homozygotes may have a more active immune system through increased glucocorticoid resistance as demonstrated by the association with autoimmune disease, notably rheumatoid arthritis [30]. Although no data on insulin sensitivity or tissue glucose concentrations are available for the population studied, the lack of association between fasting blood glucose and the exon 9beta polymorphism suggests that a lack of glucocorticoid-induced immune suppression is the most likely explanation for the association observed here. Furthermore, where glucocorticoid receptor mediated gene activation mostly acts through DNA binding, one of the key regulatory pathways of the immune system, the strong anti-inflammatory effect of glucocorticoids is the result of glucocorticoid receptor mediated regulation of pro- / anti-inflammatory genes through differential activity of the pro-inflammatory nuclear transcription factor kappa B (NF-kappa B) [21,22]. Lys-carriers of the Arg23Lys polymorphism show a relative glucocorticoid resistance due to a lesser transactivation in combination with normal transinhibition of NF-κB [43,44]. We hypothesize that the higher cortisol levels in these carriers lead to an increased transinhibition and thus to suppression of the immune system. However, too little is currently known on the physiological basis of the (combined) effects of glucocorticoid receptor gene polymorphisms, and future research should lead to better insight in the underlying mechanisms. In addition, it has recently been demonstrated that glucocorticoids, in conjunction with pro-inflammatory cytokines, affect the expression of toll-like receptor 2 (TLR-2), the main pattern recognition molecule for gram positive pathogens such as *S. aureus* [45-47]. Whether the various glucocorticoid receptor gene polymorphisms studied here are (partially) defective in supporting this biological activity is currently unknown, but, again, warrants further investigation. These further investigations would form a de novo field of research focusing on *S. aureus* carriage and glucocorticoid/ glucocorticoid receptor gene dependent innate immune determinants.

In conclusion, this study identifies the first human genetic factor as a (co-)determinant of individual *S. aureus* nasal carriage. Apparently, polymorphisms and combinations thereof in the glucocorticoid receptor gene affect the human colonization resistance against *S. aureus*. This knowledge should be taken

into account when designing future studies into the fundamentals of staphylococcal colonization and infection.

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8

Effect of *Staphylococcus aureus* Nasal Carriage on Mortality & Antibiotic Use in the Community

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Abstract

Context *S. aureus* nasal carriage is a risk factor for nosocomial and community-acquired infections. No data are available on overall health effects in the community.

Objective To determine if in the community *S. aureus* nasal carriage is associated with mortality and antibiotic use.

Design Prospective population-based cohort study.

Setting The Rotterdam Study.

Participants 3,851 individuals, 61 years of age and older.

Outcome Measures Between April 1st 1997 and December 31st 1999 *S. aureus* nasal carriage status was determined. Non-or-intermittent and persistent carriage were distinguished. Follow-up ended on December 1st 2003. The influence of potential prognostic factors on mortality was evaluated using Cox proportional hazards model. To evaluate the influence of potential prognostic factors on antibiotic use we used Poisson regression analysis.

Results 678 (17.6%) persons were persistent carriers. 2510 (65.2%) persons used antibiotics and 516 (13.4%) persons died. Male gender (hazard ratio [HR], 1.88; 95% confidence interval [CI], 1.54-2.30), age (HR per 10 years increase 2.86; 95% CI, 2.52-3.24), smoking (HR 1.75; 95% CI, 1.33-2.29), fasting glucose level (HR per mmol/L increase 1.09; 95% CI, 1.04-1.14), and diastolic blood pressure (HR per 10 mm Hg increase 1.01; 95% CI, 1.01-1.02) were significantly associated with mortality. Persistent *S. aureus* nasal carriage was not associated with increased mortality (HR 1.03; 95% CI, 0.82-1.30).
Persistent *S. aureus* nasal carriage was associated antibiotic use (incidence rate ratio [IRR], 1.09; 95% CI, 1.03-1.14), as were female gender (IRR, 1.29; 95% CI, 1.23-1.34), age (IRR per 10 years increase, 1.10; 95% CI, 1.07.-1.13), current smoking (IRR, 1.17; 95% CI, 1.11.-1.24), eczema (IRR, 1.10; 95%CI, 1.03-1.17) and history of skin infections (IRR, 1.20; 95% CI, 1.04-1.40).

Conclusions Among elderly persons in the community, persistent *S. aureus* nasal carriage was not associated with all-cause mortality. Persistent *S. aureus* nasal carriage was, however, associated with a slight, though significant increase in antibiotic use.

Introduction

Staphylococcus aureus is the cause of a variety of infections ranging from mild, including skin infections and food poisoning, to life-threatening, including pneumonia, sepsis, osteomyelitis, and infective endocarditis.[1] Over the last 20 years the incidence of both community-acquired and hospital-acquired *S. aureus* infections has increased, accounting for about 13 percent of nosocomial infections in U.S. hospitals.[2-4] In Europe, the EPIC study demonstrated that more than 30% of infections in the intensive care unit (ICU) were due to *S. aureus.*[5] In Canada, severe community-acquired blood stream infections (BSI) necessitating ICU admission (incidence 15.7 per 100,000 per year) were caused by *S. aureus* in 20% of all cases.[6] Infections by *S. aureus* are the cause of serious attributable morbidity and mortality, especially in the elderly.[6-10]

The nose (i.e. the anterior nares) has been shown to be the primary ecological reservoir of *S. aureus* in humans.[11] Three *S. aureus* nasal carriage patterns can be distinguished: persistent, intermittent and non-carriage.[12] Persistent *S. aureus* nasal carriage is the major risk factor for the development of *S. aureus* infections, the majority being of endogenous origin.[12-14]

Eradication of *S. aureus* nasal carriage by application of topical mupirocine results in a reduction in endogenous infections in various populations at risk.[15-18] However, the absolute risk of developing a nosocomial *S. aureus* infection among nasal carriers is only 2 to 5%.[13,14]

In contrast to the numerous studies on consequences of *S. aureus* nasal carriage in the hospital setting, limited data are available about the risks of *S. aureus* carriage in the community.[19-21]

Recently, we demonstrated that persistent nasal carriage decreases with increasing age.[22] This could be due to decreased survival of nasal carriers, as the elderly are at highest risk of serious *S. aureus* infections.[6,10]

To examine this hypothesis we performed a prospective population-based study on the relation between *S. aureus* nasal carriage, mortality and antibiotic use in a cohort of elderly persons.

Material & Methods

Study sample

The Rotterdam Study population

The present study was conducted as part of the Rotterdam Study, a prospective, population-based cohort study on the occurrence and determinants of disease and disability in elderly persons.[23] In 1990, all inhabitants of Ommoord, a suburb of Rotterdam in The Netherlands, who were 55 years of age or older and had lived in the district for at least one year were invited to participate in the study. Of the 10,275 eligible persons, 7983 (78%) participated. Participants gave informed consent and permission to retrieve information from medical records. At baseline, between 1990 and 1993, trained interviewers administered extensive questionnaires during a home interview. During subsequent visits to the study center, additional interviewing, laboratory assessments, and clinical examinations were performed.

The second follow-up was performed between April 1st 1997 and December 31st 1999 among 4,797 remaining participants. Using two quantitative nasal swab cultures with one-week interval, the *S. aureus* nasal carrier state was determined in 3,851 (80%) persons: 2,224 females and 1,627 males. All 3,851 were followed from the moment of culturing (between April 1st 1997 and December 31st 1999) until they died, or reached the end of the study at December 1st 2003. The Medical Ethics Committee of the Erasmus MC, Rotterdam, The Netherlands, approved the study.

Exposure Definition

Defining the S. aureus nasal carrier state

S. aureus nasal carriage was defined according to an earlier validated culture rule.[24] In brief, this culture rule used qualitative data (i.e. nasal swab cultures being positive or not) combined with quantitative data (i.e. number of colony forming units (CFUs) in cultures). Persons were classified as persistent carriers when both nasal swab cultures were positive with an earlier defined number of *S. aureus* CFUs.[24] All other persons were classified as non-or-intermittent carriers. The *S. aureus* nasal carrier state is thought to be a characteristic of the individual and for the present study we assumed it to be stable over time.[25]

Microbiological procedures

Nasal swabs were cultured as described earlier.[25] In brief, samples were obtained using sterile cotton-wool swabs (Transwab, Medical Wire & Equipment Co. Ltd., Corsham, United Kingdom). Both the left and right anterior nares were sampled and swabs were immediately placed in Stuart's transport medium and kept at 4 ^{0}C until further processing (within 24 hours). Swabs were cultured quantitatively on selective media: phenol-red mannitol salt agar (PHMA) and phenol red mannitol salt broth (PHMB). Identification of *S. aureus* was based upon colony morphology on the PHMA medium. Suspected colonies were cultured overnight on Columbia blood agar plates (Becton-Dickinson B.V., Etten-Leur, The Netherlands). A catalase test (positive) and a latex agglutination test (Staphaurex Plus^R, Murex, Dartford, UK), positive in case of *S. aureus*, were then performed.

Outcome Definition

The outcomes of interest included all-cause mortality and overall antibiotic use as a parameter of morbidity. Information on vital status was obtained at regular time intervals from the municipal authorities in Rotterdam. Antibiotic use was recorded as the number of filled antibiotic prescriptions during follow-up (count data). In the research area, there are 7 fully computerized pharmacies that are linked into a single network. During the study, all participants obtained their medication in 1 of these 7 pharmacies. Data on all dispensed drugs between 1 January 1991 and October 1st 2003 were available in computerized format on a day-to-day basis. The data include the date of prescribing, the total amount of drug units per prescription, the prescribed daily number of units, product name, and the Anatomical Therapeutic Chemical (ATC) code.[26,27]

Co-factors

The following baseline patient characteristics were individually assessed as potential confounders: age, gender, current smoking, diabetes mellitus, fasting serum glucose level and skin diseases (eczema, boils or other infections). Diabetes mellitus was defined as the use of glucose-lowering medication or a fasting serum glucose level of equal to or greater than 7.0 mmol/L (126 mg/dl).[28] Impaired fasting glucose was defined as a fasting serum glucose level equal to or higher than 6.1 mmol/L (110mg/dl) but lower than 7.0 mmol/L (126 mg/dl).

Statistical Analysis

Kaplan-Meier curves and the log-rank test were used to evaluate the difference in survival between the carrier states. We used a Cox proportional hazards model to assess the effect of *S. aureus* nasal carriage on mortality.[29] Antibiotic use was evaluated as the number of antibiotic prescriptions using Poisson regression. In these analyses follow-up ended either at time of death, or end of the study period. The non-or- intermittent *S. aureus* nasal carrier state was the reference category in all regression models. Gender, age, smoking habit, a history of eczema and fasting blood glucose level were included in all regression models to correct for potential confounding, since these were recently shown to be independent determinants of *S. aureus* nasal carriage. Other variables were additionally included to assess their prognostic contribution. Some of these variables had missing data, which never exceeded 2.5% of participants. To enable inclusion of these individuals in analyses, we created a missing value indicator when applicable. Results are reported as hazard ratios with 95 percent confidence intervals. Two-sided P values of less than 0.05 were considered to indicate statistical significance.

Results

Table 1 shows baseline characteristics for persistent *S. aureus* nasal carriers and non-or-intermittent carriers. The majority of persons enrolled in the study was living independently, together with a partner. More than 80% did not currently smoke, while 34% had never smoked. The prevalence of diabetes mellitus was 12% and 43% of the persons had experienced one or more skin diseases prior to the study.

Mortality

Total duration of follow-up included 18,359 person-years. Five-hundred and sixteen participants died during follow-up (13.4%): 98 (14.5%) in the persistent carrier group and 418 (13.2%) in the non-or-intermittent carrier group. The Kaplan-Meier calculated mean survival in the persistent carrier group was 6.08 years versus 6.11 years in the non-or-intermittent carrier group (*P* =

0.48; log-rank test). Through Cox proportional hazards analyses only male gender, age, current smoking, fasting glucose level, and diastolic blood pressure were identified as significantly contributing to mortality in this elderly population (Table 2). Persistent *S. aureus* nasal carriage was not associated with mortality (HR 1.03; 95% CI, 0.82-1.30, from a Cox model containing all above variables).
A secondary analysis was performed within gender and smoking habit subgroups. However, also in these analyses persistent *S. aureus* nasal carriage was not associated with mortality.

Antibiotic use

Of a total of 3,851 participants in this study, 2,510 (65%) were prescribed at least one course of antibiotics. Follow-up for antibiotic use analysis ended at death or end of the medication registration period (October 1st 2003). The antibiotic use follow-up included 17,717 person-years. Poisson regression analysis demonstrated persistent *S. aureus* nasal carriage, female gender, age, a history of skin infections or eczema and current smoking to be significantly associated with antibiotic use in this elderly population (Table 2).
As with the mortality analysis, a secondary analysis was performed within gender and smoking habit subgroups. No gender differences were found. However, within smoking habit subgroups persistent *S. aureus* nasal carriage was only significantly associated with increased antibiotic use in the past-or-never smokers group (IRR, 1.11; 95%, 1.05-1.17) and not anymore in the current smokers group (IRR, 0.99; 95% CI, 0.88-1.13).

Discussion

In our large prospective population-based study among elderly persons we found no relation between persistent *S. aureus* nasal carriage and all-cause mortality. However, persistent *S. aureus* nasal carriage was associated with a slight, but significant increase in antibiotic use. When analyzing subgroups, the effect of persistent *S. aureus* nasal carriage was only substantiated in the past-or-never smokers. No effect of persistent carriage on antibiotic use was seen in current smokers.

Table 1 *Baseline Characteristics of Persistent S. aureus Nasal Carriers and the Total Study Sample.**

Characteristic	Persistent *S. aureus* Nasal Carriers (N=678)	Total Participants (N=3,851)	Participants with Missing Values
Gender, *n*			0 (0)
Men	333 (20.5)	1,627	
Women	345 (15.5)	2,224	
Age, *y*	71.9 ± 6.8	72.3 ± 6.8	0 (0)
Subgroups, *n*			
61-64 years	118 (19.4)	608	
65-74 years	343 (17.5)	1,962	
75-84 years	189 (17.0)	1,113	
≥ 85 years	28 (16.7)	168	
Living with partner, *n*			20 (0.5)
Yes	481 (18.3)	2,624	
No	193 (16.0)	1207	
Institutionalized, *n*			20 (0.5)
Yes	64 (14.6)	438	
No	610 (18.0)	3,393	
Smoking, *n*			24 (0.6)
Current	96 (14.3)	670	
Past	348 (18.7)	1,865	
Never	230 (17.8)	1,292	
Diabetes mellitus, *n*			83 (2.2)
Diabetes mellitus	99 (22.2)	446	
Impaired fasting glucose	110 (18.9)	582	
Normal fasting glucose	459 (16.8)	2,740	
Fasting Serum Glucose, *mmol/L*	6.1 ± 1.80	5.9 ± 1.52	92 (2.4)
Eczema, *n*			49 (1.3)
Yes	100 (27.5)	364	
No	567 (16.5)	3,438	
Boils, *n*			36 (0.9)
Yes	254 (20.0)	1,270	
No	419 (16.5)	2,545	
Skin infections other than boils, *n*			27 (0.7)
Yes	62 (22.9)	271	
No	611 (17.2)	3,553	

Table 2 *Antibiotic Use Incidence Rate Ratios (Poisson Regression) and Mortality Hazard Ratios (Cox Regression) including S. aureus nasal carrier state and the significant predicting factors and/or potential confounders.*

Variable	Participants Antibiotic Prescriptions, *n*	Incidence Rate Ratio (95% CI)
Antibiotic Use	2510	
Female Gender		1.29 (1.23-1.34)
Age (per 10 years increase)		1.10 (1.07-1.13)
Current Smoking		1.17 (1.11-1.24)
History of Skin Infections		1.20 (1.04-1.40)
History of Eczema		1.10 (1.03-1.17)
Persistent *S. aureus* nasal carriage		1.09 (1.03-1.14)
	Participants who Died, *n*	**Hazard Ratio (95% CI)**
Mortality	516	
Male Gender		1.88 (1.54-2.30)
Age (per 10 years increase)		2.86 (2.52-3.24)
Current Smoking		1.75 (1.33-2.29)
Fasting Serum Glucose Level (per 1 mmol/L increase)		1.09 (1.04-1.14)
Diastolic Blood Pressure (per 10 mm Hg increase)		1.01 (1.01-1.02)
History of Eczema		0.85 (0.61-1.19)
Persistent *S. aureus* nasal carriage		1.03 (0.82-1.30)

Earlier retrospective cohort or case-control studies have demonstrated increasing age, male gender, alcoholism, lung disease, cancer, diabetes mellitus and end stage renal failure and dialysis to be risk factors for community-acquired *S. aureus* infections necessitating hospital admission.[6,30-32] These factors have also been identified earlier as determinants of *S. aureus* nasal carriage in case-control or cross-sectional studies.[12] As reported before, we found male gender, age, smoking, higher fasting glucose levels and higher diastolic blood pressure to be independent predictors of mortality. Earlier we demonstrated that male gender and higher fasting glucose levels were positively, and increasing age and current smoking negatively associated with persistent *S. aureus* nasal carriage[22] indicating that these factors should indeed be considered as confounders in the present analyses.
Next to persistent *S. aureus* nasal carriage, female gender, increasing age, smoking and skin infections were shown to be independent predictors of (any) antibiotic use. This finding was anticipated since women are known to have a higher risk of urinary tract infections,[33-35] smoking is associated with higher risk of upper and lower respiratory tract infections[36-38] and persons with a history of skin infections are likely to have a higher rate of skin infections.
In the present cohort, males are significantly more often current smokers (21.6% versus 14.5%), and report boils (58.6% versus 41.4%) and other skin infections (8.2% versus 6.2%) significantly more often than women. These are all factors associated with antibiotic use as well. The findings from the subgroup analyses suggests that the impact of persistent *S. aureus* nasal carriage on antibiotic use is higher in past-or-never smokers than in current smokers.

The major limitation of our study is that we studied all-cause mortality and overall antibiotic use and not infection-related mortality or specific anti-staphylococcal antibiotic prescriptions. It remains possible that persistent *S. aureus* nasal carriage is associated with mortality from *S. aureus* infections. However, since persistent carriage did not significantly attribute to the all-cause mortality, the absolute assignable risk for *S. aureus* nasal carriage-related mortality due to infection will, if any, be low. Also, the vast majority of antibiotics prescribed in the community are for urinary tract and respiratory tract infections,

and not for the major community-acquired *S. aureus* infections, mostly skin infections. Therefore, in the community, the relative contribution of *S. aureus* infections may be too small to demonstrate an large effect on overall antibiotic consumption, although a slight effect was demonstrated.

From this large population-based prospective cohort study we conclude that in the community setting, the healthy elderly persistent *S. aureus* nasal carrier does not die earlier, but is prescribed antibiotics more often than his or her non-or-intermittent carrier counterpart.

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9

Persistent (Not Intermittent) Nasal Carriage of *Staphylococcus aureus* is Thé Determinant of CPD-related Infections

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Abstract

Context *S. aureus* nasal carriage has been demonstrated to be a major risk factor for *S. aureus* infections in patients on continuous peritoneal dialysis (CPD).

Objective To study the impact of the different staphylococcal carriage patterns on the incidence of infections among CPD patients.

Design Retro- and prospective cohort study.

Setting Tertiary care university hospital.

Participants Patients were screened for *Staphylococcus aureus* carriage and categorized as persistent, intermittent or non *S. aureus* nasal carriers. Patients were subsequently recultured every 12 weeks for *S. aureus* and coagulase negative staphylococcal (CoNS) carriage and followed-up for CPD related infections and antibiotic resistance.

Outcome Measures Incidence of CPD-related infections, antibiotic usage and antibiotic resistance.

Results Fifty-two patients were included: 20 peristent, 10 intermittent and 22 non *S. aureus* carriers. Only persistent *S. aureus* carriage was significantly associated with an increased risk for all CPD related infections (incidence rate ratio (IRR) 3.52 [95% CI: 2.56-4.85]), exit site infections (IRR 5.59 [95% CI: 3.50-8.92]) and peritonitis (IRR 2.19 [95% CI: 1.39-3.45]), as well as increased antibiotic use (IRR 3.43 [95% CI: 2.50-4.72]), including vancomycin (IRR 2.15 [95%: 2.13-2.16]). No vancomycin resistant *S. aureus* strains were detected. However, 8 (2%) out of 407 CoNS strains isolated were vancomycin intermediately susceptible. In all 5 patients (4 persistent and 1 intermittent carriers) concerned this was significantly related to a higher antibiotic (including vancomycin) usage (IRR 2.65 [95% CI: 1.82-3.84]).

Conclusions Persistent - but not intermittent - *S. aureus* nasal carriage is thé major determinant of CPD related infections and associated with a significantly higher consumption of antibiotics, including vancomycin. The highly diverse population of CoNS appears to be the prime reservoir of staphylococcal vancomycin resistance. Accurate determination of the *S. aureus* nasal carriage state of CPD patients is essential to better target intervention strategies to prevent CPD related infections.

Introduction

Continuous peritoneal dialysis (CPD) is a commonly used technique in patients with chronic renal failure. CPD-catheter related infections (including peritonitis, exit-site and tunnel infections) are frequently encountered complications and result in significant morbidity, mortality and costs. The majority of these CPD related infections are caused by *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS).
S. aureus nasal carriage is a major risk factor for the development of both community-acquired and nosocomial infections, including CPD related infections.[1-5] The anterior nares are the primary reservoir of *S. aureus* in humans.[6-8] The prevalence of *S. aureus* nasal carriage has been estimated to be 50% in CPD patients,[9,10] while cross-sectional surveys of healthy adult populations have reported carriage rates between 20 and 55%.[11-13] From longitudinal studies it is known that *S. aureus* nasal carriage patterns differ between individuals, and that 10 to 35% of individuals carry *S. aureus* persistently (hence called persistent *S. aureus* nasal carriers), 20 to 75% carry *S. aureus* intermittently (intermittent carriers), and 5 to 50% never carry *S. aureus* in their nose (non-carriers).[14-18] However, data on the true status of *S. aureus* carriage based on longitudinal studies in patients on CPD are lacking.

Since *S. aureus* nasal carriage has been demonstrated to be a major risk factor for *S. aureus* infections in CPD patients,[4,19-25] several intervention studies have adopted different strategies including vaccination[26,27] and eradication of *S. aureus* using antimicrobial prophylaxis .[10,28-32] These latter studies consistently demonstrated a significant reduction in the incidence of exit site infections, but not a consistent reduction in the incidence of CPD related peritonitis. Mupirocin is the antibiotic agent commonly used in these intervention studies. However, due to its popularity, resistance to mupirocin is on the rise, compromising its usefulness as an intervention tool in the prevention of *S. aureus* infections.[33] Also, the cost-effectiveness of applying mupirocin in the CPD setting has been questioned.[34] As a consequence new intervention strategies need to be developed.
One of these strategies would be the targeting of prophylactic

antibiotics to high-risk patient groups only, instead of the whole (CPD) population. In the case of *S. aureus* nasal carriage there still is no consensus on how to define the *S. aureus* carrier states.[35] Precise determination of the *S. aureus* nasal carriage state is relevant, since earlier studies have shown the mean number of colony forming units (CFU) of *S. aureus* isolated from the anterior nares to be higher in persistent carriers than in intermittent carriers,[36,37] resulting in more extensive dispersal of the staphylococci in the environment[38] and in an increased risk of *S. aureus* infections.[39-41]

Even less is known about the long term molecular epidemiology of CoNS in CPD patients, with respect to CPD related infections caused by CoNS.[42-44] Furthermore, the long term use of glycopeptides as first line antibiotics in CPD related infections has been related to the development of glycopeptide resistance in staphylococci (mainly CoNS). Since in our medical center vancomycin treatment is part of the empiric antibiotic treatment of CPD related peritonitis, it could be questioned if short term vancomycin treatment is also a risk factor for the development of glycopeptide resistance in staphylococci.

The main objectives of the present study, therefore, were to provide more insight in the long-term molecular epidemiology of *S. aureus* and coagulase negative staphylococci (CoNS) carriage in CPD patients and to determine the value of differentiating between persistent carriage, intermittent carriage and non-carriage in predicting the subsequent risk of CPD related infections. In addition, we determined the impact of empiric use of vancomycin in CPD related peritonitis on the risk of vancomycin resistance in *S. aureus* and CoNS.

Material & Methods

Patients

The Erasmus University Medical Center Rotterdam (Erasmus MC) is a 1,250 bed tertiary care university hospital with approximately 60 adult patients in its CPD program. Table 1 sum marizes the definitions used in the present study for the different CPD related infections. The empiric antimicrobial therapy for CPD related peritonitis consisted of ceftazidime (loading dose of

500mg/L intraperitoneally, followed by 125 mg/L CPD dialysate qid) plus vancomycin (2 grams intraperitoneally once every 5-7 days). Antimicrobial therapy was adjusted according to culture results and given for at least two weeks. Exit and tunnel infections were treated according to (routine) culture results.
From January 1998 onwards all current and new patients on CPD were screened for S*. aureus* carriage with 6 consecutive nasal swab cultures (vestibulum nasi) taken at one week intervals. *S. aureus s*creening cultures of the CPD catheter exit site were performed together with the first and last nasal swab culture. Based on these culture results patients were divided into three categories (non carriers, intermittent carriers and persistent carriers) according to the definitions in table 2.
After this initial screening period, nasal swab cultures, cultures of CPD catheter exit site and cultures of contralateral (i.e. opposite to the catheter exit site) abdominal skin were taken every 12 weeks during follow-up visits to identify colonization with *S. aureus,* CoNS species and/or other microorganisms. Cultures from other sites (CPD dialysis fluid, wounds, blood, etc.) were performed on indication only. Patients were followed-up for an overall period of four years until December 31st 2001 or until the date of ending CPD treatment, whichever date came earlier.
From January 1st 1998 until December 31st 2001, data on antibiotic use and (CPD related) infections were collected prospectively. Data on the molecular epidemiology of CoNS and vancomycin resistance of the staphylococci isolated were collected prospectively between January 1st 1998 and December 31st 2000. Data on antibiotic use and (CPD related) infections before January 1998 were collected retrospectively by chart review.
Written informed consent was obtained from all patients included. This study was approved by our institutional Medical Ethics Review Committee *(*METC 165.585/1997/164).

Microbiological procedures

Phenotyping

Cultures from abdominal skin, catheter exit site, nose (vestibulum nasi), blood and CPD dialysis fluid were performed according to standard procedures.[45] Blood and CPD dialysis fluid were cultured using the BACTEC 9240 system (Becton-Dickinson Diagnostic Instrument Systems, Sparks, Md., USA). In the case of CPD catheter removal, the tip was cultured in thioglycolate broth (Brewer modified). MicroScan WalkAway-96

Table 1 *Definitions of CPD related infections.*

Peritonitis	Peritonitis was diagnosed if at least two of the following three criteria were met: ≡ turbid peritoneal effluent with more then 100 leukocytes/mm, of which more then 50% were neutrophilic granulocytes; ≡ abdominal pain or tenderness; ≡ micro-organisms cultured from the peritoneal effluent. Peritonitis was considered relapsing when it was caused by the same micro-organism as in the preceding episode, within four weeks after discontinuing antimicrobial therapy.
Exit-site infection	Exit site infection (including tunnel- and cuff infection) was defined as the presence of redness, pain, swelling and/or induration of the catheter exit site and/or of the overlying tissues, with or without purulent discharge or abscess formation, together with a positive exit site culture.
Bacterial colonization	Bacterial colonization was defined as micro-organisms present on the skin or mucosal surfaces without local signs or symptoms of infection.

Table 2 *Definitions of the different S. aureus carrier states.*

Non-carriers	Non carriers had zero out of 6 nasal screening cultures positive for *S. aureus*.
Persistent carriers	Persistent carriers had either 6 out of 6 nasal screening cultures positive for *S. aureus*, or 4 or 5 out of six nasal screening cultures positive for *S. aureus* and at least one of the exit site cultures positive for *S. aureus* at screening. Genotyping showed all these *S. aureus* isolates to be identical.
Intermittent carriers	Intermittent carriers had either less than 4 out of six nasal screening cultures positive for *S. aureus*, or 4 or 5 out of six nasal screening cultures positive for *S. aureus* but no positive exit site culture or genotypically non-identical *S. aureus* strains.

(Dade International Inc., West Sacramento, CA, USA**)** and Vitek equipment **(**bioMérieux Vitek, Hazelwood, Mo, USA**)** were used for the identification of microorganisms.
Swabs from nose, abdominal skin and catheter exit site were also cultured quantitatively on phenol-red mannitol salt agar (PHMA) and in phenol red mannitol salt broth (PHMB) for *S. aureus* according to a standard operating procedure as described earlier.[35,37] Culture results were recorded as 0 (no *S. aureus*), 1 (*S. aureus* only from PHMB), 2 (2-9 CFU), 3 (10-99 CFU), 4 (100-999 CFU), or 5 (≥ 1000 CFU) (CFU= colony forming units). Identification of *S. aureus* was based upon colony morphology on the PHMA. Suspected colonies were cultured overnight on Columbia blood agar plates (Becton-Dickinson B.V., Etten-Leur, The Netherlands). A catalase test and a latex agglutination test (Staphaurex Plus^{R}, Murex, Dartford, UK) were then performed on all staphylococcal isolates. The ID32Staph test (bioMérieux, Lyon, France) was used for further speciation of the CoNS. All staphylococcal isolates were stored at -70 ^{0}C in glycerol containing liquid media.

Susceptibility testing

MicroScan WalkAway-96 and Vitek equipment were also used for susceptibility testing. Based on the NCCLS breakpoints, strains were categorized as resistant (R), intermediately sensitive (I), or sensitive (S) to the antibiotic used.[45-47] Further MIC determinations were done using E-test (AB Biodisk, Solna, Sweden). Methicillin susceptibility of staphylococci was tested using the disc diffusion method. Testing for vancomycin intermediate sensitivity (VISA or VISE) or vancomycin resistance (VRSA or VRSE) was done according to the method described by Hiramatsu.[48]

Genotyping (analysis of genetic relatedness)

Pulsed-field gel electrophoresis (PFGE) was performed based on protocols as previously described.[49] Bacteria were embedded in a agarose plugs and treated with lysostaphin (Sigma Aldrich, Germany), proteinase K (Merck, Germany) and the restriction enzyme *Sma*I (Boehringer Mannheim, Mannheim, Germany) prior to electrophoresis. The electrophoresis program consisted of two blocks (10 hrs 5-15 seconds switch time followed by 10 hrs 15-45 seconds switch time) and was performed at 14^{0}C and 6 V/cm. Gels were photographed after ethidium bromide staining and banding patterns were interpreted according to Tenover et al.[50]

Statistical procedures

Percentages were compared by the Chi-square test (Fisher's

exact test in case of 2x2 tables). To compare the number of CPD-related infections and number of antibiotic courses (including vancomycin) used during follow-up between the different *S. aureus* carrier states, Poisson regression analysis was employed. The influence of potential prognostic factors on the incidence of CPD related infections and antibiotic usage was evaluated using Poisson regression as well. Kaplan-Meier curves and the log-rank test were used to evaluate differences in survival between the carrier states. We used a Cox proportional hazards model to assess the effect of *S. aureus* nasal carriage on mortality.[51] In these analyses follow-up ended either at death, date of renal transplantation or switch to intermittent hemodialysis, or end of the study period. The non-or- intermittent *S. aureus* nasal carrier state was the reference category in all multiple regression models. Sex and age were included in all multiple regression models to correct for potential confounding.
All statistical tests were two tailed and performed at the 0.05 significance level. SAS statistical software (The SAS system for Windows, release 8.0; SAS Institute inc., Cary, NY, USA) was used for Poisson regression analyses. All other statistical tests were done using SPSS statistical software (SPSS for Windows, version 12.0.1; SPSS inc., Chigago, IL, USA).

Results

Demographics

Fifty-two patients were included in the study. Demographic data and prior history before start of the current CPD episode are summarized in table 3. Nearly twice as many males as compared to females participated (33 vs. 19).

S. aureus nasal carriage patterns

The distribution of the different *S. aureus* nasal carriage groups, based on six weekly nasal swab cultures, is shown in table 4. There were 22 non-, 10 intermittent and 20 persistent carriers. No difference in distribution between males and females was found. Of the intermittent carriers, 9 had only one nasal screening culture positive and one had 3 cultures positive but with two genotypically different strains and with negative exit site cultures. Of the 20 persistent carriers 13 had 6 out of 6 nasal screening cultures positive. All of these 13 persons had positive

Table 3 *Characteristics of the CPD population at inclusion.*

Participants (n)	Males	33
	Females	19
Age (median-range)	Males	50.0 (22.6-76.9)
	Females	47.4 (19.0-70.4)
Cause of end stage renal disease (n)	Vascular disease	11
	Diabetes Mellitus	9
	Glomerulonephritis	12
	Urinary tract infections	10
	TTP*/HUS†	2
	Other	8
Previous kidney transplantation(s) (n)	No	38
	1	8
	2	4
	3	2
Previous chronic intermittent hemodialysis (n)	No	36
	Yes	16
Number of CPD catheters (n)	1st	42
	2nd	8
	3rd	1
	4th	1
Diabetes Mellitus (n)	No	37
	Insulin dependent	10
	Non-insulin dependent	5
Immunosuppressive medication (n)	No	47
	Corticosteroids	3
	Other	2

* TTP = thrombotic thrombocytopenic purpura; †HUS = hemolytic uraemic syndrome

exit site cultures on at least one occasion and all strains isolated at screening were genotypically identical. The other 7 persistent carriers had 4-5 positive nasal screening cultures. In all of these patients, also, exit site cultures were positive on at least one occasion and all strains isolated at screening were genotypically identical.

Neither the cause of end stage renal disease, a history of renal transplantation, a history of intermittent hemodialysis, the presence of diabetes mellitus, nor the use of corticosteroids or other immunosuppressive drugs were significantly associated with *S. aureus* nasal carriage.

Follow-up

Total duration of follow-up was 2,260 months, with a median of 33.6 months (mean 43.5; range 1.6-204). There was no difference in follow-up between the three *S. aureus* nasal carriage groups (table 5). Eighteen patients were still on CPD at the end of the study. In 34 patients CPD therapy was stopped during the study (16 non-, 5 intermittent and 13 persistent carriers; *P*=ns). Fifteen patients received a kidney transplantation (5 non-, 3 intermittent and 7 persistent carriers; *P*=ns). In 6 patients persisting or relapsing CPD peritonitis necessitated removal of the CPD catheter (3 non- and 3 persistent carriers; *P*=ns): 3 were caused by *Candida albicans*, 1 caused by *C. albicans* in combination with *Escherichia coli* and 2 caused by a perforation of the colon.

Thirteen patients died during follow-up (8 non-, 2 intermittent and 3 persistent carriers; *P*=ns). Three patients died of CPD related infections (1 caused by *C. glabrata* in combination with *Enterobacter cloacae,* 1 caused by *S. aureus* and 1 by a perforation of the colon), 2 died of non-CPD related infections (1 of fasciitis necroticans and 1 of pneumonia) and 8 died of non-infectious causes (atherosclerosis related cardiac and neurological events). No CPD catheter had to be removed because of (recurrent) exit-site or tunnel infections. Persistent *S. aureus* nasal carriage was not associated with mortality (hazard ratio1.276 [95% confidence interval (CI): 0.658-2.475]).

CPD related infections

CPD related infections occurred in all three carriage groups and are summarized in table 5. As compared to non- and intermittent carriers, persistent carriers patients had significantly higher incidences of all-cause CPD related infections (overall 0.13 /month [P<0.001], exit site infections 0.09/month [P<0.001], peritonitis 0.05/month [P=0.019]) and S. aureus CPD related

infections (overall 0.08 /month [P<0.001], exit site infections 0.07/month [P<0.001], peritonitis 0.01/month [P=0.026]; Table 5). Intermittent carriers behaved like non-carriers as their incidence rates for CPD related infections were similar (Table 5).

To calculate incidence rate ratio's, the non- and intermittent carrier groups were merged and compared with the persistent carrier group. Age, sex, cause of end stage renal disease, history of intermittent hemodialysis and use of corticosteroids or other immunosuppressive drugs were not associated with any category of CPD related infections in Poisson regressions analysis. Only persistent S. aureus nasal carriage and the presence of diabetes mellitus were found to be significantly associated with CPD related infections. Persistent carriers had a more than 3-fold higher risk of all-cause CPD related infections (incidence rate ratio (IRR) 3.52 [95% CI: 2.56-4.85]) and a more than 9-fold higher risk of S. aureus CPD infections (IRR 9.54 [95% CI: 5.25-17.33]) compared to non-or-intermittent carriers. The IRR for exit site infections, S. aureus exit site infections, all peritonitis, S. aureus peritonitis and CoNS peritonitis were 5.59 [95% CI: 3.50-8.92], 10.75 [95% CI: 5.45-21.19], 2.19 [95% CI: 1.39-3.45], 6.54 [95% CI: 1.83-23.31] and 3.37 [95% CI: 1.45-7.83], respectively (Table 5).

Patients with diabetes mellitus were at a 2-fold higher risk of all-cause CPD related infections (IRR 1.99 [95% CI: 1.44-2.75]), S. aureus CPD infections (IRR 2.02 [95% CI: 1.28-3.18]), all-cause exit site infections (IRR 2.33 [95% CI: 1.53-3.55]) and S. aureus exit site infections (IRR 2.03 [95% CI: 1.23-3.34]). However, no significant association between diabetes mellitus and peritonitis was found.

Antibiotic usage

Antibiotic usage also differed significantly between the persistent carrier group and the combined non-or-intermittent carrier group: the number of antibiotic courses per month for all infections was 0.13 [95% CI: 0.11-0.16] in the persistent carrier group versus 0.04 [95% CI: 0.03-0.07] in the non-or-intermittent carrier group (IRR 3.43 [95% CI: 2.50-4.72]; *P*<0.001). Also, vancomycin consumption was higher in persistent carriers compared to non-or-intermittent carriers: 203 milligrams of vancomycin per month [95% CI: 202-204] in persistent carriers versus 103 milligrams [95% CI: 102-104] in non-or-intermittent carriers (IRR 2.15 [95%: 2.13-2.16]; *P*<0.001). Diabetes mellitus was significantly associated with higher antibiotic (including

Table 4 *S. aureus carriage patterns of CPD patients on screening.*

	non	intermittent	persistent	Total
nasal carriage (n)				
male	13 (39%)	6 (18%)	14 (42%)	33 (100%)
female	9 (47%)	4 (21%)	6 (32%)	19 (100%)
Total	22 (42%)	10 (19%)	20 (39%)	52 (100%)

vancomycin) consumption as well. Diabetics used nearly twice as much antibiotics (IRR 1.95 [95% CI: 1.41-2.54], while vancomycin use was increased with 36% (IRR 1.36 [95% CI: 1.35-1.37] compared to patients without diabetes mellitus.

Molecular epidemiology of staphylococcal isolates

In this study a total of 407 different *S. aureus* isolates were cultured, consisting of 44 genotypically (PFGE) distinct strains. During follow-up 16 of the 20 persistent carriers remained persistently colonized with the same resident S*. aureus* strain as found during the initial screening phase. In four persistent carriers there was a change in colonizing *S. aureus* strain during follow-up. These changes were all related to antecedent anti-staphylococcal antibiotic treatment for *S. aureus* exit-site infection or peritonitis. In 2 of these patients exit site and nasal *S. aureus* isolates were different by PFGE. Except for one case of *S. aureus* peritonitis, all 78 *S. aureus* infections in the persistent carriers were of endogenous origin, i.e. with the same genotype strain as had been found on screening. Four genotypically identical *S. aureus* strains were isolated from 2 patients each, while all other strains were distinct genotypes.
Only one intermittent carrier patient developed *S. aureus* infections: two exit site infections with genotypically non-identical strains. Six non-carriers suffered from *S. aureus* infections (9 exit site infections and 3 peritonitis episodes) during follow-up. Two of them turned into a persistent carrier,

becoming colonized at the CPD catheter exit-site as well as in their nose with an identical *S. aureus* strain in each case, causing their *S. aureus* infections. Two non-carriers became intermittently colonized at the exit-site, but their nasal swab cultures remained negative. The two remaining non-carriers with *S. aureus* infections had nasal swab and exit-site cultures that were always negative for *S. aureus.*

All 52 patients had one or more cultures positive for coagulase-negative staphylococci (CoNS). A total of 407 different CoNS isolates were cultured, consisting of 189 genotypically (PFGE) distinct strains. Per patient a median of 5 genotypically distinct CoNS were isolated from the nose, exit-site and/or abdominal skin. Results varied widely per culture and per culture site. Moreover, CoNS strains involved in CPD peritonitis were different from those found in the screening and follow-up cultures within patients. Thus, no CoNS carriage 'pattern' could be detected in individual patients. The source of CoNS strains causing infections was not the patients' microflora on the abdominal skin or vestibulum nasi.

Antibiotic resistance

All 407 *S. aureus* strains isolated were methicillin sensitive *S. aureus* (MSSA), no methicillin resistant *S. aureus* (MRSA) was cultured in this period in this patient group.
A total of 135 *S. aureus* isolates were tested for vancomycin (intermediate) resistance. These included all 92 isolates associated with CPD related infections and the 43 genotypically distinct 'colonizing' strains. None of these *S. aureus* isolates were vancomycin intermediately susceptible or resistant.
In contrast to the *S. aureus* strains, methicillin resistance was demonstrated in 280 (69%) and intermediate vancomycin susceptibility (MIC 8 μg/L) was detected in 8 (2%) out of all 407 CoNS strains tested. The 8 vancomycin intermediately susceptible CoNS strains were all genotypically distinct *S. epidermidis* strains and were isolated from 5 different patients. In one person 3 distinct strains were isolated on different time points, in one person 2 distinct strains were isolated on different time points and in 3 persons each one distinct strain was isolated only once. None of these 8 vancomycin intermediately susceptible CoNS strains were implicated in (CPD related) infection. Four of the 5 patients from which a vancomycin intermediately susceptible CoNS strain was isolated were persistent carriers, one patient was an intermittent carrier

(*P*=0.066 when comparing persistent carriage with non-carriage/intermittent carriage).
Antibiotic usage was significantly higher in patients with a vancomycin intermediately susceptible CoNS strain than in patients without: 0.19 (95% CI: 0.14.-0.27) versus 0.07 (95% CI: 0.04-0.15) antibiotic courses per month for all causes (IRR 2.65 [95% CI: 1.82-3.84]; *P*<0.001). Also, the presence of a vancomycin intermediately susceptible CoNS strain was significantly associated with increased vancomycin consumption: 390 (95% CI: 388-393) versus 123 (95% CI: 121-125) milligrams of vancomycin per month (IRR 3.17 [95%: 3.15-3.20]; *P*<0.001). When adjusting for age, sex, diabetes mellitus and *S. aureus* nasal carrier state, carrying a vancomycin intermediately susceptible CoNS strain was still significantly associated with antibiotic usage (IRR 2.07 [95% CI: 1.37-3.12]) and vancomycin consumption (IRR 2.53 [95% CI:2.51-2.55]).

Discussion

In the study presented here 52 patients on CPD were followed-up closely for a total of 2,260 months. More than 2,000 cultures were performed, resulting in the isolation of 407 *S. aureus* and 407 CoNS strains. All 814 staphylococcal isolates were genotyped using PFGE and all CoNS plus 135 *S. aureus* strains were tested for vancomycin resistance according to Hiramatsu.[48] This is the first large scale study to combine the careful assessment of *S. aureus* and CoNS carriage patterns and dynamics with their clinical impact on CPD related morbidity and mortality.

S. aureus nasal carriage has been found to be a major risk factor for infections in CPD patients before, mainly associated with exit infections but not CPD peritonitis and the incidence rates found in this study are comparable with these earlier studies.[4,19-25] Not surprisingly, intervention studies consistently demonstrated a significant reduction in the incidence of exit site infections, but not a consistent reduction in the incidence of CPD related peritonitis.[10,28-32] These studies have led to the widespread use of antimicrobial prophylaxis, mainly with mupirocin, in CPD

patients to prevent *S. aureus* infections, often irrespective of the individual carrier state.

However, in these studies only one or two nasal swab or exit site cultures were used to determine the *S. aureus* carrier state (carrier versus non-carrier) and genotyping techniques were not routinely applied. Given the lack of accepted definitions for the different *S. aureus* [35] and given the fact that precise determination of the *S. aureus* nasal carriage state seems relevant,[36-41] we earlier derived and validated a culture rule to adequately determine the true carrier state with the least effort.[37] In healthy individuals it is possible to predict the carrier state with only two nasal swab cultures. Since CPD patients are treated with antibiotics regularly, we anticipated that two cultures would not be enough, and we extended our screening to six nasal swab cultures together with genotyping all strains isolated. Here we demonstrate that differentiation between the non-carrier, intermittent carrier and persistent carrier state in CPD patients is feasible and of clinical importance. Persistent carriage, but not intermittent carriage, is thé major determinant of CPD related infections, including CPD peritonitis of all microbial causes and CPD peritonitis by CoNS. Persistent carriage is also associated with significantly higher antibiotic consumption, including vancomycin. Targeting interventions to prevent CPD related infections is thus possible, thereby eliminating unnecessary prophylactic and therapeutic antibiotic use and resistance development.[33] Intermittent carriers behave like non-carriers as their risk for CPD related infections is equally low (0.04 infections per patientmonths at risk). In our opinion, therefore, neither intermittent nor non-carriers should be offered antibiotic prophylaxis for the prevention of *S. aureus* CPD related infections. In contrast, prophylaxis should be reserved for patients shown to be persistent carriers, since only they have a high rate of CPD related infections (0.13 per patientmonths at risk).

Two out of 22 non-carriers became persistent carriers, while 2 others converted to the intermittent carrier state during follow-up. Thus, in a proportion of CPD patients, colonization with *S. aureus* is a dynamic process. Consequently, screening for *S. aureus* carriage should not be limited to the start of CPD treatment, but regular (e.g. once a year) determination of the current carrier state should be performed and action taken accordingly.

Eighty percent of persistent carriers were persistently colonized with their own resident *S. aureus* strain during follow-up, which is in concordance with earlier studies.[35,52] Anti-staphylococcal antibiotic treatment for *S. aureus* exit-site infection or peritonitis induced a change in *S. aureus* genotype. As reported earlier, nearly all *S. aureus* infections in persistent carriers were of endogenous origin.[3,53] In total 44 distinct *S. aureus* strains were identified. In contrast to other *S. aureus* infections such as impetigo, no clonal spread of strains was observed.[54] No significant cross-contamination between patients occurred, since only 4 genotypically identical *S. aureus* strains were isolated from 2 patients each, while all other strains were patient unique. Whether *S. aureus* strains causing CPD related infections harbour specific virulence factors necessary for successful colonization and infection, as was reported in other clinical settings,[54-56] is currently not known, but will need to be the subject of future research.

The molecular epidemiology of CoNS proved to be extremely complex and dynamic. CoNS were cultured in all 52 patients, but the great variety and variability of strains within individual patients and the lack of concordance between colonizing and infecting strains, made it impossible to establish a CoNS carriage 'pattern'. In contrast to other settings, no clonal spread was observed.[57] Thus, the exact source of CoNS strains causing CPD related infections could not be determined.

No vancomycin resistance was detected in our *S. aureus* isolates, despite the fact that vancomycin intraperitoneally is part of the empiric treatment of CPD peritonitis together with intraperitoneal ceftazidime. However, antibiotic treatment is adjusted according to culture results within 72 hours. Since the prevalence of methicillin resistant *S. aureus* (MRSA) is lower than 0.1% in our hospital (including our CPD population), vancomycin is nearly always changed to flucloxacilline in the case of *S. aureus* peritonitis. Only peritonitis by CoNS has to be treated with vancomycin intraperitoneally since about 70% of the CoNS strains are methicillin resistant. Exit- and tunnel infections by *S. aureus* are treated with flucloxacilline or clindamycine orally, since there is no need for vancomycin therapy.
However, among the 407 genotypically highly diverse (189 different genotypes) CoNS strains collected, 8 (2%)

demonstrated intermediate susceptibility to vancomycin. On a strain level, this rate is comparable to the rate found in a survey of hospital wide CoNS blood culture isolates in 1991 and 1997.[58] But, more importantly, this implies that on a patient level 5 out of 52 (or 9.6%) were colonized at least once with a vancomycin intermediately susceptible CoNS strain between January 1st 1998 and December 31st 2000. In all 5 patients (4 persistent carriers and 1 intermittent carrier) concerned this was significantly related to a higher antibiotic (including vancomycin) usage as compared to the other 47 CPD patients ($P<0.001$). This implicates that even in a setting with relative low vancomycin consumption and low prevalence of MRSA, the CoNS population serves as a potential reservoir of vancomycin resistance.

In summary, we conclude that persistent, and not intermittent, nasal carriage of *S. aureus* is thé major determinant of CPD related (*S. aureus)* infections and that persistent carriage of *S. aureus* associated with a higher consumption of antibiotics, including vancomycin. Accurate determination of the *S. aureus* nasal carriage state makes it possible to better target future prophylactic strategies. Not only to prevent CPD related infections, but also to reduce antibiotic consumption and the further emergence of (vancomycin) resistance staphylococci.

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Table 5 *Infection incidence rates by S. aureus nasal carriage patterns.*

	Non		**Intermittent**		**Persistent**		**Total**				
		95% CI		95% CI		95% CI		95% CI	*P*-value*	IRR†¶	95% CI
Follow-up (months at risk)											
Mean	41.1	[28.1-54.1]	41.9	[15.3-68.6]	46.9	[26.0-67.7]	43.5	[33.3-53.7]	0.87		
Total (n)	904		419		937		2,260				
Patients in follow-up (n)	22		10		20		52				
CPD related infections (n)											
All	41		16		126		183				
Exit site infections	17		8		81		106				
Peritonitis	24		8		45		77				
CPD related infections incidence (nr/personmonths at risk)											
All	0.05	[0.03-0.08]	0.04	[0.02-0.08]	0.13	[0.11-0.16]	0.08	[0.07-0.09]	<0.001	3.52	[2.56-4.85]
Exit site infections	0.02	[0.01-0.04]	0.02	[0.01-0.05]	0.09	[0.07-0.11]	0.05	[0.04-0.06]	<0.001	5.59	[3.50-8.92]
Peritonitis	0.03	[0.01-0.06]	0.02	[0.01-0.05]	0.05	[0.04-0.06]	0.03	[0.03-0.04]	0.019	2.19	[1.39-3.45]
***S. aureus* CPD related infections** (n)											
All	12		2		78		92				
Exit site infections	9		2		65		76				
Peritonitis	3		0		13		16				
***S. aureus* CPD related infections incidence** (nr/personmonths at risk)											
All	0.01	[0.01-0.03]	0.01	[0.00-0.02]	0.08	[0.07-0.10]	0.04	[0.03-0.05]	<0.001	9.54	[5.25-17.33]
Exit site infections	0.01	[0.00-0.03]	0.01	[0.00-0.02]	0.07	[0.05-0.09]	0.03	[0.03-0.04]	<0.001	10.75	[5.45-21.19]
Peritonitis	0.00	[0.00-0.02]	0		0.01	[0.01-0.02]	0.01	[0.00-0.01]	0.026	6.54	[1.83-23.31]

* *P*-value for the univariate comparison of incidence rates in non, intermittent and persistent carriers using Poisson regression.
† IRR is incidence rate ratio.
¶ IRR comparing incidence rates in persistent carriers versus in non-or-intermittent carriers in a Poisson regression model adjusting for age, sex and diabetes mellitus.

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10

Staphylococcus aureus Carriage Patterns & the Risk of Infections in Continuous Peritoneal Dialysis

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Abstract

Context *S. aureus* nasal carriage has been demonstrated to be a major risk factor for *S. aureus* infections in patients on continuous peritoneal dialysis (CPD).

Objective To study the epidemiology and risks of *S. aureus* carriage in CPD patients.

Design Retrospective cohort study.

Setting Tertiary care referral hospital.

Participants From January 1995 until June 1999, cultures were taken routinely from CPD exit site and vestibulum nasi. All *S. aureus* isolates from these cultures as well as from cultures taken during infectious complications were genotyped (pulsed-field gel electrophoresis). Vancomycin resistance was tested in vitro. Data on CPD related infections, antibiotic use and clinical course were collected retrospectively.

Outcome Measures Incidences of CPD-related infections and antibiotic usage associated with the various *S. aureus* carriage patterns.

Results Seventy-five patients (on a total of 98) with at least one culture positive for *S. aureus* in this period were included. Fifty-three patients had more than 2 cultures positive for *S. aureus*: 43 had genotypically identical *S. aureus* strains in over 80% of cultures and were classified as persistent carriers; 10 were classified as chronic carriers since their cultures were mostly positive for *S. aureus,* but all isolates were genotypically different. Twenty-two patients were intermittent carriers. Persistent carriage was associated with a 2.91 [95% confidence interval (CI): 2.17-3.90] higher risk for all CPD related infections and 6.06 [95% CI: 5.99-6.14] higher vancomycin consumption compared to intermittent and chronic carriers. Chronic carriers and intermittent carriers had similar risks for all CPD related infections analyzed. No vancomycin resistance was detected.

Conclusions Forty-four percent of CPD patients were persistent carriers of *S. aureus,* and at a 3-fold higher risk for CPD related infections compared to intermittent and chronic carriers. Precise determination of the *S.aureus* carrier state of CPD patients is possible and needed to adequately target prevention strategies.

Introduction

Continuous peritoneal dialysis (CPD) is commonly used in patients with end stage renal failure. CPD-catheter related infections, including peritonitis, exit-site and tunnel infections are, however, major and frequent complications and the cause of significant morbidity and mortality. Most of these infections are caused by the ubiquitously present staphylococci.[1]. It is thought that these bacteria are part of the patients own endogenous bacterial flora, although most of the evidence is circumstantial. Earlier cross-sectional studies, have found *S. aureus* nasal carrier rates to be about 50% in CPD patients. The same studies demonstrated *S. aureus* nasal carriage to be a major risk factor for the development of *S. aureus* infections.[2-4] The risk for exit-site infections for example is 3-6 times higher in carriers than in non-carriers. Typing of causative strains has revealed that frequently the strain isolated from the infection site and the strain that colonizes the nose are identical.[3,5] Therefore, prophylactic interventions aiming at the elimination of nasal carriage may be effective.

In order to effectively intervene in the infectious process, additional insight in the long term epidemiology of staphylococcal carriage in the specific CPD patient group is required. In addition, the more fundamental aspects of nasal carriage of *S. aureus* in CPD patients and the effect of frequent use of glycopeptides as first line antibiotics against CPD infections needs further analysis as well. Prolonged use of these antibiotics has been associated with the development of glycopeptide resistance in staphylococci.[6,7]

Since *S. aureus* carriage has already been established as a major risk factor for the development of CPD related infections compared to non-carriers,[4] we studied the long-term epidemiology of *S. aureus* carriage within carriers only. Thus, we aimed to identify subgroups of *S. aureus* carriers in CPD patients and their associated risks for CPD related (*S. aureus*) infections. Furthermore, we wanted to investigate if glycopeptide resistance developed in *S. aureus* strains from CPD patients in a single tertiary care institution, where glycopeptides were not used as the first line antibiotics for CPD related (staphylococcal) infections.

Material & Methods

Patients

The Jeroen Bosch Hospital is a 600 bed tertiary care teaching hospital with about 50 adult patients on CPD. CPD patients were followed-up every 6 to 8 weeks. Between January 1995 and December 1998, cultures of the nose (vestibulum nasi) and CPD catheter exit site were performed routinely during follow-up visits. Cultures from other sites (CPD dialysis fluid, wounds, blood, etc.) were done on indication only. Based on these culture results patients were subsequently divided in four categories (non-carriers, intermittent carriers, chronic carriers and persistent carriers), according to the definitions stated in table 1.

The intermittent, chronic and persistent carriers were further analyzed and data on CPD related infections, antibiotic use and clinical course were collected retrospectively by chart review. CPD related infections were defined according international standards [8]. The empiric antimicrobial therapy for CPD related peritonitis consisted of cephalotin plus tobramycin intraperitoneally. Antimicrobial therapy was adjusted according to culture results and given for at least two weeks. Exit and tunnel infections were treated according to (routine) culture results.

This study was approved by our institutional medical ethics review committee (METC 165.585/1997/164).

Microbiological procedures

Phenotyping

Cultures from skin, exit site, nose (vestibulum nasi), blood and CPD dialysis fluid were performed according to standard procedures.[9] For blood and CPD dialysis fluid culturing, the BACTEC 9240 system (Becton-Dickinson Diagnostic Instrument Systems, Sparks, Md., USA) was used. After removal of the CPD catheter the tip was cultured in thioglycolate bouillon (Brewer modified medium). MicroScan WalkAway-96 (Dade International Inc., West Sacramento, CA, USA) and Vitek equipment (bioMérieux Vitek, Hazelwood, Mo, USA) were used for the identification of microorganisms. Staphylococcal isolates were identified using the catalase test, followed by a latex agglutination test (Staphaurex Plus®, GenProbe, Massachusetts, USA). The ID32Staph test (bioMérieux) was used for further

Table 1 *Definitions of the different S. aureus carrier states.*

Non-carriers	Non-carriers had zero cultures positive for *S. aureus*.
Intermittent carriers	Intermittent carriers had at least one but less than 80% of cultures positive for *S. aureus.*
Chronic carriers	Chronic carriers had more than 80% of cultures positive for *S.* aureus with genotypically different serial *S. aureus* isolates.
Persistent carriers	Persistent carriers had more than 80% of cultures positive for *S.* aureus with genotypically identical *S. aureus* isolates.

speciation of coagulase negative staphylococci (CoNS). All staphylococcal isolates were stored at -70 ^{0}C in glycerol containing liquid media.

Susceptibility testing MicroScan WalkAway-96 (Dade International Inc.) and Vitek equipment (bioMérieux) were also used for susceptibility testing. Strains were categorized as resistant (R), intermediately resistant (I), or sensitive (S) to the antibiotic used based on the NCCLS breakpoints.[10] Further MIC determinations were done using E-test (AB Biodisk, Skolna, Sweden). Methicillin susceptibility of staphylococci was tested using the disc diffusion method. All staphylococcal isolates in this study were subsequently tested for vancomycin intermediate sensitivity (VISA or VISE) or vancomycin resistance (VRSA or VRSE) according to the method as described by Hiramatsu.[11,12] This required that 10^8 bacteria were inoculated on Vancomycin Screen agar plates (Becton Dickinson, USA). These media contain 6 µg/mL of vancomycin. The plates were visually inspected for bacterial growth after 24 and 48 hrs of incubation. In the case that colonies grew on the agar, the MIC was determined by agardilution and the individual colonies showing a MIC of ≥ 8 µg/mL were inoculated onto a fresh bloodagar plate

an reinoculated every day for nine consecutive days. After this passaging procedure the MICs were determined again. Heterogeneous resistance was defined when the isolate maintained its resistance level after passaging.

Bacterial genotyping

Pulsed-field gel electrophoresis (PFGE) was subsequently performed based on protocols as previously described.[13-15] Bacteria were embedded in agarose plugs and treated with lysostaphin (Sigma-Aldrich, Münich, Germany), proteinase K (Merck, Darmstadt, Germany) and the restriction enzyme *Sma*I (Boehringer Mannheim, Mannheim, Germany) prior to electrophoresis. The electrophoresis program consisted of two blocks (10 hrs 5-15 seconds switch time followed by 10 hrs 15-45 seconds switch time) and was performed at 14^0C and 6 V/cm. Gels were photographed after ethidium bromide staining and banding patterns were interpreted according to Tenover et al.[16]

Statistical procedures

Percentages were compared by the Chi-square test (Fisher's exact test in case of 2x2 tables). To compare the number of CPD-related infections and number of antibiotic courses (including vancomycin) used during follow-up between the different *S. aureus* carrier states, Poisson regression analysis was employed. The influence of potential prognostic factors on the incidence of CPD related infections and antibiotic usage was evaluated using Poisson regression as well. Sex and age were included in all multiple regression models to correct for potential confounding. All statistical tests were two tailed and performed at the 0.05 significance level. SAS statistical software (The SAS system for Windows, release 8.0; SAS Institute inc., Cary, NY, USA) was used for Poisson regression analyses. All other statistical tests were done using SPSS statistical software (SPSS for Windows, version 12.0.1; SPSS inc., Chigago, IL, USA).

Results

Epidemiology of S. aureus carriage

A total of 98 patients were treated with CPD at the Bosch Medicenter in this 5-year period, 60 males and 38 females. In 23 (23%) patients none of the cultures performed were positive for *S. aureus*. These patients were classified as non-carriers and not followed-up any further. Seventy-five (76%) patients had at least

Table 2 *Characteristics of the CPD study population.*[$]

Participants (n)	Males	46
	Females	29
Age (median-range)	Males	49 (18-75)
	Females	53 (36-74)
Cause of end stage renal disease (n)	Vascular disease	22
	Diabetes Mellitus	18
	Glomerulonephritis	16
	Urinary tract infections	15
	TTP*/HUS[†]	2
	Other	2
Duration of end stage renal disease prior to start of study (months; median-range)		3 (0-120)
Duration of CPD prior to start of study (months; median-range)		1 (0-119)
Number of CPD catheters (n)	1st	60
	2nd	14
	4th	1
Diabetes mellitus (n)	No	49
	Insulin dependent	20
	Non-insulin dependent	6
Prior kidney transplantation (n)	No	70
	1	4
	4	1

[$] Intermittent, chronic and persistent carriers only
* TTP = thrombotic thrombocytopenic purpura;
[†] HUS = hemolytic uraemic syndrome

one culture positive for *S. aureus* and were included in this study. Total duration of follow-up in these 75 patients was 2,402 months, with a median of 27.2 months (mean 32.0; range 6.7-60). Table 2 summarizes the main characteristics of this study population.

Twenty-two patients (22%) carried *S. aureus* in their nose and/or exit site only now and then, exchanging culture-positive with culture-negative periods (Table 3). These patients were classified as intermittent carriers.

Fifty-three (54%) patients had 3 or more cultures positive for *S. aureus* on 2 or more outpatient visits. Forty-three of these patients had genotypically identical strains in all cultures during their follow-up, while in ten of them sometimes a second *S. aureus* strain could be isolated in one or more cultures. These 43 (44%) patients were classified as persistent carriers. Overall carriage rates among males and females were not significantly different (Table 3).

One of the 53 patients switched *S. aureus* strains on two occasions during his 4 year follow-up, both times related to antibiotic treatment for CPD related infections. However, in this patient, nasal and exit site cultures yielded concordant results, showing genotypically identical strains at all points in time. The remaining 9 (out of 53) patients were chronically colonized with *S. aureus*, but all *S. aureus* strains were genotypically different when their serial culture isolates were compared. Together, the latter 10 (10%) patients were classified as chronic carriers.

All *S. aureus* isolates were unique to each of the patients as defined by the PFGE analysis, suggesting that cross-colonization and –infection were not a problem in this CPD population during the monitoring period.

CPD related infections

CPD related infections occurred in all three carrier groups analyzed and are summarized in table 4. Compared to chronic and intermittent carriage, persistent *S. aureus* carriage was associated with significantly higher incidences of CPD related infections from all causes (0.17/month, $p<0.0001$), *S. aureus* CPD related infections (0.13/month, $p<0.0001$), exit site infections from all causes (0.10/month, $p<0.0001$), *S. aureus* exit site infections (0.09/month, $p<0.0001$), CPD peritonitis from all causes (0.07/month, $p=0.0011$) and *S. aureus* CPD peritonitis (0.04/month, $p=0.0023$). Chronic carriers and intermittent carriers had similar risks for all CPD related infections analyzed. The relative risk for all CPD related infections for persistent

carriers as compared to the combined intermittent-or-chronic carrier group was 2.91 [95% confidence interval (CI): 2.17-3.90]. Likewise, the relative risk for *S. aureus* CPD related infections for persistent carriers as compared to the combined intermittent-or-chronic carrier group was 3.42 [95% CI: 2.40-4.88].

Glycopeptide use & resistance

The overall use of vancomycin during this study was 196 grams. More vancomycin was consumed in the persistent carrier group (166 grams) than in the chronic (10 grams) or intermittent groups (20 grams). When adjusted for the duration of follow-up, the use of vancomycin still was 6-fold higher in the persistent carrier group (145 milligrams/month) than in the chronic (23 milligrams/month) and intermittent carrier groups (24 milligrams/month), which was highly statistically significant ($p<0.0001$).
Neither high-level nor intermediate glycopeptide resistance was detected in a collection of 446 *S. aureus* strains isolated from the group of 98 CPD patients. In 34 strains a small number of colonies were found on the Vancomycin Screen agar plate, but after subsequent subculturing the existence of vancomycin resistant phenotypes could not be confirmed.

Table 3 *S. aureus carriage patterns of CPD patients.*

	non	**intermittent**	**chronic**	**persistent**	**total**
***nasal carriage* (n)**					
male	14 (23%)	13 (22%)	5 (8%)	28 (47%)	60 (100%)
female	9 (24%)	9 (24%)	5 (13%)	15 (39%)	38 (100%)
Total	23 (23%)	22 (22%)	10 (10%)	43 (44%)	98 (100%)

Discussion

Forty-four percent of all CPD patients were persistently colonized with a single unique *S. aureus* strain, indicating that long term persistent *S. aureus* carriage is common in this group of patients. This prevalence of persistent carriage in itself is not clearly different from the rates observed in both healthy individuals and other patient cohorts,[3,17] but persistent carriage was clearly associated with a 3-fold increased risk for CPD related infections.

Ten percent of patients were chronically colonized with *S. aureus,* but by genotypically different strains at subsequent occasions and 20% were only intermittently colonized by *S. aureus*. Whether the chronic carrier group should also be defined as persistent carriers is a matter of definition and debate.[18] Although apparently these patients carry *S. aureus* for prolonged periods of time, their risk of invasive *S. aureus* infections was similar to that of the intermittent carrier group. As such, chronic carriers should in our opinion be viewed as 'high level' intermittent carriers and be separated from persistent carriers.

We did not present comparative data from the non-carrier group in this CPD population. However, as we demonstrated before, among CPD patients intermittent carriers behave like non-carriers, as they are not at an increased risk for CPD related infections.[17] Now we also demonstrate that it is only the genuinely persistent *S. aureus* carrier who is at an increased risk of CPD related infections. Thus, accurate determination of the true *S. aureus* carrier state would enable us to improve the prevention of *S. aureus* infections in CPD patients and thus limit antibiotic (including vancomycin) usage. Prevention of *S. aureus* infections can be achieved by procedures such as antimicrobial prophylaxis including mupirocin application,[19-21] vaccination,[22,23] or even bacterial interference.[24] In a recent pilot study we demonstrated that interference with *S. aureus* 502A in persistent carrier CPD patients resulted in successful replacement of the wild type *S. aureus* strain from the nose in all and eventually from the CPD catheter exit site in most patients by the interfering strain.[25] Earlier, interference with *S. aureus* 502A has been shown to be successful in the prevention of *S. aureus* infections in neonates and patients with recurrent

furunculosis.[24]
Although the mechanisms of resistance differ from those encountered in vancomycin-resistant enterococci,[11,12,26,27] the risk that a strain of *S. aureus* could ultimately acquire the genetic elements for high level resistance is considered significant[28] and indeed the first vancomycin resistant MRSA strains have already been identified.[29] Little is as yet known about the precise mechanisms by which staphylococci acquire low level resistance against vancomycin and other glycopeptides. Most probably, several factors cooperate in this event, cell wall thickness and the presence of cell wall biosynthesis enzymes with reduced affinity for glycopeptides are important features.[30] No glycopeptide resistance was demonstrated in *S. aureus* in a center where glycopeptides are not routinely used in the empiric treatment of CPD related infections. This suggests that the epidemiology of glycopeptide resistance in *S. aureus* is different from that in enterococci. Where in enterococci glycopeptide usage in the environment (hospitals and veterinary industry) is related to glycopeptide resistance development,[27] in staphylococci resistant strains seem to emerge after frequent and long term exposure of glycopeptides in the individual patiënt.[6,12,31] Recent studies by our group revealed that in a university hospital where vancomycin was used in the first-line empiric treatment of CPD related infections, exposure to large quantities of vancomycin was a risk factor to become colonized with low level vancomycin resistant coagulase-negative staphylococci.[17] In that study also no resistance, either low of high level, to glycopeptide was demonstrated in *S. aureus.* Our current finding suggests that the routine use of glycopeptide therapy in a group of patients that is quite susceptible to staphylococcal infection should be avoided, since, in a setting were glycopeptides are not routinely used, patients do not seem to be at an increased risk of acquiring glycopeptide resistant staphylococcal strains.

In conclusion, 44% of CPD patients were persistent carriers of *S. aureus,* and at a 3-fold higher risk for CPD related infections as compared to intermittent and chronic carriers. Precise determination of the *S.aureus* carrier state including bacterial genotyping is possible, makes sense and is needed to adequately target prevention strategies. No vancomycin resistance in *S. aureus* was encountered in this setting of low level glycopeptide use. However, continued screening for the

emergence of glycopeptide resistant isolates of *S. aureus* remains a prudent strategy.

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Table 4 *Infection incidence rates by S. aureus nasal carriage patterns.*

	intermittent		**chronic**		**persistent**		**total**				
		95% CI		95% CI		95% CI		95% CI	*P* value*	RR†¶	95% CI
Follow-up (months at risk)											
mean	37.6	[29.9-45.2]	43.0	[31.2-54.8]	26.6	[21.5-31.7]	32.0	[27.9-36.1]			
total	827		430		1,145		2,401				
Patients in follow-up (n)	22		10		43		75				
CPD related infections (n)											
All	48		21		193		262				
Exit site infections	30		12		117		159				
Peritonitis	18		9		76		103				
CPD related infections incidence (nr/person months at risk)											
All	0.06	[0.04-0.09]	0.05	[0.03-0.09]	0.17	[0.15-0.19]	0.11	[0.10-0.12]	< 0.0001	2.91	[2.17-3.90]
Exit site infections	0.04	[0.02-0.07]	0.03	[0.01-0.06]	0.10	[0.09-0.12]	0.07	[0.06-0.08]	< 0.0001	2.82	[1.94-4.11]
Peritonitis	0.02	[0.01-0.05]	0.02	[0.01-0.05]	0.07	[0.05-0.08]	0.04	[0.03-0.05]	0.0011	3.04	[1.92-4.85]
***S. aureus* CPD related infections** (n)											
All	32		13		149		194				
Exit site infections	26		10		100		136				
Peritonitis	6		3		49		58				
***S. aureus* CPD related infections incidence** (nr/person months at risk)											
All	0.04	[0.02-0.07]	0.03	[0.01-0.06]	0.13	[0.11-0.15]	0.08	[0.07-0.09]	< 0.0001	3.42	[2.40-4.88]
Exit site infections	0.03	[0.02-0.06]	0.02	[0.01-0.05]	0.09	[0.07-0.11]	0.06	[0.05-0.07]	< 0.0001	2.84	[1.89-4.27]
Peritonitis	0.01	[0.00-0.02]	0.01	[0.00-0.03]	0.04	[0.03-0.06]	0.02	[0.02-0.03]	0.0023	5.76	[2.74-12.10]

* *P*-value for the univariate comparison of incidence rates in intermittent, chronic and persistent carriers using Poisson regression.
† RR is relative risk (or incidence rate ratio to be more precise).
¶ RR comparing incidence rates in persistent carriers versus in intermittent-or-chronic carriers in a Poisson regression model adjusting for age and sex

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11

Bacterial Interference with *Staphylococcus aureus* 502A for Eradication of Wild Type *S. aureus*

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Abstract

Context The incidence of *Staphylococcus aureus* infections has steadily increased, coinciding with the widespread dissemination of *S. aureus* strains resistant to multiple antibiotics. These developments again necessitate the search for new strategies to combat *S. aureus* infections.

Objective To study the dynamics of inoculation with *S. aureus* 502A (SA-502A) in volunteers (group I, n=8), chronic peritoneal dialysis (CPD) patients (group II, n=4) and furunculosis patients or methicillin-resistant *S. aureus* (MRSA) carriers (group III, n=3).

Design Prospective cohort study (pilot).

Setting Tertiary care university hospital.

Participants Participants were pretreated with mupirocin (group II and III only) and systemic antibiotics (group III). They were subsequently inoculated with 10^8 bacteria only once (group I), or for 3 consecutive days and every 2 weeks thereafter (groups II and III). Cultures were performed weekly in group I and every 4 weeks in groups II and III.

Outcome Measures Eradication of wild type *S. aureus.*

Results In group I SA-502A could be co-cultured with the resident strain in all for one week. At week 6 all persons had lost SA-502A, while retaining their resident strain. In group II, all persons became colonized in the nose with SA-502A. After 11 months SA-502A had replaced the resident strain at the exit site in 2 persons, was co-cultured with the resident strain in one, while in one person only the resident strain remained present. In group III, the person with furunculosis became successfully colonized by SA-502A in the nose and skin. Ultimately, she lost SA-502A and remained without furunculosis. In the persistent MRSA nasal carrier, it was successfully replaced by SA-502A. In the throat carrier of MRSA, inoculation with SA-502A failed. No side effects were noted.

Conclusions Inoculation with SA-502A in persistent carriers can replace wild type *S. aureus* strains in a several patient categories provided that their resident strain is eradicated first.

Introduction

Staphylococcus aureus is an important agent of human disease and the cause of a variety of infections.[1] The nose (i.e. the anterior nares) has been shown to be the primary ecological reservoir of *S. aureus* in humans[2] and *S. aureus* nasal carriage is a major risk factor for *S. aureus* infections,[3,4] the majority being of endogenous origin.[5] Host factors,[4,6] as well as environmental factors contribute to the *S. aureus* nasal carrier state.[7-9] Recent studies have suggested that *S. aureus* nasal carriage is a process of permissive colonization, resulting from failing local inflammatory responses.[10,11]

Bacterial interference has been hypothesized to be a major determinant of the *S. aureus* carrier state.[12-15] The concept of bacterial interference has been successfully taken into clinical testing, employing artificial nasal inoculation with *S. aureus* 502A (SA-502A) or *Corynebacterium* species.[16-21] However, the practice of artificial nasal inoculation with SA-502A was abandoned after alleged complications,[22-24] and the advent of newer anti-staphylococcal antibiotics.

Since then, the incidence of *S. aureus* infections has steadily increased,[25] coinciding with the widespread dissemination of *S. aureus* strains resistant to multiple antibiotics including methicillin and, more recently, vancomycin.[26] These developments again necessitate the search for new strategies to combat *S. aureus* infections. The logical starting point would be the prevention of endogenous *S. aureus* infections by eradication of (nasal) carriage of wild type *S. aureus* strains in individual persons at increased risk of infection. In the study presented here we investigated the risks and dynamics of artificial colonization with SA-502A in three groups of adults with persistent *S. aureus* carriage This effort was undertaken to establish the optimal strategy for bacterial interference as a tool in the eradication of *S. aureus* carriage and the prevention of *S. aureus* infections.

Material & Methods

Selection of Volunteers

Three different groups of adults with persistent *S. aureus* carriage were studied: healthy volunteers (group I, n=8), chronic peritoneal dialysis (CPD) patients (group II, n=4) and otherwise healthy persons suffering from either recurrent furunculosis or persistent carriage with methicillin-resistant *S. aureus* (MRSA) (group III, n=3). Written informed consent was obtained from all participating persons and the study protocol was approved by the Medical Ethics Review Committee of the Erasmus MC, Rotterdam, The Netherlands (METC: 156.137/1996/186 and 165.585/1997/164).

Group I

In 1988 a cohort of healthy volunteers (staff members of the departments of Medical Microbiology & Infectious Diseases and Virology of the Erasmus MC) was formed to investigate bacterial and human factors associated with *S. aureus* nasal carriage.[27] The composition of this volunteer cohort was flexible, in that outgoing personnel were considered lost to follow-up and replaced by incoming personnel. All participants were screened initially with 12 consecutive quantitative nasal swab cultures taken with one-week intervals. After this initial establishment of *S. aureus* nasal carriage status, volunteers were re-screened regularly with 4 quantitative nasal swab cultures with one-week intervals. Long term persistent carriers (i.e. in follow-up for at least 2 years and with at least 16 nasal swab cultures positive with a genotypically identical *S. aureus* strain) were invited to participate.

Group II

Approximately 60 adult patients are taken care for in the chronic peritoneal dialysis (CPD) program of the Erasmus MC, a 1,250 bed tertiary care university hospital. From January 1998 onwards all current and new patients on CPD were screened for S*. aureus* carriage with 6 consecutive nasal swab cultures (vestibulum nasi) taken with one week intervals. *S. aureus s*creening cultures of the CPD catheter exit site were performed together with the first and last nasal swab culture. CPD patients with all 6 initial nasal swab and both CPD exit site cultures positive with a genotypically identical *S. aureus* strain were invited to participate.

Group III

This group consisted of one patient and two health care workers visiting the Infectious Diseases outpatient clinic of the Erasmus MC. One patient was seen for recurrent furunculosis for which she had been treated elsewhere for 5 years. Treatment had consisted of drainage of skin abscesses, multiple courses of antibiotics, local and nasal mupirocin treatment and multiple hygienic measures (chloorhexidine and povidon-iodine washings, thorough house cleaning, washing of underwear and bed sheets at high temperatures). However, eradication of *S. aureus* nasal carriage had failed and the furunculosis problem persisted. When she was seen at our outpatient clinic, 6 nasal swab and 6 skin cultures with one-week intervals were performed. All cultures were positive with a genotypically identical *S. aureus* strain, and she was thus confirmed to be a persistent *S. aureus* carrier. The two other participants in this group were both healthy health care workers documented to be persistently carrying MRSA (one person was a persistent nasal carrier, the other a persistent throat carrier). In The Netherlands personnel carrying MRSA are not allowed to work in hospitals as long as they remain MRSA positive. MRSA carrying hospital personnel is referred for MRSA eradication therapy. The two participants described here, were persistent carriers of MRSA, despite multiple eradication attempts using nasal ointments (mupirocin, fusidic acid) in combination with systemic antibiotic combination therapy and hygienic measures. Also, all household or intimate contacts were screened for MRSA carriage and treated accordingly if positive. All eradication efforts had failed in these persons and all agreed to participate in the present study.

Exclusion criteria

Participants were excluded if they suffered from diabetes mellitus, non-staphylococcal skin diseases, chronic obstructive pulmonary disease, and cardiac valve abnormalities or were taking immunosuppressive agents.

Inoculation and Follow-up

From all participants blood was drawn by venepuncture at inoculation for the determination of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), leukocyte count, leukocyte differentiation and anti-staphylococcal antibodies (ASTA) titer. These tests were repeated in case of (suspected) side effects and at the end of the study. In all participants inoculation was

performed using cotton-wool swabs drenched in PHMB containing 10^9 CFUs/mL of SA-502A. Per nostril, one swab was firmly applied against the inner side of both anterior nares and turned around 4 times. In this way, strains were inoculated in a total amount of 10^8-10^9 CFUs.
Pre-inoculation treatment, inoculation frequency and follow-up differed per study group:

Group I In order to assess colonization resistance, no pre-inoculation antimicrobial treatment was used in this group. Participants were inoculated only once. After inoculation nasal swab cultures were taken weekly for 6 consecutive weeks. At week 6 post-inoculation the study was ended.

Group II Before inoculation participants were pretreated with mupirocin nasal ointment two times daily for 5 days in both nostrils. One week later they were inoculated on 3 consecutive days. Inoculation was repeated every 2 weeks until the end of the study. Nasal swab cultures and cultures of CPD catheter exit site were taken every 6 weeks during follow-up visits. Cultures from other sites (CPD dialysis fluid, wounds, blood, etc.) were performed on indication only. Patients were followed-up from January 1st 1998 until December 31st 2001 or until the date of ending CPD treatment, whichever date came first.

Group III Before inoculation participants were pretreated with mupirocin nasal ointment two times daily for 5 days (two participants) or fusidic acid nasal ointment two times daily for 7 days (one participant carried a mupirocin resistant, fusidic acid sensitive MRSA strain in his throat), systemic antibiotic therapy for 14 days (co-trimoxazol 960 mg bid + rifampin 600 mg oid, ciprofloxacin 750 mg bid + rifampin 600 mg oid and clindamycine 600 mg tablets tid + rifampin 600 mg oid), combined with hygienic measures during 2 weeks. These measures included chlorhexidine scrub body washings daily, chlorhexidine hair washings twice weekly, chlorhexidine lozenges 2.5 mg tablets every 2 hours during daytime, chlorhexidine solution 10 ml (=20mg) bid, daily washing of underwear and bed sheets at high temperatures and thorough professional housecleaning. One week after finishing this treatment, participants were inoculated on 3 consecutive days. Inoculation was repeated every 2 weeks for 3 months. Cultures of the vestibulum nasi, throat and perineal skin were taken every 2 weeks. The study was ended 12 weeks

after the last inoculation.

Microbiological procedures

Phenotyping

Nasal swab cultures were performed as described earlier.[27] Swabs were cultured quantitatively on selective media: phenol-red mannitol salt agar (PHMA) and phenol red mannitol salt broth (PHMB). The number of CFUs was recorded quantitatively. Initial identification of *S. aureus* was based upon colony morphology on the PHMA. All morphologically different colonies were recultured overnight on Columbia blood agar plates (Becton-Dickinson B.V., Etten-Leur, The Netherlands) and a catalase- and latex-agglutination test (Staphaurex Plus^{R}, Murex, Dartford, UK) were performed. Per culture taken at each point in time for each of the participants, all morphologically different *S. aureus* isolates, were stored at -70 ^{0}C in glycerol containing liquid media.
Cultures from skin, CPD catheter exit site, blood and CPD dialysis fluid were performed according to standard procedures.[28] Blood and CPD dialysis fluid were cultured using the BACTEC 9240 system (Becton-Dickinson Diagnostic Instrument Systems, Sparks, Md., USA). In the case of CPD catheter removal, the tip was cultured in thioglycolate broth (Brewer modified). Vitek equipment **(**bioMérieux Vitek, Hazelwood, Mo, USA**)** was used for the identification of microorganisms.

Genotyping

To obtain bacterial DNA for strain typing purposes, *S. aureus* isolates were grown overnight at 37 ^{0}C on Brucella blood agar. Between 2 and 5 colonies were suspended in 150 μL of 25 mmol/L Tris-HCl pH 8.0, 10 mmol/L EDTA, and 50 mmol/L glucose (TEG buffer). To prepare spheroplasts, 75 μL of a lysostaphin solution (100 μg/ml in water) was added. After incubation at 37 ^{0}C for 1 hour, DNA was isolated.[29] DNA (10 ng per ml of 10 mmol/L Tris-HCl pH 8.0 and 1 mmol/L EDTA) was stored at -20 ^{0}C. Restriction fragment length polymorphisms (RFLP) of the coagulase and protein A genes were determined for identification purposes for SA-502A and all resident *S. aureus* strains before inoculation.[30] Furthermore, all *S. aureus* strains isolated two and twelve weeks after inoculation and/or from the last positive culture were genotyped using RFLP of the coagulase and protein A genes. Pulsed-field gel electrophoresis (PFGE) was performed to confirm the results obtained by RFLP, as described earlier.[31]

Results

Group I

The inoculation of SA-502A was initially successful in all participants. SA-502A could be demonstrated in all 8 persons until at least two weeks after inoculation, albeit together with the individuals' resident *S. aureus* strain. Thereafter, participants gradually lost the SA-502A strain. At week four, SA-502A was recovered in 2 persons only and at week six all persons had lost SA-502A, while still retaining their resident strain (Table 1).

Group II

SA-502A was retained in the nares of all four persons after repeated inoculations. In contrast, cultures from their CPD exit site continued to grow their resident strain for many months after start of the study. After a mean of 11 months of follow-up SA-502A had replaced the resident strain at the exit site in two persons (after 6 months in person 4015 and after 18 months in person 4030), or could be co-cultured with the resident strain in one person (after 9 months in person 1026). In the remaining person (1031) the resident strain remained present as the single *S. aureus* strain on the exit site (Table 2).
Mean follow-up was 18 months and a total number of 12 CDP related infections occurred (mean incidence 0.22 per person months at risk [95% CI: 0.08-0.35]). Eight infections were caused by *S. aureus* (four exit site infections and four peritonitis), two by *Serratia marcescens* (one exit site infection and one peritonitis) and two by coagulase-negative staphylococci (both peritonitis). All of the *S. aureus* infections encountered were caused by the resident *S. aureus* strain as cultured from these persons nose and exit site prior to inoculation. Of note, SA-502A was not involved in any CDP related infection.

Group III

The person with recurrent furunculosis became successfully colonized by SA-502A in her nose, as well as on her skin, replacing her resident strain. After stopping the inoculation after 3 months, she rapidly lost SA-502A from her nose and skin. Thereafter, she remained a non-carrier and did not experience a recurrence of furunculosis. In one persistent nasal carrier of MRSA, the MRSA strain was successfully replaced by SA-502A after three months of inoculation. In the other person, a throat carrier of MRSA, inoculation with SA-502A failed to eradicate MRSA carriage from this niche (Table 3).

No clinical side-effects such as skin lesions, abscesses or other infections were noted in all 15 persons. Laboratory parameters at inoculation and at the end of the study were all within normal limits.

Table 1 *Artificial inoculation with S. aureus 502A in healthy persistent S. aureus nasal carriers.*

ID	***Age (yrs)***	***Sex***	***S. aureus nasal isolate before inoculation***	***S. aureus nasal isolate – week 2***	***S. aureus nasal isolate – week 4***	***S. aureus* nasal isolate - week 6**
1	48	female	**R1**[¶]	**R1 + 502A**[§]	**R1 + 502A**	**R1**
2	47	male	**R2**	**R2 + 502A**	**R2 + 502A**	**R2**
3	29	male	**R3**	**R3 + 502A**	**R3**	**R3**
4	33	male	**R4**	**R4**	**R4**	**R4**
5	34	male	**R5**	**R5 + 502A**	**R5**	**R5**
6	25	male	**R6**	**R6 + 502A**	**R6**	**R6**
7	28	female	**R7**	**R7 + 502A**	**R7**	**R7**
8	34	female	**R8**	**R8 + 502A**	**R8**	**R8**

[¶] R1 to R8 refer to the original resident *S. aureus* strains of the volunteers 1-8 before artificial inoculation with *S. aureus* 502A
[§] 502A is *S. aureus* 502A

Discussion

Bacterial interference therapy using nasal inoculation with SA-502A in persistent *S. aureus* carriers is safe and successful in replacing wild type resident *S. aureus* strains in a variety of patient categories. The principle of bacterial interference and its clinical application in the control of outbreaks of *S. aureus* infections dates back to the early 1960s, and was explored at that time by Shinefield and colleagues.[32-36] They elegantly demonstrated that when an ecological niche is already occupied by certain bacteria, other bacteria do not seem to have the means to replace this resident bacterial population.[12-15] We also found that in order to successfully 'interfere' with the resident flora, this resident flora must be reduced or eliminated.[13] Failure to eradicate the resident flora will lead to failure of the interference therapy, as we clearly noticed in our group I. The same volunteers were on a later occasion also inoculated with a *S. xylosis* strain producing RNAIII inhibiting protein (RIP, by courtesy of Dr. N. Balaban, Tufts University School of Veterinary Medicine Department of Biomedical Sciences, Infectious Diseases, North Grafton, Massachusetts, USA[37]) without pre-inoculation antimicrobial treatment. Again, interference therapy failed, and the *S. xylosis* strain could not be retrieved from any of the eight volunteers two weeks after inoculation (data not shown). The analysis of the carriage status of the CPD patients (group II) and the throat MRSA carrier (group III), revealed that eliminating and replacing the wild type strain in the nose does not necessarily imply that colonization of other body sites by the wild type strain is simultaneously eliminated or replaced. Probably, the CPD catheter exit site and throat act as separate niches that need to be specifically addressed when attempting to replace resident wild type *S. aureus*.[38] The data from the CPD group also suggest, however, that there is continuous interference by SA-502A from the nose to the skin and CPD exit site, which over time may lead to replacement of the original resident strain on the exit site in a significant proportion of persons. To test if in CPD patients replacement of the wild type resident *S. aureus* strain in the nose and at the CPD exit site by SA-502A will lead to a reduction of (*S. aureus*) CPD related infections, further studies are needed. From this preliminary study, we conclude that nasal application of SA-502A in CPD

patients persistently colonized with wild type *S. aureus* is safe in the short-, as well as the longer-term and can successfully replace the resident *S. aureus* strain.
As mentioned already, inoculation with SA-502A has been successfully used earlier in nurseries during out-breaks of *S. aureus* infections in the 1960s and for treatment of patients with recurrent furunculosis,[16-20]. However, the early practice of artificial inoculation with SA-502A was abandoned after alleged complications,[22-24] and the advent of newer anti-staphylococcal antibiotics in the early 1970s. At that time, the authors reporting these side effects explicitly stated that, despite the potential (serious) side-effects of artificial inoculation with SA-502A, the 'benefits far outweigh the hazards', claiming that it is an effective tool to prevent serious or recurring *S. aureus* problems from wild type strains.[23,24] From our present study we conclude that artificial inoculation with SA-502A is safe when applied to individuals known to be long-term persistent carriers of *S. aureus*. In a previous study, we demonstrated that when long-term persistent *S. aureus* carriers were inoculated with a mixture of *S. aureus* strains, none suffered from side-effects from the inoculation.[6] In that study also individuals known to be free of wild type *S. aureus* were inoculated, and two out of eight of those long-term non-carriers suffered from minor self-limiting skin lesions after artificial inoculation.[6] Thus, we claim that determining the exact *S. aureus* carrier state is an essential part of performing artificial inoculation and that bacterial interference by artificial inoculation with SA-502A should be restricted to individuals known to be long-term persistent *S. aureus* carriers.

In conclusion, artificial inoculation with SA-502A in persistent *S. aureus* carriers is safe and can be used to successfully to replace wild type resident *S. aureus* strains in a variety of patient categories provided that the resident strain is eradicated first. This strategy holds promise for the prevention of *S. aureus* infections and may help limit the spread of wild type, potentially more virulent and hard-to-treat *S. aureus* strains including MRSA.

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Table 2 *Artificial inoculation with S. aureus 502A in CPD patients.*

ID	***Age*** *(yrs)*	***Sex***	***Follow-up*** *(months)*	***Reason to stop study***	***CPD related infection incidence*** *(nr/personmonths)*	***S. aureus isolate before inoculation***		***S. aureus isolate – week 24***		***S. aureus isolate – week 48***		***S. aureus* isolate – week 72**	
1026	52	male	12	RTX*	0.34	*n*‡	**R26**¶	*n*	**502A**§	*n*	**502A**	*n*	nd$
						e#	**R26**	*e*	**R26**	*e*	**502A + R26**	*e*	nd
1031	40	male	26	RTX	0.08	*n*	**R31**	*n*	**502A**	*n*	**502A**	*n*	**502A**
						e	**R31**	*e*	**R31**	*e*	**R31**	*e*	**R31**
4015	47	male	11	RTX	0.35	*n*	**R15**	*n*	**502A**	*n*	**502A**	*n*	nd$
						e	**R15**	*e*	**502A**	*e*	**502A**	*e*	nd
4030	38	female	21	IHD†	0.09	*n*	**R30**	*n*	**502A**	*n*	**502A**	*n*	**502A**
						e	**R30**	*e*	**R30**	*e*	**R30**	*e*	**502A**

* RTX is renal transplantation
† IHD is intermittent hemodialysis
‡ *n* is nose
# e is CPD catheter exit site
¶ R15 to R30 refer to the original resident *S. aureus* strains of these CPD patients before artificial inoculation with *S. aureus* 502A
§ 502A is *S. aureus* 502A
$ nd means not done because patients underwent renal transplantation before week 72

Table 3 *Artificial inoculation with S. aureus 502A in a patient with recurrent furunculosis and two persistent MRSA carriers.*

ID	***Age*** *(yrs)*	***Sex***	***S. aureus isolate before inoculation***		***S. aureus isolate – week 6***		***S. aureus isolate – week 12***		***S. aureus* isolate – week 24**	
F1	33	female	n[†]	**F1**[¶]	n	**502A**[§]	n	**502A**	n	-
			t[‡]	-	t	-	t	-	t	-
			p[#]	**F1**	p	-	p	-	p	-
M2	27	female	n	**M2**[¶¶]	n	**M2 + 502A**	n	**502A**	n	**502A**
			t	-	t	-	t	-	t	-
			p	-	p	-	p	-	p	-
M3	48	male	n	**M3**[¶¶]	n	**M3+ 502A**	n	**M3+ 502A**	n	**M3**
			t	**M3**	t	**M3**	t	**M3+ 502A**	t	**M3**
			p	-	p	-	p	-	p	-

[†] *n* is nose
[‡] *t* is throat
[#] *p* is perineal skin
[¶] F1 refers to the original resident *S. aureus* strains of this furunculosis patient
[¶¶] M2 and M3 refer to the original MRSA strain of these two MRSA carriers
[§] 502A is *S. aureus* 502A

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12

General Discussion, Summary & Future Prospects

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Determinants

S. aureus nasal carriage has been identified as the key to preventing *S. aureus* infections. As stated earlier, to fully exploit this key we will first have to unlock the fundamental mechanisms underlying it. Much research so far has focused on bacterial factors in trying to explain carriage. Often in vitro data could not be corroborated in vivo, not to mention real life. So far, no single common genetic or phenotypic characteristics segregating successful from less- or non-successful colonizing *S. aureus* strains have been identified.[1-5] However, lipoteichoic acid and clumping factor B (ClfB)[6] have been implicated as essential bacterial factors for *S. aureus* nasal colonization recently.[7] Furthermore, most studies on host factors associated with carriage have been performed in the hospital setting. We, therefore, decided to study the host as the main determinant of *S. aureus* nasal carriage in the community setting.

Since the underlying mechanisms determining persistent and intermittent carriage differ,[8-15] adequate differentiation between persistent and intermittent carriage is relevant for epidemiological studies. In the Rotterdam Study an efficient and reliable way to assess *S. aureus* nasal carriage was obligatory. It would be impossible to perform 10-12 weekly nasal swab cultures in all participants. Thus, we developed a 'culture rule' to discriminate reliably between persistent-carriage and non-or-intermittent carriage, with a minimum of nasal swab cultures (chapter 2). Combining qualitative results with quantitative data is, in our opinion, conceptionally the best choice. Incorporating quantitative data makes it possible to refine associations between potential determinants and *S. aureus* nasal carriage, since not only carriers are compared with non-carriers, but also carriers with low CFUs can be compared with carriers with high CFUs in their anterior nares. Incorporating quantitative data will also make it possible to refine associations between carriage state and morbidity and mortality. However, in large-scale epidemiologic studies, simplicity will often prevail, because of logistic reasons and resources. It is therefore reassuring that in the validation cohort the simple qualitative culture results performed as good as the more complicated 'culture rule'. Two nasal swab cultures taken at a one-week interval were shown to

provide sufficient information to indeed adequately predict the *S. aureus* nasal carriage state. Using only one nasal swab culture to predict the carriage state, as is often done, cannot be recommended on the basis of our data since it will lead to misclassification of the carriage state. On the other hand, the addition of a 3rd or 4th quantitative nasal swab culture only minimally improved test performance. Importantly, no persons with the first 2 cultures positive were found to be non-carriers. One negative 'screening' culture virtually excludes persistent carriage. Predicting the non-carrier state from 2 nasal swab cultures is more difficult since at least 7 nasal swab cultures would be needed to distinguish intermittent from non-carriers. Apart from its role in the Rotterdam study, we hope that the presented 'culture rule' will prove to be a helpful tool in identifying determinants of *S. aureus* nasal carriage and infections, as well as in identifying high-risk patient populations and the implementation of new methods in the prevention of *S. aureus* infections.

From the other studies presented in the first section (chapters 3-7) of this thesis we conclude that indeed the human host that is a, if not thé major factor in determining the individual carrier state. After artificial inoculation (chapter 3) with a mixture of *S. aureus* strains, median nasal survival of *S. aureus* was significantly longer in persistent carriers than in non-carriers. Half of non-carriers became non-carriers again within 2 weeks after inoculation. Only one non-carrier became a persistent carrier, coinciding with minor self-limiting skin lesions. These data suggest that host characteristics significantly co-determine the *S. aureus* carrier state and that optimal fit between host and bacteria seems to be important.
We found a striking difference in the amount of *S. aureus* in the nose of persistent carriers between young healthy volunteers and healthy elderly participants (chapter 2). No prior data are available regarding age and the number of CFUs in the noses of persistent carriers. From the Rotterdam study (3851 persons) the high numbers of CFUs (median geometric mean 2.8) in elderly persistent carriers are confirmed, but the underlying mechanisms of this finding remain to be elucidated.
Furthermore, the main findings in this population-based study (chapter 4) are the positive association of fasting blood glucose levels with persistent *S. aureus* nasal carriage, and the negative association or 'protective' effect of current smoking on the rate

and density of *S. aureus* nasal carriage. We confirm that age, sex and a history of skin disease are independent determinants of *S. aureus* nasal carriage. As for diabetes mellitus and blood glucose levels, in earlier studies only insulin dependent diabetics were found to have a higher prevalence of persistent carriage.[16,17] We now found that, apart from the diabetic state and its treatment, fasting blood glucose levels, even within the normal range, are associated with persistent carriage. The pathophysiological basis of this association remains to be discovered, but we hypothesize that phagocytic activity of neutrophils and macrophages, as well as the amount of killing of intracellular micro-organisms (including *S. aureus*) are pivotal.[18]
Another major finding is the negative association of smoking and persistent carriage. In a population-based cohort of children and adolescents it was recently also shown that cigarette smoking lowers the prevalence of *S. aureus* nasopharyngeal carriage.[19] Past smokers have the same prevalence of persistent carriage as those who have never smoked. Therefore, the ''protection against carriage'' conferred by smoking seems rapidly inducible and transient in nature. The fact that within the cohort of persistent carriers, current smoking was associated with a significantly lower density of *S. aureus* CFUs in the nares also points toward a direct effect of (cigarette) smoke on carriage. Cigarette smoking is known to induce airway inflammation.[20,21] Cole *et al*. reported that nasal secretions obtained from *S. aureus* carriers contained higher levels of inflammatory proteins including defensins, but lacked antimicrobial activity in vitro, while nasal fluid from non-carriers was bactericidal.[3] From these and our data, we hypothesize that *S. aureus* colonization of the anterior nares is less likely to occur in smoking persons because of a toxic effect of smoke on *S. aureus* combined with smoke induced airway mucosa infiltration of inflammatory cells.
Environmental factors were also shown to play an important role in determining the *S. aureus* nasal carrier state (chapter 5). The risk of sharing identical carrier states was 2 to 4-fold increased for members of the same household, while first degree relatives did not 'share' identical carrier states. Apparently, the repeated (non-) exposure to *S. aureus* in the household environment is a more important determinant of *S. aureus* nasal carriage than the genetic background of first degree family members.
The vitamin D endocrine system (chapter 6) has been shown to influence immune response and polymorphisms in the vitamin D receptor (VDR) gene have been associated with susceptibility to

infectious diseases. VDR polymorphisms have been associated with susceptibility to infections.[22-26] VDR gene polymorphisms may determine the likelihood of persistent infection or colonization with intracellular micro-organisms (including *S. aureus)*, since Vitamin D metabolites have been shown to play a role in macrophage activation and differentiation.[27] However the prevalence of persistent *S. aureus* nasal carriage was not different by VDR genotype, suggesting that VDR gene variation is not associated with *S. aureus* carriage.

We also determined whether functional polymorphisms in the glucocorticoid receptor (GR) gene (chapter 7) were associated with persistent nasal carriage of *S. aureus*. We demonstrated that genetic variantion in the GR gene are associated with *S. aureus* nasal carriage: GG-homozygotes of the exon 9beta polymorphism had a 67% reduced risk of persistent *S. aureus* nasal carriage (odds ratio 0.33; 95% confidence interval, 0.15-0.74), while heterozygous carriers of the haplotype containing the 9beta G-allele and the codon 23 Lys-allele had a 80% increased risk (odds ratio 1.80; 95% confidence interval, 1.08-3.00). Our data show that glucocorticoid regulation influences the risk of persistent *S. aureus* nasal carriage, either through modulation of the pro/anti inflammatory cytokine balance or through changes in insulin sensitivity. The 9beta GG-homozygotes may have a more active immune system through increased glucocorticoid resistance as demonstrated by the association with autoimmune disease, notably rheumatoid arthritis.[28] In addition, as a result of relative glucocorticoid resistance 9beta GG homozygotes could have an increased insulin sensitivity leading to lower insulin and/or overall glucose levels. However, too little is currently known on the physiological effect of GR mutation combination and future research should resolve this apparent controversy. In addition, it has recently been demonstrated that glucocorticoids, in conjunction with pro-inflammatory cytokines, affect the expression of toll-like receptor 2 (TLR-2), the main pattern recognition molecule acting on gram positive pathogens such as *S. aureus.*[29-31] Whether the various GR gene polymorphisms studied here are (partially) defective in supporting this biological activity is currently unknown, but again warrants further investigation. We thus report the first genetic factor as a determinant of individual *S. aureus* nasal carriage.

Combined these data suggest that glucose metabolism, insulin sensitivity and glucocorticoid sensitivity interact in eliminating

or tolerating *S. aureus* in its niche, the anterior nares. Whether other 'infection' genes (e.g. IL-4, IL-10, TNF-alpha, TGFbeta) are involved is currently unknown, but the subject of future research. Also further research is needed into the contribution of the innate immunity, since defensins and also Toll-like receptors (e.g. TLR-2) are thought to play an important role.[2,29,30,32]

Risks

Most of the studies on the risks associated with *S. aureus* nasal carriage have been performed in the hospital setting and limited data are available on the health effects of *S. aureus* nasal carriage in the community setting. Moreover, studies in populations at risk, such as patients on continuous peritoneal dialysis (CPD), have been hampered by lack of adequate definition of the different *S. aureus* nasal carrier states, thereby impeding the adequate targeting of prophylactic measures. Accurate determination of the true *S. aureus* carrier state would, theoretically, enable us to improve the prevention of *S. aureus* infections in CPD patients.

In a community setting of elderly persons (chapter 8), persistent *S. aureus* nasal carriage was not associated with all-cause mortality. However, persistent *S. aureus* nasal carriage was associated with a slight though significant increase in antibiotic use. This association was even more pronounced in the subgroup of non-smokers. The major limitation of this study was that we studied all-cause mortality and overall antibiotic use and not infection-related mortality or specific anti-staphylococcal antibiotic prescriptions. It remains possible that persistent *S. aureus* nasal carriage is associated with mortality from *S. aureus* infections. However, since persistent carriage did not significantly attribute to the all-cause mortality, the absolute assignable risk for *S. aureus* nasal carriage-related mortality due to infection will, if any, be low. Also, the vast majority of antibiotics prescribed in the community are for urinary tract and respiratory tract infections, and not for the major community-acquired *S. aureus* infections, mostly skin infections. Therefore, in the community, the relative contribution of *S. aureus* infections

may be small, explaining the only slight increase in overall antibiotic consumption.

In both studies in CPD patients (chapters 9 & 10) we clearly demonstrated that persistent, but not intermittent, *S. aureus* nasal carriage is thé major determinant of CPD related infections and associated with a significantly higher consumption of antibiotics, including vancomycin. Nearly half of all CPD patients were persistently colonized with a single unique *S. aureus* strain, indicating that long term persistent *S. aureus* carriage is common in this group of patients. This prevalence of persistent carriage in itself was not different from the rates observed in both healthy individuals and other patient cohorts,[33] but persistent carriage was associated with a more than 3-fold increased risk for CPD related infections.
In the second CPD study (chapter 10) 10% of patients were chronically colonized with *S. aureus,* but by genotypically different strains at subsequent occasions and 20% were only intermittently colonized by *S. aureus*. Whether the chronic carrier group should also be defined as persistent carriers is a matter of definition and debate.[34] Although apparently these patients carry *S. aureus* for prolonged periods of time, their risk of invasive *S. aureus* infections was similar to the intermittent carrier group. As such, chronic carriers should in our opinion be viewed as 'high level' intermittent carriers and be separated from persistent carriers.
The highly divers population of CoNS appears to be the prime reservoir of staphylococcal vancomycin resistance, although no vancomycin resistance in *S. aureus* was encountered. However, continued screening for the emergence of glycopeptide resistant isolates of *S. aureus* remains a prudent strategy.
From our CPD studies we conclude that only persistent carriers of *S. aureus* have a significantly increased risk for CPD related infections as compared to non-carriers. Intermittent and chronic *S. aureus* carriage is not associated with increased infection risk. Moreover, in a recent large multicenter study it was clearly demonstrated that routine culture for *S. aureus* nasal carriage at admission and subsequent mupirocin application does not provide effective prophylaxis against nosocomial *S. aureus* infections in non-surgical patients.[35] Precise determination of the *S.aureus* carrier state is possible, makes sense and is needed to adequately target prevention strategies to high risk patient groups.

Dynamics

S. aureus remains an important pathogen in human disease.[36] The incidence of both community-acquired and hospital-acquired *S. aureus* infections is still increasing and, despite antibiotic therapy, the cause of serious morbidity and high mortality.[37,38] That *S. aureus* is capable of developing resistance to all available antibiotics has been clearly demonstrated again with the dramatic advent of community-acquired MRSA and vancomycin-resistant *S aureus* (VRSA) strains.[39-42] In the era of *S. aureus* 'multi-resistance' prudent use of antibiotics in prevention and therapy is highly needed. Also, alternative strategies, including interference therapy,[43-45] vaccination,[46-48] phage-therapy and non-antibiotic therapies such as honey, to combat *S. aureus* are necessary.

We studied the dynamics of inoculation with *Staphylococcus aureus* 502A in volunteers, CPD patients, furunculosis patients or methicillin-resistant *S. aureus* carriers. inoculation with SA-502A has been successfully used earlier in nurseries during out-breaks of *S. aureus* infections in the 1960s and for treatment of patients with recurrent furunculosis.[49-53].However, the early practice of artificial inoculation with SA-502A was abandoned after alleged complications,[54-56] and the advent of newer anti-staphylococcal antibiotics in the early 1970s. At that time, the authors reporting these side effects explicitly stated that, despite the potential (serious) side-effects of artificial inoculation with SA-502A, the 'benefits far outweigh the hazards', claiming that it is an effective tool to prevent serious or recurring *S. aureus* problems from wild type strains.[55,56] From our present study we conclude that artificial inoculation with SA-502A is safe when applied to individuals known to be long-term persistent carriers of *S. aureus*. In a previous study, we demonstrated that when long-term persistent *S. aureus* carriers were inoculated with a mixture of *S. aureus* strains, none suffered from side-effects from the inoculation.[57] In that study also individuals known to be free of wild type *S. aureus* were inoculated, and two out of eight of those long-term non-carriers suffered from minor self-limiting skin lesions after artificial inoculation.[57] Thus, we claim that determining the exact *S. aureus* carrier state is an essential part of performing artificial inoculation and that bacterial

interference by artificial inoculation with SA-502A should be restricted to individuals known to be long-term persistent *S. aureus* carriers.
In conclusion, artificial inoculation with SA-502A in persistent *S. aureus* carriers is safe and can be used to successfully to replace wild type resident *S. aureus* strains in a variety of patient categories provided that the resident strain is eradicated first. This strategy holds promise for the prevention of *S. aureus* infections and may help limit the spread of wild type, potentially more virulent and hard-to-treat *S. aureus* strains including MRSA.

Conclusions & Future Developments

Worldwide, MRSA rates have increased dramatically during the last decades. The threat of development of resistance to vancomycin, the only antimicrobial agent effective against MRSA, and the emergence of community-acquired MRSA (CA-MRSA) is alarming. The worldwide use of vancomycin has increased dramatically over the past years.
Optimization of preventive strategies is needed to control staphylococci. Therefore, new strategies have to be developed. The ability to control staphylococcal infections in the future will depend on the development of new therapeutic agents and the optimization of infection control measures.
In this thesis we have demonstrated that the human host is the prime determinant of the *S. aureus* nasal carrier state. 'To tolerate or to eliminate?' that's the question! *S. aureus* nasal carriage is definitively a multifactorially defined status, with bacterial and environmental factors involved as well. More in depth research will be necessary to elucidate the host-pathogen interaction. The results from the studies presented in this thesis set the stage for future research to be directed towards host genetic factors and in vivo host-bacterial interaction studies. The completion of both the human genome project and the *S. aureus* genome project opens new possibilities to pinpoint individuals at risk, as well as 'risky' bacteria, and could thus 'personalize' our preventive options.

For now, mupirocin is the most effective drug available to achieve eradication of carriage. However, resistance to mupirocin is increasing, and it must be asked for how long this agent will be effective. One strategy that has been used successfully in the past is bacterial interference. This alternative approach to controlling staphylococcal infections could offer new opportunities if strains with minimal virulence and maximal competition for the binding sites in the nose could be developed.

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13

Algehele Discussie, Samenvatting & Toekomstperspectief

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Determinanten

Neusdragerschap van *S. aureus* wordt wel beschouwd als de sleutel tot het voorkomen van *S. aureus* infecties. Om deze sleutel optimaal te kunnen benutten is meer onderzoek nodig naar de fundamentele mechanismen van dragerschap. Veel onderzoek heeft zich voornamelijk gericht op bacteriële factoren. Keer op keer konden in vitro bevindingen niet in vivo worden bevestigd. Daardoor zijn er tot op heden geen bacteriële factoren essentieel voor *S. aureus* dragerschap geïdentificeerd.[1-5] Recent werden lipoteichoïne zuur (LTA) en clumping factor B (ClfB) als essentiële factoren voor kolonisatie beschreven.[6,7] Echter, ook van deze factoren moet in het 'echte' leven nog blijken of zij inderdaad zo essentieel zijn.
Tevens is het merendeel van de studies naar gastheerfactoren geassocieerd met *S. aureus* dragerschap verricht in het ziekenhuis of de polikliniek. Derhalve besloten wij om de gastheer als primaire determinant van *S. aureus* neusdragerschap te bestuderen in de open bevolking.

Het adequaat kunnen onderscheiden van de verschillende dragerschapcategorieën (nooit, intermitterend en persisterend dragerschap) is essentieel voor het kunnen doen van epidemiologisch onderzoek.[8-15] Om binnen ERGO de associatie tussen *S. aureus* neusdragerschap en gastheerfactoren te kunnen bestuderen, moest er als eerste een methode ontwikkeld worden om op een zo efficiënt en betrouwbaar mogelijke manier de *S. aureus* nesudragerschapstatus vast te kunnen stellen. Het zou namelijk onmogelijk geweest zijn om bij iedere van de bijna 4000 ERGO deelnemers gedurende 10-12 weken een neuskweek af te nemen. Door kwalitatieve gegevens (= aantal positieve neuskweken) te combineren met kwantitatieve gegevens (= aantal bacterie kolonies in een neuskweek) kon een 'kweekregel' worden ontworpen, waarbij middels 2 neuskweken met één week tussenpoos met een 94% betrouwbaarheid de dragerschapstatus van de ERGO deelnemers adequaat kon worden vastgesteld (hoofdstuk 3). Het toevoegen van een 3^{e} of 4^{e} kweek had geen significante verbetering tot gevolg. Wel bleek dat het gebruik van slechts één neuskweek, zoals vaak in klinische studies wordt gedaan, ter vaststelling van de dragerschapstatus onvoldoende was. Wel

sluit één negatieve kweek persisterend dragerschap vrijwel uit. Naast het gebruik in ERGO, zou deze 'kweekregel' ook in andere omstandigheden kunnen worden gebruikt in studies naar determinanten van *S. aureus* dragerschap en infecties en de implementatie van nieuwe strategieën in de preventie van infecties.

Uit de gegevens van de studies beschreven in de hoofdstukken 3-7 concluderen wij dat de gastheer inderdaad een belangrijke, zo niet dé belangrijkste, determinant is van *S. aureus* neusdragerschap. Na artificiële inoculatie met een mengsel van *S. aureus* stammen (hoofdstuk 4), bleek de overleving van *S. aureus* in de neus significant langer te zijn bij persisterend dragers dan bij niet-dragers. Bij de helft van de niet-dragers waren binnen 2 weken de geïnoculeerde stammen alweer verdwenen uit de neus. Slechts één voorheen niet-drager werd na inoculatie een persisterend drager, gepaard gaande met milde huidlesies. Deze gegevens suggereren dat gastheerfactoren een belangrijke rol spelen in het bepalen van het type dragerschap. Tevens lijken gast en gastheer elkaar 'uit te zoeken' in de zin dat persisterend dragers vaak langdurig drager zijn van hun eigen *S. aureus* stam.
Opvallend is het, tot nu toe niet eerder beschreven en ook nog onverklaarde, verschil in de hoeveelheid *S. aureus* bacteriën in persisterend dragers tussen jongeren en ouderen. Oudere persisterend dragers hebben 10-maal zoveel bacteriën in de neus dan jongeren (hoofdstuk 2).
De belangrijkste bevindingen uit ERGO zijn de positieve associatie tussen nuchtere glucose waarde en persisterend *S. aureus* neusdragerschap en het beschermende effect van roken. Verder wordt bevestigd dat geslacht (mannen vaker drager dan vrouwen), leeftijd (des te ouder des te minder dragers) en huidaandoeningen (met name eczeem) geassocieerd zijn met *S. aureus* dragerschap. In eerdere studies werd alleen een associatie gevonden tussen insuline-afhankelijke diabetes mellitus en *S. aureus* dragerschap.[16,17] Echter uit onze ERGO studie blijkt dat de nuchtere glucose waarde, zelfs in het normale gebied, is geassocieerd met *S. aureus* dragerschap. Het pathofysiologische mechanisme hierachter is (nog) niet bekend, maar waarschijnlijk spelen neutrofiele granulocyten en macrofagen en hun intracellulaire dodend vermogen hierbij een belangrijke rol.[18]
Dat roken de kans op dragerschap verminderd komt overeen

met de resultaten van een recente studie onder kinderen.[19] Het feit dat personen die gestopt zijn met roken een vergelijkbare kans op persisterend dragerschap hebben als nooit rokers, doet vermoeden dat het 'beschermende' effect snel induceerbaar en reversibel is. Binnen de groep van persisterend dragers hadden rokers ook lagere aantallen bacteriën in de neus, wat ook wijst in de richting van een direct (toxisch) effect van (sigaretten) rook. Eerdere studies hebben laten zien dat roken gepaard gaat met een toename van ontstekingsverschijnselen in de luchtwegen[20,21]. Cole *et al*. vonden dat neussecreet van persisterend *S. aureus* dragers hogere concentraties onstekingsmediatoren bevatten (o.a. defensines), maar in antibacteriële tekort schoten, in vergelijking met niet-dragers.[3] Onze hypothese is dan ook dat roken een beschermend effect tegen persisterend *S. aureus* neusdragerschap heeft door enerzijds een direct toxisch effect van de rook op de bacterie en anderzijds door een toegenomen ontstekingsactiviteit in de neus.

Omgevingsfactoren blijken ook een belangrijke rol te spelen in *S. aureus* neusdragerschap. Personen behorend tot eenzelfde huishouden hadden een 2-4 maal verhoogde kans op dezelfde dragerschapstatus als hun partners. Oftewel: persisterend dragers wonen met persisterend dragers en niet-dragers wonen bij niet-dragers (hoofdstuk 6). Dit in tegenstelling tot 1^{e}-graads familieleden, waarbij geen concordantie in dragerschapstatus kon worden gevonden. Blijkbaar speelt het bij herhaling worden blootgesteld aan *S. aureus* (of juist niet) binnen het huishouden een belangrijkere rol in het bepalen van de individuele *S. aureus* dragerschapstatus dan de genetische achtergrond van 1^{e}-graads familieleden.

Van het vitamine D endocriene systeem (hoofdstuk 6) is aangetoond dat het de immuunrespons beïnvloedt. Polymorfismen in het vitamine D receptor (VDR) gen zijn geassocieerd met een verhoogde gevoeligheid voor infecties.[22-26] VDR gen polymorfismen lijken vooral een rol de spelen in de afweer tegen intracellulaire micro-organismen (waaronder *S. aureus*), daar vitamine D metabolieten een rol spelen in macrofaag activatie en differentiatie.[27] Echter, wij konden geen associatie aantonen tussen VDR gen polymorfismen en de kans op persisterend *S. aureus* neusdragerschap.
Naast het VDR gen, hebben we, ook binnen ERGO, gekeken of

functionele polymorfismen in het glucocorticoïd receptor (GR) gen geassocieerd waren met *S. aureus* neusdragerschap (hoofdstuk 7). We konden aantonen dat het G-allel in het exon 9beta polymorfisme en het haplotype bestaande uit de combinatie van het G-allel in het exon 9beta polymorfisme samen met een Lys-allele in codon 23, significant geassocieerd waren met persisterend *S. aureus* neusdragerschap: GG-homozygoten in het exon 9beta polymorfisme hadden 67% lagere kans op persisterend dragerschap, terwijl heterozygote dragers van het G-allel in het exon 9beta polymorfisme samen met een Lys-allele in codon 23 een 80% hogere kans op persisterend dragerschap hadden. Deze gegevens zijn compatibel met de hypothese dat glucocorticoïd regulatie het risico op *S. aureus* dragerschap beïnvloed door middel van verandering in de lokale pro/anti-inflammatoire cytokine balans, dan wel via veranderingen in insuline gevoeligheid. De 9beta GG-homozygoten lijken een actiever immuunsysteem te hebben, getuige ook de associatie met reumatoïde artritis.[28] Verder hebben 9beta GG-homozygoten een relatieve glucocorticoïden resistentie, gepaard gaande met een hogere insuline gevoeligheid, leidend tot lagere insuline en/of glucose spiegels. Tevens werd van glucocorticoïden recent aangetoond dat deze, in samenspraak met pro-inflammatoire cytokinen, de expressie van Toll-like receptor 2 (TLR-2), het belangrijkste 'pattern recognition molecuul' voor gram-positieve micro-organismen zoals *S. aureus,* beïnvloeden.[29-31] Of dat de verschillende, door ons onderzochte, GR gen polymorfismen daadwerkelijk een rol spelen in de regulatie/expressie van TLR-2 is op dit moment onbekend. Kortom, met de twee genoemde GR gen polymorfismen rapporteren wij de eerste genetische determinanten van *S. aureus* neusdragerschap.

Gecombineerd, suggereren bovengenoemde gegevens, dat glucose metabolisme, insuline gevoeligheid en glucocorticoïd gevoeligheid interacteren in het elimineren dan wel tolereren van *S. aureus* in zijn niche, de neus. Of en in welke mate andere 'infectie' genen (bijvoorbeeld IL-4, IL-10, TNF-alpha, TGFbeta) en de aangeboren immuniteit (defensines, Toll-like receptoren etc.) hieraan bijdragen is het onderwerp van toekomstige studies.[2,29,30,32]

Risico's

De meeste studies betreffende de risico's van *S. aureus* dragerschap zijn verricht in de ziekenhuis setting en slechts weinig data zijn beschikbaar over de gezondheidseffecten van dragerschap in de open bevolking. Hiernaast wordt de interpretatie van veel studies naar de effecten van dragerschap bij risico patiënten, zoals CAPD (continue ambulante peritoneaal dialyse) patiënten, bemoeilijkt door inadequate definities van *S. aureus* dragerschap. Accurate vaststelling van de reële *S. aureus* dragerschapstatus is noodzakelijk voor de studie van zowel determinanten van dragerschap als het ontwikkelen van preventieve maatregelen tegen *S. aureus* infecties in risico patiënten.

Persisterend *S. aureus* dragerschap was in de open bevolking van ERGO niet geassocieerd met mortaliteit (hoofdstuk 8). Echter persisterend dragerschap was wel geassocieerd met een lichte, maar significante toename in antibiotica gebruik. De toename in antibiotica gebruik was het meest uitgesproken in de subgroep van niet-rokers. Het is niet uitgesloten dat er een associatie bestaat tussen persisterend *S. aureus* dragerschap en (*S. aureus*) infectiegerelateerde mortaliteit. Echter, gezien het ontbreken van een effect op de overall mortaliteit, zal het eventuele effect op infectiegerelateerde mortaliteit gering zijn. De indicaties voor het merendeel van de voorgeschreven antibiotica in de bevolking zijn urineweg- en luchtweginfecties, en niet zozeer community-acquired *S. aureus* infecties, meestal huidinfecties. Daarom zal de relatieve bijdrage van *S. aureus* infecties aan het totaal antibiotica gebruik klein zijn.

In beide studies in CAPD patiënten (hoofdstukken 9 en 10), bleek dat alleen de echte persisterend *S. aureus* drager (en niet de intermitterend drager!) een 3-voudig verhoogd risico op CAPD gerelateerde infecties heeft vergeleken met niet-dragers. Persisterend dragerschap was tevens geassocieerd met een hoger antibiotica gebruik, inclusief vancomycine. Ongeveer de helft van de CAPD patiënten is over een langere periode persisterend gekoloniseerd met één en dezelfde *S. aureus* stam. Patiënten die intermitterend drager van *S. aureus* zijn en patiënten die weliswaar chronisch gekoloniseerd zijn met *S.*

aureus maar met steeds (genotypisch) andere stammen, lopen géén verhoogd risico op CAPD gerelateerde infecties. De infectie-incidentie in deze twee laatste categorieën dragers is gelijk aan die van niet-dragers. De door ons zogenoemde chronische dragers zijn dus eigenlijk 'high-level' intermitterend dragers.[33,34] Wij stellen dan ook voor om bij het vaststellen van het type dragerschap gebruik te maken van genotyperings methoden zoals PFGE (pulsed-field gel electropheresis) ter differentiering van persisterend en andere *S. aureus* dragers, zeker omdat dat klinische consequenties heeft.

In een centrum waar vancomycine deel uit maakt van de eerstelijns empirische behandeling van CAPD-peritonitis (hoofdstuk 9) bleek 10% van de patiënten op enig moment gedurende de studie gekoloniseerd met voor vancomycine verminderd gevoelige coagulase-negatieve stafylokokken. Blijkbaar is de zeer diverse populatie coagulase-negatieve stafylokokken het primaire reservoir van vancomycine verminderd gevoelige stafylokokken. In een centrum waar vancomycine geen deel uit maakt van de eerstelijns empirische behandeling van CAPD-peritonitis, werden geen voor vancomycine verminderd gevoelige coagulase-negatieve stafylokokken gevonden. Er werd geen vancomycine resistentie gevonden in *S. aureus.*

In een recente multicenter studie werd geen effect aangetoond van het met één neuskweek screenen op *S. aureus* dragerschap en applicatie van mupirocine in niet-chirurgische patiënten op het voorkomen van nosocomiale *S. aureus* infecties.[35] Voor het ontwikkelen van effectieve preventie strategieën is precieze vaststelling van het type dragerschap mogelijk, zinvol en noodzakelijk, zodat deze strategieën kunnen worden gericht op die patiëntengroep die er het meest baat van heeft.

Dynamica

In het tijdperk van de multiresistente *S. aureus* is het afgewogen gebruik van antibiotica in de behandeling, maar ook de preventie van *S. aureus* infecties noodzakelijk.[36,37,38,39-42] Ook het nader uitwerken van alternatieve strategieën, zoals interferentie therapie,[43-45] vaccinatie,[46-48] faag therapie en niet antibiotische behandelingen (honing, lysostaphine) is hoogst gewenst.

Daarom bestudeerden wij de dynamica van artificiële inoculatie met *Staphylococcus aureus* 502A (SA-502A), een verzwakte *S. aureus* stam, als interferentie therapie voor de eradicatie van wild type *S. aureus* in vrijwilligers, patiënten met recidiverende furunculose en methicilline-resistente *S. aureus* (MRSA) (hoofdstuk 11). SA-502A is in de jaren 60 en begin jaren zeventig met succes gebruikt ter preventie en behandeling van *S.aureus* infecties bij pasgeborenen en patiënten met recidiverende furunculose.[49-53] Echter, door de ontwikkeling van nieuwe antibiotica en het optreden van een aantal complicaties, werd interferentie therapie met SA-502A verlaten.[54-56,55,56] Onze studie laat zien dat, indien toegepast bij goed gedefinieerde persisterend *S. aureus* dragers, artificiële inoculatie met SA-502A veilig en effectief is. Interferentie met SA-502A kan succesvol gebruikt worden ter vervanging van de residente wild type *S. aureus* mits de residente stam eerst kan worden geëradiceerd.

Conclusies & Toekomstperspectief

Wereldwijd is de incidentie van MRSA de afgelopen decades dramatisch toegenomen. De dreiging van vancomycine resistente *S. aureus* stammen en de opkomst van community-acquired MRSA (CA-MRSA) zijn alarmerend.
Preventie van *S. aureus* infecties en de ontwikkeling van resistentie is alleen mogelijk als de huidige preventieve strategieën worden geoptimaliseerd en nieuwe kunnen worden ontwikkeld. Precieze vaststelling van het type *S. aureus*

dragerschap is daarvoor essentieel. De waarde van sneldiagnostiek met nieuwe ontwikkelingen als real-time PCR en DNA-chip analyse hierbij, zal moeten worden vergeleken met conventionele kweek- en genotyperingsmethoden.

In dit proefschrift hebben we kunnen aantonen dat de gastheer een zeer belangrijke, zo niet de belangrijkste determinant van *S. aureus* neusdragerschap is. 'Tolereren of elimineren?' is de vraag! *S. aureus* neusdragerschap is multifactorieel bepaald, waarbij ook bacteriële en omgevingsfactoren betrokken zijn. Natuurlijk is meer onderzoek noodzakelijk om de gast-gastheer interactie verder te ontrafelen, maar de resultaten beschreven in dit proefschrift zijn richtinggevend voor nader onderzoek naar onderliggende genetische factoren bij de gast en in vivo gast-gastheer interacties. Zodat we uiteindelijk risico-patiënten en risico-bacteriën kunnen aanwijzen en zodoende onze preventie opties kunnen individualiseren.

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Dankwoord

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Dankwoord

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Curriculum Vitae

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Curriculum Vitae

Jan Leendert Nouwen werd geboren op 22 juli 1963 te Hendrik-Ido-Ambacht. Na het behalen van het VWO diploma aan de Openbare Scholengemeenschap Walburg te Zwijndrecht, begon hij 1981 begonnen met de studie geneeskunde aan de Faculteit der Geneeskunde & Gezondheidswetenschappen van de Erasmus Universiteit te Rotterdam (het huidige Erasmus MC - Faculteit). Gedurende de periode 1983-1987 was hij vier jaar bestuurslid van de Medische Faculteitsvereniging Rotterdam (MFVR) en viel hij in 1985 als secretaris als een blok voor de toenmalige voorzitster Angélique Zondag, nu nog steeds zijn levenspartner. In januari 1989 legde hij het artsexamen af. In de periode april 1989 tot juli 1990 was hij werkzaam als AGNIO Interne Geneeskunde in het toenmalige Bergwegziekenhuis (het huidige IJsselland ziekenhuis) bij Dr. G.J.H. den Ottolander. In juli 1990 startte hij met de opleiding tot internist in het toenmalige Refaja ziekenhuis te Dordrecht (het huidig Albert Schweitzer ziekenhuis) (opleider Dr. B.P. Hazenberg). Van juli 1992 tot januari 1995 vervolgde hij zijn opleiding in het Sint Franciscus Gasthuis te Rotterdam (opleider Dr. H.S.L.M. Tjen), en van januari 1995 tot juli 1996 in het Academisch Ziekenhuis Dijkzigt (het huidige Erasmus MC – Centrumlocatie) (opleider Prof. Dr. M.A.D.H. Schalekamp). Op 16 juli 1996 volgde inschrijving als internist in het specialisten register. In september 1995 begon hij op de afdeling Medische Microbiologie & Infectieziekten (hoofd Prof. Dr. H.A. Verbrugh) met de opleiding voor het aandachtsgebied Infectieziekten (opleider Dr. S. de Marie). In mei 1997 volgde registratie als internist-infectioloog. Tijdens deze opleiding begon hij met zijn wetenschappelijk onderzoek, aanvankelijk met als focus (catheter-gerelateerde) infecties bij de immuungecompromitteerde patiënt (Dr. S. de Marie), later met als focus het huidige promotie onderwerp 'Determinanten, Risico's en Dynamica van *Staphylococcus aureus* neusdragerschap' (Prof. Dr. H.A. Verbrugh, Prof. Dr. A. Hofman en 'last but not least' Prof. Dr. Dr. A. van Belkum). In het kader van dit onderzoek volgde hij tussen augustus 1998 en augustus 2000 de Master of Science opleiding 'Clinical Epidemiology'. Sinds april 2001 is hij onderwijscoördinator van de afdeling Medische Microbiologie & Infectieziekten en sinds december

2001 themacoördinator van het tweede jaars thema Infectie- & Immuunziekten binnen het curriculum Erasmusarts 2007 aan de Faculteit der Geneeskunde & Gezondheidswetenschappen. In deze hoedanigheid is hij ook lid van Centraal Coördinatoren Overleg (CCO). In juni 2004 werd hem door de medisch studenten de MORE onderwijsprijs toegekend en werd hij gekozen tot docent van het jaar. Hij is getrouwd met Angélique Zondag. Samen hebben zij twee dochters, Anouk (1995) en Lonneke (1997).

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