Clinical Epidemiological Studies on Methicillin Resistant and Susceptible Staphylococci

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Clinical Epidemiological Studies on Methicillin Resistant and Susceptible Staphylococci

Klinisch epidemiologisch onderzoek naar methicilline resistente en gevoelige staphylococcen

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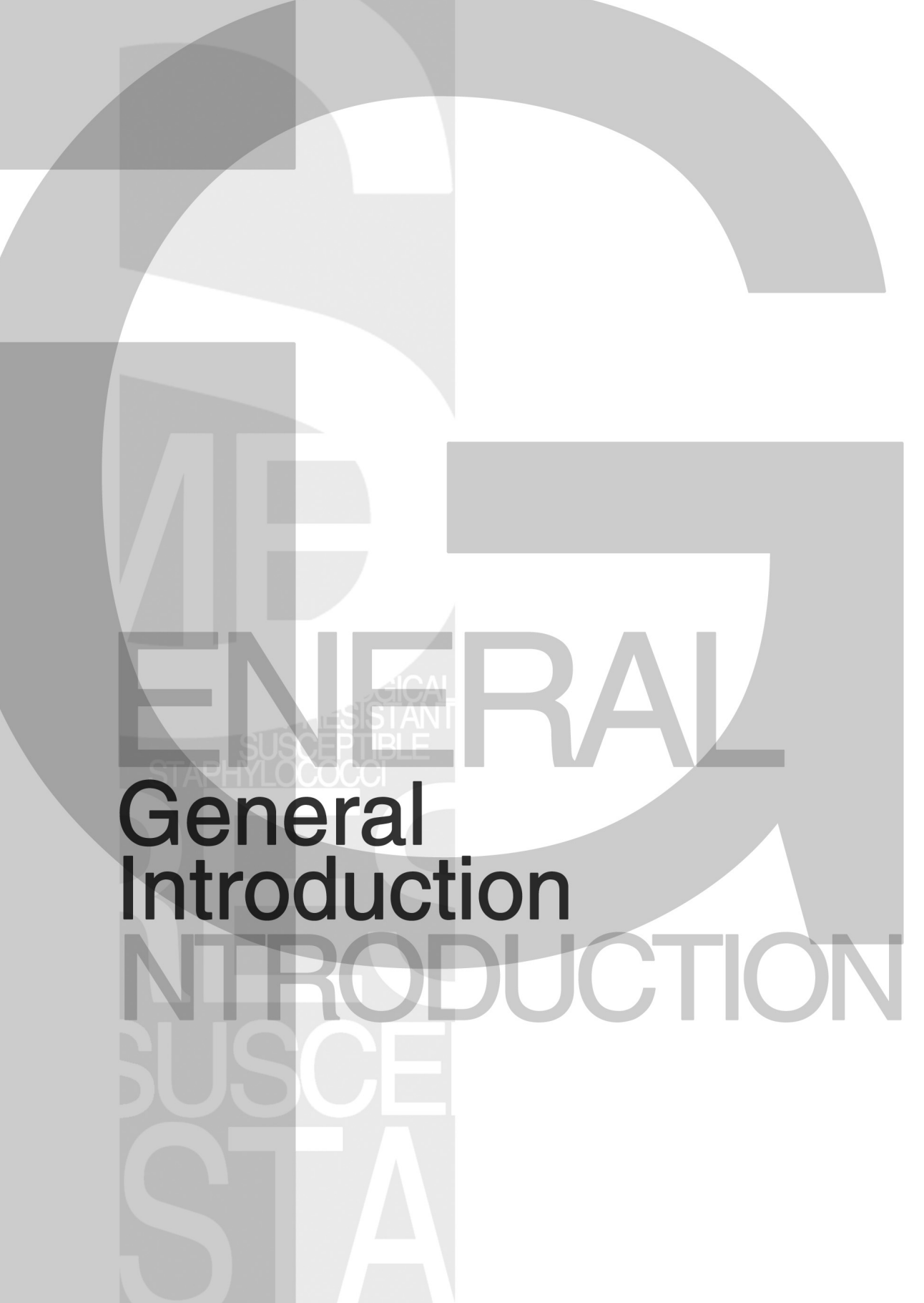
Contents



General Introduction

Chapter 1

Introduction and outline of the thesis

General introduction

Staphylococcus aureus was discovered for the first time in the 1880s [1]. Since then, *S. aureus* has been shown to be a major pathogenic Gram-positive bacterium, causing relatively mild superficial infections (e.g., furuncles or boils, post-operative wound infections) to life-threatening invasive infections (e.g., sepsis, endocarditis) [2]. In the early 1940s, prior to the introduction of penicillin, the mortality rate of individuals with a severe *S. aureus* infection was about 80% [3]. The emerge of strains with penicillin resistance was reported for the first time in 1948 [4]. Since 1960, approximately 80% of all *S. aureus* strains became resistant to penicillin. Therefore, methicillin was introduced in 1959 to treat infections caused by this penicillin-resistant *S. aureus* [5]. In 1961, two years after the introduction of methicillin, there were reports from the United Kingdom of *S. aureus* isolates that had acquired resistance to methicillin (methicillin-resistant *S. aureus*, MRSA) [6]. The cause of the development of methicillin resistance was the acquisition of the *mecA* gene [7].
During the last decades, MRSA has become the most prevalent antibiotic-resistant pathogen in hospitals in many parts of the world and a growing number of reports describe the increasing prevalence in various populations in the community [8-10]. In addition, various healthcare-associated methicillin-resistant *S. aureus* (HA-MRSA) clones disseminated worldwide [11]. Furthermore, since the 1990s, virulent community-associated MRSA (CA-MRSA) clones, characterized by the presence of the toxin Panton-Valentine Leukocidin (PVL), have spread worldwide, first in the community, but now they are also emerging in healthcare facilities [11, 12]. The CA-MRSA prevalence worldwide remains low, but an increasing prevalence has been reported [10, 13] and, some people tend to believe that the distinction between CA-MRSA and HA-MRSA is beginning to fade [2, 14]. A CA-MRSA clone which is frequently isolated is the socalled USA-300 strain. This strain has proved to be able to transmit between individuals and causes outbreaks of skin infections and boils [15, 16].

The *mecA* gene is not only present in methicillin-resistant strains of *S. aureus,* but can be found more often in coagulase-negative staphylococci (MR-CoNS) [17]. Although, coagulase-negative staphylococci have long been regarded as apathogenic, their important role as nosocomial pathogens has been recognized and studied in detail in the last two decades [18, 19]. The increased use of medical devices, such as intravascular catheters in serious ill and immunocompromised patients, is an important contributing factor for the increased isolation of (methicillin-resistant) CoNS [18]. In addition, the emergence of MR-CoNS has limited therapeutic options in case of infection and increases the risk of therapy failure [18, 20].

Colonization with bacteria

In the epidemiology of (antibiotic-resistant) bacteria an important distinction should be made between colonization and infection with a pathogen. Colonization is a prolonged presence of a pathogen in or on a host. This pathogen may multiply or grow in or on the host, but no (sub-) clinical effects are observed when the pathogen is isolated [21]. Every human being becomes colonized with different micro-organisms during and after birth until the normal human, or commensal, flora is established. This commensal flora is a dynamic process and different events, like hospitalization, may cause changes in the pathogens isolated.

Infection with a pathogen is characterized by damage of the host tissue that may result in a mild but sometimes severe infection. The latter occurs when a (antibiotic-resistant) bacteria contaminates wounds, other sterile tissues or enters the bloodstream and produces a systemic inflammatory response [21]. Therefore, colonization is a prerequisite in the pathogenesis of healthcare-associated and endogenous infections [22].

Assessing colonization with methicillin-resistant and susceptible staphylococci

As mentioned before, colonization is an important step in the pathogenesis of infections and therefore it is important to assess colonization with (antibiotic-resistant) bacteria in humans. The carriage, or colonization, rate of MRSA in the Netherlands is, together with the Nordic countries, among the lowest in the world [23]. The maintenance of such low prevalence has been ascribed to the active 'Search-and-Destroy' policy that was implemented in these countries in combination with a prudent antibiotic use [23-25]. The 'Destroy' part of this policy is important, as this eliminates two out of the three known reservoirs by MRSA eradication therapy: carriage or colonization in patients and in healthcare workers, whereas the third reservoir is long-term contamination of innate objects and surfaces in the environment. As carriage of MRSA precedes endogenous MRSA infections [24, 26-28] and plays an important role in transmission of this organism within healthcare facilities and into the community [29-34], it seems essential to eradicate MRSA from carriers. However, some regard MRSA eradication therapy as controversial because it is not evidence based and the need is questioned whether carriers with MRSA colonization but without clinical infections should be treated with antibiotics.

Methicillin-resistant CoNS together with (methicillin-resistant) *S. aureus,* are both opportunistic pathogens which are colonizing the human skin and mucous membranes and are the most prevalent pathogens causing healthcare-associated, or so-called nosocomial infections [2, 19, 35]. Healthcare-associated infections contribute to a large part of the infection rate that is observed in hospitals and are leading to an increased morbidity and mortality among patients. Furthermore, they may contribute to healthcare-associated costs because

of increased need for additional medical treatment and increased length of hospital stay [36-39]. According to isolation guidelines of the Centers for Disease Control and Prevention (CDC) and the Dutch national Working Party of Infection Prevention (www.wip.nl), nursing in single hospital rooms may be an effective method to prevent cross-transmission of pathogens that can cause nosocomial infections [40, 41].

It has been demonstrated by several studies that (methicillin-resistant) CoNS clones can spread within a hospital [42-48] and can even have geographic dissemination [49]. Despite the importance of MR-CoNS as a cause of healthcare-associated infections, limited data is available on the incidence and risk of acquisition and transmission of MR-CoNS skin carriage during hospital admission.

Although different body sites such as the throat, perineum or skin can be colonized, *S. aureus* most frequently colonizes the nasal cavity [27, 50]. In the past, three different *S. aureus* nasal carriage patterns have been distinguished: persistent carriage, intermittent carriage and non carriage [27]. Recently a reclassification has been made which revealed that there are only two different carriage types: persistent carriers and others [51]. Persistent *S. aureus* carriage rates vary between different populations studies and mechanisms leading to persistent *S. aureus* nasal carriage appear to be multifactorial [27, 50, 52-54]. Several subgroups of patients e.g. intravenous drug users, diabetic patients, and patients with hemodialysis or continuous ambulatory peritoneal dialysis (CAPD) have been associated with an increased rate of persistent nasal carriage compared to healthy individuals [27]. Human immunodeficiency virus (HIV) infected patients are also thought to be more prone on becoming persistent *S. aureus* nasal carriers and subsequently are more at risk of *S. aureus* infections [55-57]. Earlier uncontrolled studies demonstrated high *S. aureus* carriage rates among HIV infected patients with a concordant high risk of developing *S. aureus* infections [2, 27, 52-54, 58-60]. An indirect relationship between persistent nasal carriage and the immune system or a direct effect of antiretroviral therapy have been suggested [55]. In addition, HIV infected patients with low CD4 cell counts have been demonstrated to have an increased risk of becoming infected with *S. aureus* [56]. However, the exact mechanism(s) underlying these high *S. aureus* carriage and infection rates still remain to be elucidated.

Outline of this thesis

The aims of the studies described in this thesis are to explore the clinical epidemiology of methicillin-resistant and susceptible staphylococci carriage, transmission and eradication. It is essential to reduce colonization of methicillin-resistant and susceptible staphylococci to prevent infections with these pathogens. Therefore, in **Part I** we want to gain insight in

the colonization and transmission rate of methicillin-resistant staphylococci and we want to determine the effectiveness of MRSA eradication.

As the contribution of transmission in households to the MRSA burden has so far not been studied, and because of lack of data and well-calculated scenarios, no evidence based policy for this reservoir has been developed. In **Chapter 2** we perform a prospective observational study to gain insight in the rate of, and risk factors for transmission of MRSA to household contacts.

In **Chapter 3** we describe an observational study in which we assess the outcomes of MRSA eradication therapy, the determinants of MRSA eradication therapy failure, and the minimal number of follow-up culture sets needed after completion of MRSA eradication therapy to accurately assess the effectiveness of MRSA eradication therapy.

With the prospective cohort study described in **Chapter 4** we want to gain insight in the incidence and possible risk factors for MR-CoNS skin carriage acquisition during hospital admission. Furthermore, we want to gain insight in the dynamics of MR-CoNS acquisition of patients assigned to single bed versus those assigned to stay in hospital rooms with four beds.

In **Part II** of this thesis, we want to gain insight in the *S. aureus* colonization rate in HIV infected patients, as this population is known to be at an increased risk to develop *S. aureus* infections.

In **Chapter 5** we describe a case-control study in which we aim to identify determinants of *S. aureus* and *S. pneumoniae* carriage in an outpatient population of HIV infected individuals as compared to healthy controls. Within the HIV infected patients, we specifically want to test if CD4 cell levels and antiretroviral therapy are associated with *S. aureus* and *S. pneumoniae* carriage.

In sub-Saharan Africa, where the prevalence of HIV is high, the burden of disease caused by staphylococci is significant, also among HIV uninfected individuals. In **Chapter 6** we set out to assess the prevalence and determinants of persistent *S. aureus* carriage, as well as the prevalence of antibiotic resistance in *S. aureus* strains in an outpatient HIV infected adult population in rural Zambia.

The results presented in the previous chapters are discussed in **Chapter 7** and future perspectives are addressed.

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PART I

Methicillin Resistant Staphylococci

Chapter 2

Transmission of MRSA to household contacts

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Abstract

The frequency and risk factors for MRSA transmission of a MRSA index person to household contacts were assessed in this prospective study.

Between January 2005 and December 2007, 62 newly diagnosed MRSA index persons (46 patient and 16 healthcare workers) and their 160 household contacts were included in the study analysis.

Transmission of MRSA from an index person to household contacts occurred in nearly half of the cases (47%; n=29). These 29 index persons together had 84 household contacts of which two-thirds (67%; n=56) became MRSA positive. Therefore, the reproductive number R_0 is < 1. Prolonged MRSA exposure time to MRSA at home was a significant risk factor for MRSA transmission to household contacts. In addition, MRSA colonization at least in the throat, younger age and eczema in index persons were significantly associated with MRSA transmission; the presence of wounds was negatively associated with MRSA transmission. Furthermore, an increased number of household contacts and being the partner of an MRSA index person were household related risk factors for MRSA acquisition of the index person. No predominant PFGE type was observed which transmitted more frequently compared to other PFGE types.

To date, screening household contacts and, to those found positive, providing MRSA eradication therapy simultaneously with the index person is not included in the 'Search-and-Destroy' policy. We suggest including both in MRSA prevention guidelines, as this may reduce further spread of MRSA.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is currently the most prevalent antibiotic-resistant pathogen in hospitals in many parts of the world and there are a growing number of reports describing the increasing prevalence in various community populations [1-3]. MRSA is an important cause of infections and MRSA infections are increasing in both healthcare centers and the community. Compared to methicillin-sensitive *Staphylococcus aureus* (MSSA), infections with MRSA are more difficult to treat and tend to have a poorer outcome [4, 5].
Carriage of MRSA is a prerequisite for most MRSA infections and plays an important role in the dissemination of this organism within healthcare facilities and the community [6-9]. In the Netherlands, due to the 'Search-and-Destroy' infection control policy and a strict antibiotic policy, the number of patients colonized with MRSA is still very limited [10-12]. The 'Destroy' part of this policy is important, as it eliminates two out of the three known reservoirs: carriage in patients and carriage in healthcare workers, whereas the third reservoir is the environment. But even in low-prevalence countries like the Netherlands, the emergence of community-acquired MRSA has caused a change in MRSA epidemiology and an increasing number of MRSA cases [10].

In the past, it has been shown that carriers of *Staphylococcus aureus* and MRSA can be a source of transmission of these pathogens to their household contacts [13-17]. The exact risk factors for transmission of MRSA to household contacts has not been studied properly, but close contact, the environment or being a healthcare worker (HCW) are thought to be plausible risk factors for transmission [18-20].

The contribution of transmission in households to the MRSA burden has so far not been studied and because of lack of data and well-calculated scenarios, no evidence based policy for this reservoir has been developed. For this reason, being a household contact of a MRSA carrier has not yet been established as a risk group for MRSA by the Dutch 'Search-and-Destroy' policy.

The aims of this study are to gain insight in the frequency of, and risk factors for transmission of MRSA to household contacts and therefore into the community.

Patients and Methods

Data collection

All newly diagnosed MRSA positive persons between January 2005 and December 2007 being admitted, treated in the outpatient clinic or being a healthcare worker (HCW) at the Erasmus University Medical Center (Rotterdam, The Netherlands) or in a general hospital in Rotterdam, The Netherlands (Maasstad Hospital) and their household contacts were invited to participate in this prospective observational study. Index persons without household contacts were not included in this study. Informed consent was obtained from all persons and their household contacts.

Household contacts were screened for MRSA to determine whether transmission from index to household contacts had taken place.

Definitions

Index persons were patients or HCWs with newly diagnosed MRSA. Household contacts were defined as persons living in the same house as the initial MRSA index person or having frequent contact in the same house (more than 2 hours per day) with the index person. MRSA transmission was defined as a positive MRSA swab from the anterior nares, throat, perineum, wounds or skin lesions when present, in one of the household contacts during the period of exposure to the index person. The MRSA strain from the household contact had to have the same PFGE pattern as that of the index person.

The basic reproductive ratio (R_0) is the number of secondary MRSA cases generated from a single MRSA index person introduced into a susceptible population of household contacts.
The total exposure time of MRSA positivity between the index and household contacts was defined as the time between the first positive MRSA culture of the index and the swabs taken from the household contacts. Exposure time at home was defined as the time between hospital discharge and swabs obtained from household contacts.

Data to determine risk factors for transmission from index to household contacts were collected by means of a standard questionnaire. Questions addressed whether the index person was a healthcare worker or a patient, whether they had current skin problems, non-intact skin (due to wounds, skin lesions or indwelling devices) or whether they had indwelling devices (e.g. drains or catheters) in situ. Household related data concerned household composition, relation of the household contacts to the index patient, age, sex, and number of hours of contact with the index person.

Microbiology methods

Cultures for MRSA were taken from the throat, the anterior nares and the perineum. Existing skin lesions, wounds or invasive devices (drains, catheters, and external osteosynthesis material) present at the time of MRSA detection were also simultaneously cultured.

MRSA screening swabs were tested and identified using a PCR assay as described earlier [21].

Pulse-field gel electrophoresis (PFGE)

All MRSA isolates were molecularly typed by pulse-field gel electrophoresis (PFGE). To verify the relatedness of MRSA from index patients and household contacts, the isolates from index and household contacts were compared. Intrafamilial transmission, from index person to a household contact, was considered when PFGE types were identical [22].

Statistical analysis

All analyses were performed using SPSS 15.0 for Windows (SPSS Inc. Chicago, IL, USA). Proportions were compared by the Chi-square test (Fisher's exact test in case of small numbers) and continuous data with Mann-Whitney U test. Results are reported as odds ratios (OR) with 95 percent confidence intervals (95% CI). The statistical tests were 2-tailed, a p-value of less than 0.05 was considered statistically significant.

Results

Between January 2005 and December 2007, 62 MRSA positive persons (46 patients and 16 healthcare workers) and their 160 household contacts agreed to participate and were included in the analysis. The median age of the MRSA positive persons was 33 years (range 0-87 years) and 58% (n=36) were male. The median age of the household contacts was 28 years (range 0-77 years) and 49% (n=77) were male. The general characteristics of the index persons are shown in Table 1. The diversity of MRSA colonization sites of the index persons are depicted in Figure 1.

In 33 of the 62 (53%) index cases, no transmission to their household contacts was observed. These 33 index persons together had 76 household contacts. Transmission of MRSA from an index to household contacts occurred in 29 out of the 62 (47%) index persons. These 29 index persons together had 84 household contacts. The attack rate of MRSA transmission in the 84 household contacts was 67%, as 56 household contacts became MRSA positive. Furthermore, the basic reproductive ratio R_0<1.

Table 1: General characteristics of MRSA index persons at time of MRSA detection

Characteristics	MRSA index persons n=62 (%)
Site of acquisition	
• Erasmus Medical Center	52 (84)
• Maasstad Hospital	10 (16)
Type of person	
• healthcare worker (HCW)	16 (26)
• patient	46 (74)
Gender	
• male	36 (58)
• female	26 (42)
Age	
• median (range)	33 (0-87)
• age categorical in yr	
○ 0 – 10 yr	15 (24)
○ 11 – 20 yr	1 (2)
○ 21 – 60 yr	39 (63)
○ > 61 yr	7 (11)
MRSA colonization [□]:	
median number of colonized sites (range)	2 (1-5)
○ nose	54 (87)
○ throat	42 (68)
○ perineum	23 (37)
○ other [a]	28 (45)
• nasal only	8 (13)
• extra nasal only	8 (13)
• nasal and extra nasal	46 (74)
Potential risk factors	
• non-intact skin [b]	31 (50)
• skin problems	14 (23)
○ eczema (n=58)	4 (7)
• wounds	22 (36)
• indwelling device	14 (23)
○ iv catheter	10 (16)
○ urine catheter	3 (5)
○ drain	7 (11)
○ osteosynthesis material	2 (3)
Exposure	
• median number of household members/index person (range)	3 (1-10)
• household composition	
○ with partner only	21 (34)
○ with partner + children	16 (26)
○ with parents	10 (16)
○ with parents + siblings	11(18)
○ with children only	1 (2)
○ other	4 (5)

[□] combination of more than one colonization site possible

[a] urine n=4; exit sites n=6; wound n=17; other n=1

[b] non-intact skin due to wounds, skin problems or indwelling devices

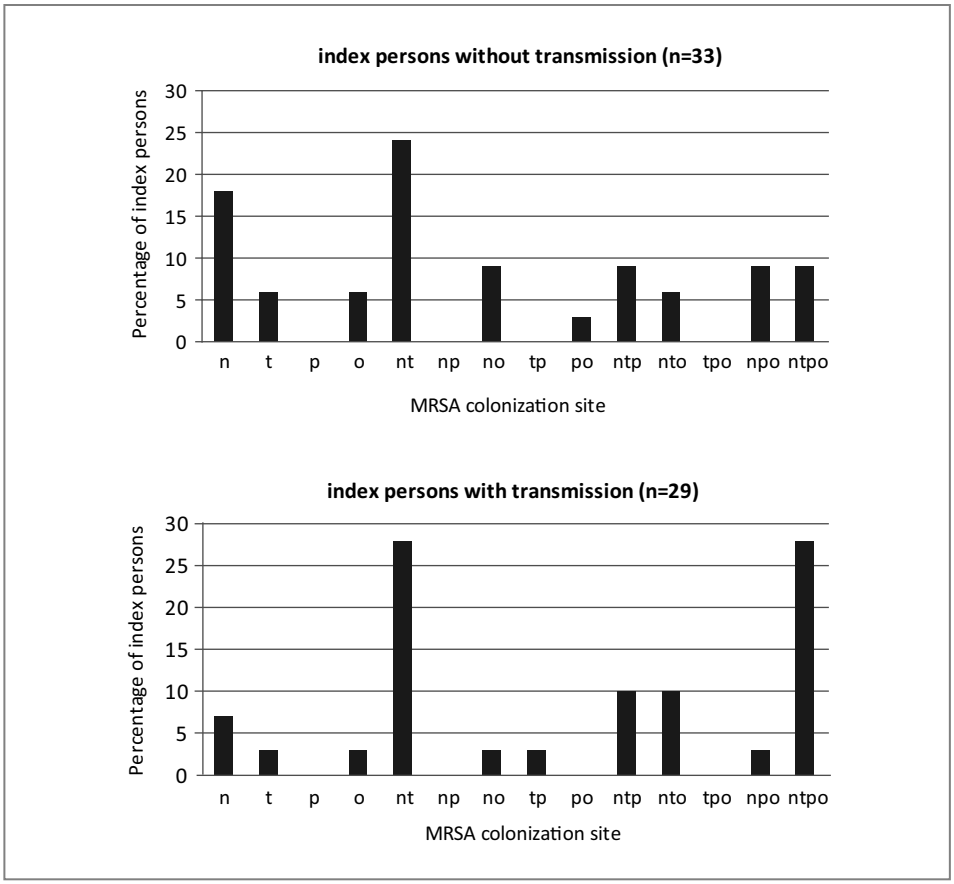

Figure 1: MRSA colonization sites in 62 MRSA positive index persons
n=nose only; t=throat only; p=perineum only; o=other only; nt=nose+throat; np=nose+perineum; no=nose+other; tp=throat+perineum; po=perineum+other; ntp=nose+throat+perineum; nto=nose+throat+other; tpo=throat+perineum+other; npo=nose+perineum+other; ntpo=nose+throat+perineum+other

Risk factors for MRSA transmission to household contacts are shown in Table 2. Of the index persons without transmission (n=33) the median total exposure time was 14.5 days (range 2 – 330). In the group with transmission to the household contacts, the median total exposure time was 29 days (range 1 – 1134). Duration of MRSA exposure time at home was significantly associated with MRSA transmission to household contacts (41 days vs 15 days; p=0.04). Younger age, MRSA colonization at least in the throat and eczema of the index person were significantly associated with MRSA transmission. Interestingly, the presence of wounds was negatively associated with MRSA transmission (Table 2).

Table 2: Index person related potential risk factors for MRSA transmission

Potential risk factors	transmission of MRSA (%) n=29	no transmission of MRSA (%) n=33	p-value	OR 95% CI
Type of person				
• healthcare worker	5 (17)	11 (33)	0.15	0.42 (0.13-1.39)
Gender				
• male	17 (59)	19 (58)	0.93	1.04 (0.38-2.87)
Age				
• median age in yr (range)	25 (0-87)	45 (0-80)	0.05 [a]	
• age categorical in yr				
◦ 0 – 10 yr	8 (28)	7 (21)	0.93	
◦ 11 – 20 yr	1 (3)	0 (0)	1.00	
◦ 21 – 60 yr	17 (59)	22 (67)	0.52	0.68 (0.21-2.23)
◦ > 61 yr	3 (10)	4 (12)	0.65	0.66 (0.11-4.00)
MRSA colonization				
• median number of MRSA colonized sites (range)	3 (1-5)	2 (1-5)	0.15[a]	
• colonization at least in:				
◦ nose	26 (90)	28 (85)	0.71	1.55 (0.34-7.13)
◦ throat (n=61)	24 (83)	18 (56)	0.03	3.73 (1.14-12.27)
◦ perineum (n=61)	13 (45)	10 (21)	0.28	1.79 (0.63-5.09)
◦ other	14 (48)	13 (39)	0.48	1.44 (0.52-3.94)
• MRSA throat colonization only	1 (3)	2 (6)	0.63	0.55 (0.05-6.44)
• MRSA extra throat colonization only	5 (17)	15 (46)	0.02	0.25 (0.08-0.82)
• MRSA throat and extra throat	24 (83)	18 (55)	0.02	4.00 (1.23-13.05)
Potential risk factors				
• non intact skin	12 (41)	19 (58)	0.20	0.52 (0.19-1.43)
• skin problems	8 (28)	6 (18)	0.38	1.71 (0.52-5.71)
◦ eczema (n=58)	4 (14)	0 (0)	0.05 [b]	
• wounds	6 (21)	16 (42)	0.02	0.28 (0.09-0.86)
• indwelling devices	4 15)	10 (30)	0.12	0.37 (0.10-1.34)
Exposure				
• median number of household members (range)	3 (1-7)	1 (1-10)	0.007 [a]	
• exposure time to MRSA in days (median, range) (n=56)	29 (1-1134)	14.5 (2-330)	0.07 [a]	
• exposure time to MRSA at home in days (median, range) (n=51)	41 (1-1134)	15 (1-330)	0.04 [a]	

[a] Mann-Whitney U test
[b] Fisher's exact test

Households with transmission of MRSA had significantly higher median number of household contacts compared to households without transmission (3.0 vs 1.0, p=0.007). The risk of MRSA transmission to household contacts was highest among partners of the index person (p = 0.02, OR 5.20, 95% CI 1.10 – 24.52). The hours of contact between household members and the index persons were not associated with an increased risk on transmission. Household related determinants of transmission are shown in Table 3.

Table 3: Risk factors for MRSA transmission in household members

	transmission of MRSA n=56 (%)	no transmission of MRSA n=28 (%)	p-value	OR (95% CI)
Female	28 (50)	12 (43)	0.54	1.33 (0.54-3.22)
Median age in yr (range)	23 (0-77)	18 (4-50)	0.77 [a]	
Relation to index				
• partner	16 (29)	2 (7)	0.02	5.20 (1.10-24.52)
• child	11 (20)	8 (29)	0.36	0.61 (0.21-1.75)
• parent	16 (29)	9 (32)	0.74	0.84 (0.32-2.26)
• sibling	8 (14)	4 (14)	1.00 [b]	
• other	5 (9)	5 (18)	0.29	0.45 (0.12-1.71)
Contact with MRSA index in hrs/day [x]				
• <1	2(5)	2 (11)	0.39	1.00
• 1-4	3 (8)	4 (22)	0.82	0.75 (0.06-8.83)
• 5-10	11 (30)	8 (28)	0.49	1.10 (0.24-20.40)
• >10	21 (57)	7 (39)	0.31	3.00 (0.35-25.46)

[a] Mann-Whitney U test
[b] Fisher's exact test
[x] Hours of contact per day with the index person was only retrieved in 55/84 household contacts

PFGE strains of index persons and their MRSA positive household contacts were compared to determine if MRSA transmission took place. Thirty different PFGE patterns were identified in the 62 MRSA positive index persons. The 29 index persons that had apparently transmitted their strain to their household contacts had 20 genotypically different MRSA strains. All MRSA positive household members had the same PFGE pattern as their index person. There was no dominant PFGE type that was transmitted more frequently compared to other PFGE types.

Discussion and Conclusion

Our study shows that MRSA transmission from an index person to household contacts occurs in approximately half the cases (47%). Furthermore, when an index person transmits MRSA to household contacts, two thirds of all household contacts (67%) will acquire MRSA carriage. Risk factors for MRSA transmission were identified in both MRSA index persons and household contacts. MRSA carriage at least in the throat, the duration of MRSA exposure time at home, eczema and younger age were all significant risk factors for transmission to household contacts. Furthermore, an increased number of household contacts was associated with transmission. A household contact related risk factor for acquisition of MRSA was being a partner of the index person.

As our study was conducted in the Netherlands where the prevalence of MRSA is amongst the lowest in the world [10], the MRSA transmission rate in our study may not be representative for countries were the MRSA prevalence is higher. In these latter countries it is more difficult to determine whether the index person was the MRSA source in case of a similar PFGE strain is cultured from a household contact, or whether the strain was picked up from another source in the community. In such circumstances, it remains equivocal whether intrafamilial transmission has occurred. As our study demonstrated a large diversity of different PFGE types in combination with a very low prevalence of MRSA in our community, we can better ascertain that the household contact obtained the MRSA from the index person.

In previous studies it has been demonstrated that the environment of the MRSA index person may have acted as an intermediate source for transmission to household contacts [18, 23, 24]. In that case, the index has first contaminated the household environment and, therefore, the environment served as a MRSA source for the household contacts. In this study we did not attempt to establish the potential role of the environment on MRSA transmission as we did not collect environmental specimens in the homes of the index person. A study by Boyce et al. [23] showed that the frequency of environmental contamination in a hospital by MRSA was higher when patients had MRSA positive wounds or urine, than when MRSA was present in other body sites. Studies on MRSA contamination of the environment in a household setting have yet to be reported. Thus, when household contacts share equipment or personal items of a MRSA index person, it is plausible that they also are at risk of becoming colonized with MRSA.

The spread of MRSA among household contacts has been previously reported, but the observed MRSA transmission rates to household contacts are variable [13, 16, 25-28]. Johansson et al [13] observed that in 22 of 51 index persons (43%) MRSA was transmitted to one or more household contacts. However, Calfee et al [16] observed a lower MRSA transmission rate than in our study as 21 of the 88 index persons (24%) transmitted MRSA to their household contacts. Most studies reporting about MRSA transmission from index persons to household members differ in study methods and therefore comparison of study outcomes with that of our study cannot yield robust conclusions [25, 27-29]. These studies differ mostly in the sites cultured. Ho et al [26] observed MRSA transmission in 12 index persons with 46 household members at risk. Six (13%) of the household members became MRSA positive. In this study, swabs were taken from the anterior vestibule of the nose, axilarry skin and cutaneous or wound lesions. Thus, no perineal nor throat swabs were obtained. The lack of perineal and, especially, throat swabs has also been observed in other studies [25, 27, 28]. Therefore these studies have probably underestimated MRSA carriage of index persons and MRSA transmission to household contacts [30, 31].

Several risk factors for MRSA transmission were observed in our study. First, MRSA throat carriage of the index person significantly increases the risk for transmission to household contacts. Studies on MRSA transmission into the environment, showed that respiratory secretions can contribute to the transmission into the environment [20, 24], suggesting that transmission of MRSA can occur by the dispersing of MRSA from the throat by coughing, sneezing or kissing. For this reason, it is important to establish colonization not only by swabbing the anterior nares, but also the throat. Second, we demonstrated that the risk on transmission from index persons to household contacts depends on MRSA exposure time at home. Calfee et al [16] demonstrated that index persons, who returned home while known to be MRSA positive, gave significantly more MRSA transmission to their household members than index persons who returned to a (residential) care home. Healthcare workers had a significant shorter exposure time at home compared to the MRSA positive patients. This can be explained by the fact that healthcare workers are offered eradication therapy immediately after MRSA carriage is detected as MRSA positive HCWs are not allowed to work in the Netherlands. Furthermore, our study revealed that index persons who transmitted MRSA to one or more of their household contacts had on average more household contacts than those who did not transmit, i.e. their households were possibly more crowded. Crowding has been shown before to be a risk factor for transmission of MRSA [13, 16]. Third, people with eczema tend to give more transmission of MRSA to their household contacts, than people without eczema. A possible explanation is that eczema sites usually are not covered by skin bandages and, therefore, dispersion of MRSA on skin particles is not impeded and thus contamination of the environment with MRSA is more likely. Similarly, we showed that covered wounds or skin lesions significantly protect against transmission of MRSA from index persons to their household contacts. This is contrary to a study by Moore et al [32] showing that broken skin or chronic skin lesions contribute to the acquisition of MRSA in roommate contacts with MRSA colonization or infection. We assume that in our setting, due to strict aseptic wound care including proper coverage of wounds and hygienic precautions, the risk of MRSA transmission may be significantly lowered.
Interestingly, the median age of index persons that did experience household transmission was significantly lower than the median age of those without transmission (25 years vs. 45 years). This is confirmed by the studies performed by Johansson et al [13] and Calfee et al [16]. This age-related effect is not readily explained but may be associated with more crowded living conditions in the household in the younger age groups compared to the elderly.

The relation of a household member to an MRSA index person also was a risk factor in our study. Partners of MRSA index persons are more likely to become MRSA colonized than other household relations of the index person. A possible explanation may be that partners share bed linen and have bodily contact, which is a known risk factor for MRSA acquisition [29]. No other household related determinants for MRSA transmission could be revealed in our study because no further clinical data from household contacts was available.

Intrafamilial transmission of MRSA has raised important issues, such as whether household screening should be routinely performed to search for carriers and treat them. Failure to identify MRSA positive household contacts may cause MRSA recolonization of the index patient and also contribute to the spread of MRSA into the community. Silent recolonization of index persons can reintroduce MRSA into the hospital. Even though the $R_{0,}$ as measured in the household contacts was < 1, MRSA transmission may contribute to the spread of MRSA in specific community populations of the index person or MRSA positive household contacts [33, 34].

To date, screening household contacts and, to those found positive, providing eradication therapy in temporal fashion with the index person is not implemented in the “search-and-destroy” policy in The Netherlands or elsewhere. We suggest including both in prevention guidelines for MRSA as this may reduce further spread of MRSA strains into the community and in healthcare settings.

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Chapter 3

Treatment of MRSA carriage: how to be successful and when to be sure it is

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Abstract

In this study analysis the success rate of MRSA eradication therapy was assessed. Furthermore, the number of follow-up culture sets to ascertain the MRSA-free status of a person after MRSA eradication therapy were determined.

Between January 2005 and December 2007 MRSA was detected in 165 persons. Fifty-five of these persons were excluded from analysis because of 1) treatment elsewhere (n=26), 2) presence of relative contraindications for MRSA eradication therapy (n=10), 3) lost to follow-up after first MRSA detection (n=5), 4) non-compliance to treatment (n=2) or 5) death before treatment could be offered (n=12). Of the remaining 110 persons, 20 (18%) spontaneously cleared their MRSA and two persons were lost to follow-up after being considered for MRSA eradication therapy. Of 88 persons who received eradication therapy in 68% (n=60) the treatment was succesful. MRSA eradication therapy was successful in 81% (n=71) of persons (intent-to-treat analysis) with a mean of 1.5 eradication therapies (range 1 – 3).

Presence of wounds before the start of MRSA eradication therapy significantly hampered MRSA eradication therapy. As 31% (n=11) of the persons had a MRSA positive swab after the third consecutive negative culture set (swabs obtained from nose, throat, and perineum and non-intact skin or indwelling devices when present), five or more culture sets should be obtained to ascertain the MRSA negative status of a previously MRSA positive person.

Introduction

Carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) precedes endogenous MRSA infections and causes dissemination of this organism within healthcare facilities and into the community [1-4].

MRSA is currently the most prevalent antibiotic-resistant pathogen in hospitals in many parts of the world and a number of reports describe the increasing prevalence in various populations in the community [5-7]. The carriage rate of MRSA in the Netherlands is, like the Nordic countries, among the lowest in the world [8] because of an active 'Search-and-Destroy' policy that is being maintained in combination with a restraint antibiotic use [9, 10]. The 'Destroy' part of this policy is important, as this eliminates two out of the three known reservoirs: carriage in patients and carriage in healthcare workers, whereas the third reservoir is the environment.

Eradication of MRSA from the human reservoirs (e.g. anterior nares, throat, perineum, skin and wounds) can be achieved by a combination of topical and systemic antimicrobial agents and disinfection of the skin. Nasal application of mupirocin, with or without chlorhexidine body wash, has been reported to be very effective in eradicating nasal carriage and moderately effective in eradicating extra-nasal MRSA carriage [11-18]. Among others, fusidic acid, trimethoprim-sulphamethoxazole and rifampicin are systemic agents that have been used for eradication of MRSA. Varying success rates of eradication therapy are reported by different studies [19-26]. The failure of MRSA eradication therapy has often been attributed to extra-nasal sites of colonization. Because of this eradication failure, it is recommended to use a combination of nasal and systemic antibiotics in case of extra-nasal MRSA colonization [20, 26, 27].

Furthermore, it remains unclear which combination of antibiotics is most efficacious in eradicating MRSA. After MRSA eradication therapy, no consensus exists regarding the number, sites and time period of cultures that should be obtained to reliably assess the MRSA status of a previously MRSA positive individual. The Dutch national policy by the Working Party on Infection Prevention (WIP) suggests that a minimum of three follow-up culture sets should be obtained to declare a previous MRSA carrier as negative. However, this culture rule is not based on solid experimental evidence but on expert opinion [28].

The aim of this observational study was threefold: 1) to assess the success rate of MRSA eradication therapy by using our MRSA eradication therapy protocol; 2) to analyze determinants predicting MRSA eradication therapy outcome and 3) to assess the minimum number of follow-up screening moments after completion of MRSA eradication therapy needed to determine the effectiveness of MRSA eradication therapy.

Patients and Methods

Data collection

All MRSA positive healthcare workers (HCWs) or patients who were newly detected, treated and followed up by the Erasmus University Medical Center (Rotterdam, The Netherlands) between January 2005 and December 2007, were included in this observational prospective follow-up study analysis. No informed consent was obtained from persons participating in the analysis as offering MRSA eradication therapy and follow-up is standard hospital infection prevention policy.

Through medical chart review, demographic and other characteristics of each MRSA positive person were obtained. Of HCWs, essential data were recorded by infection prevention practitioners.

Definitions

At the time of first detection of MRSA (baseline measurement) as well as before the start of MRSA eradication therapy (second measurement) potential determinants of MRSA eradication therapy failure were assessed.

According to our hospital MRSA eradication protocol, the presence of indwelling devices including drains, catheters, tracheostomas, other implanted materials or non-intact skin are relative contraindications for MRSA eradication therapy [25, 29]. Therefore, persons with these relative contraindications were preferably postponed for MRSA eradication therapy until such relative contraindications had ceased to exist. In each individual MRSA positive person with these relative contraindications for therapy failure, the urgency to start MRSA eradication therapy was carefully evaluated. Therefore some persons could receive MRSA eradication therapy when relative contraindications were still present.

At both baseline and second measurement, cultures for MRSA were taken from all defined culture sites; the anterior nares, the throat and the perineum. If there were any skin lesions, wounds or indwelling devices present, these were cultured as well.

Eradication therapy

Healthcare workers (HCWs) received eradication therapy immediately after detection of MRSA. Patients, who had negative cultures for MRSA at all sites at the second measurement, were offered the ‘wait-and-see’ option. The ‘wait-and-see’ option implied postponing treatment of these patients and to start follow-up screening at regular intervals (see below).

In general, the first choice MRSA eradication therapy consisted of nasal mupirocin ointment plus chlorhexidine body wash, and in case of extra-nasal colonization this therapy was extended by a combination of oral administration of fusidic acid or trimethoprim and rifampicin. In case of perineal colonization, an oral solution of gentamicin was added. In case of resistance to one or more antibiotics of the regimen, or if the person had known adverse effects, another antibiotic combination was used. MRSA eradication regimens were categorized into five groups and are showed in Table 1.

In addition, all MRSA positive persons received hygienic instructions which included changing underwear and clothes daily and using clean washcloths and towels every day. On day one, two and five of the MRSA eradication therapy the bed linen was advised to be changed and clean pajamas and underwear had to be worn when going to sleep.

Table 1: MRSA eradication therapies offered to MRSA positive persons

	MRSA positive (n=88)
1: mupirocin (2/day, 5 d) + chlorhexidine bodywash (1/day, 5 d)	18 (21%) [a]
2: 1 + trimethoprim (2/day 200mg, 10 d) and rifampicin (1/day 600 mg, 10 d)	23 (26%)
3: 2 + gentamicin oral solution (3/day 80 mg, 10 d)	3 (3%)
4: 1 + fusidic acid (3/day 500 mg, 10 d) and rifampicin (1/day 600 mg, 10 d)	13 (15%)
5: other	31 (35%)

[a] 6 persons with extra-nasal colonization

Follow-up

All MRSA positive persons were followed-up by surveillance culture sets from all defined culture sites. The follow-up cultures were taken with a maximum frequency of two culture sets per week. MRSA eradication therapy was considered successful and follow-up was ended when six consecutive sets of MRSA cultures, starting not earlier than one week after completion of the whole course of eradication therapy, were negative. Spontaneous MRSA eradication was defined as patients with the 'wait-and-see' option and six consecutive sets of cultures with no growth of MRSA, did not receive any MRSA eradication treatment.

Treatment failure was defined as a MRSA positive culture during the follow-up period. In case of treatment failure a new treatment regimen was offered unless newly arisen relative contraindications for MRSA eradication therapy were present. Other reasons to consider postponing MRSA eradication treatment were acquired resistance against the first-line treatment, limited antibiotic treatment options remaining or the wish of the person to stop or withhold MRSA eradication treatment.

A maximum of three antibiotic treatment courses were given. If there was treatment failure after three regimens, no further attempts of MRSA eradication were made.

Microbiological methods

Bacterial cultures for MRSA were obtained by swabbing the throat, the anterior nares and the perineum. Whenever skin lesions were present they were cultured as well.
MRSA screening swabs were tested and identified using a PCR assay as described before by Kerremans et al [30].

Statistical analysis

Analyses were performed using SPSS 15.0 for Windows (SPSS Inc. Chicago, IL, USA). Proportions were compared by the Chi-square test (Fisher's exact test in case of small numbers) and continuous data with Mann-Whitney U test. Results are reported as odds ratios (OR) with 95 percent confidence intervals (95% CI). The statistical tests were 2-tailed, a p-value of less than 0.05 was considered statistically significant.

Results

Between January 2005 and December 2007 165 persons (23 HCWs, 142 patients) were newly detected with MRSA. Of the 165 persons, 55 were not included in the analysis because they received no eradication treatment in our hospital (n=26), MRSA eradication therapy was postponed to date later than December 2007 (census date for this study) because of relative contraindications for MRSA eradication therapy (n=10), lost to follow-up after first MRSA detection (n=5), a presumed high risk of non-compliance to treatment (n=2; both persons were homeless and did not have a permanent home address) or died before treatment could be offered (n=12). In total 110 MRSA positive persons were eligible for MRSA eradication therapy and follow-up and were, therefore included in our analysis. Baseline characteristics of healthcare workers, patients with spontaneous eradication and patients receiving eradication treatment are presented in Table 2. The distribution of MRSA colonization sites are shown in Figure 1.

No HCWs were offered the 'wait-and-see' policy. Twenty patients were assigned to the 'wait-and-see' policy, and spontaneously cleared their MRSA. The median number of MRSA colonization sites was significantly lower in patients with spontaneous MRSA eradication than in patients who received MRSA eradication therapy (see Table 2). Patients who received MRSA eradication therapy were more often colonized at nasal and extra-nasal or at extra-nasal sites only, compared to those with spontaneous MRSA eradication.

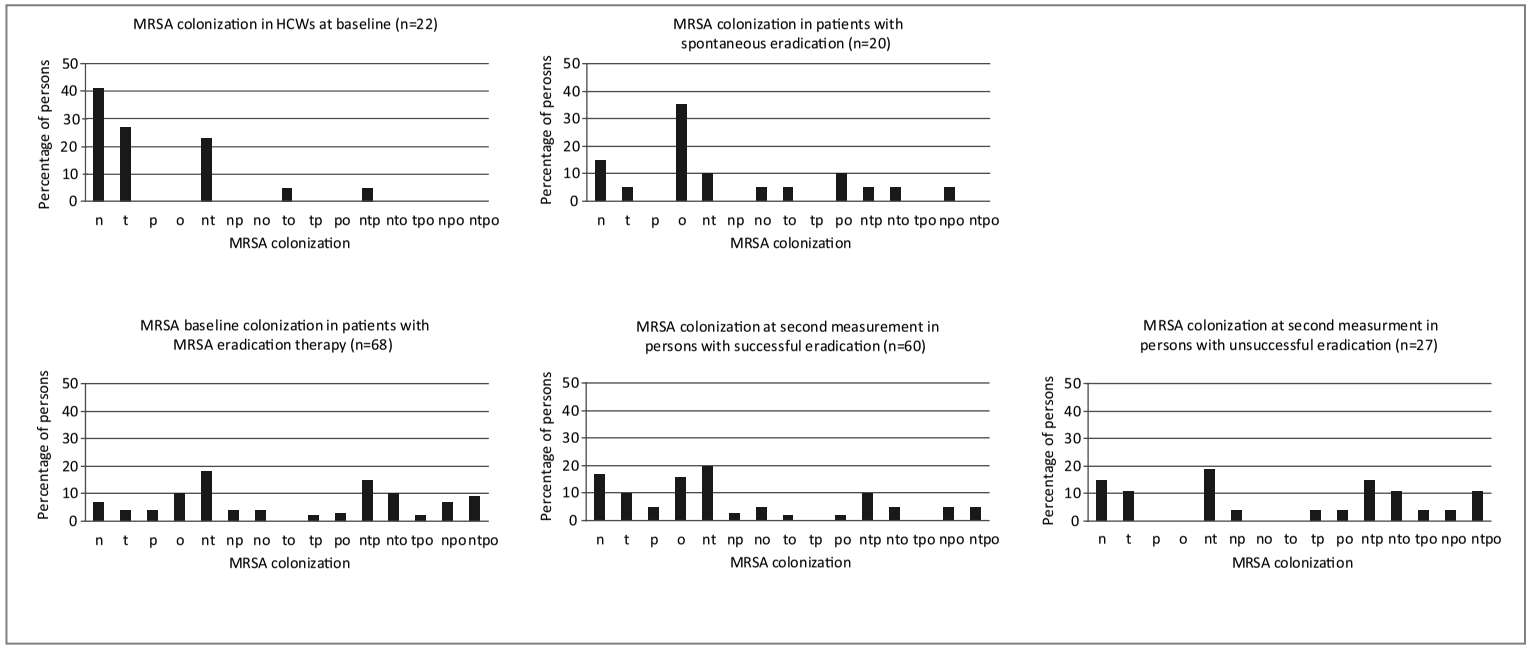

Figure 1: Distribution of MRSA colonization sites at baseline measurement in patients with spontaneous MRSA eradication, patients with eradication therapy and healthcare workers (HCWs) and at second measurement in persons with successful and unsuccessful MRSA eradication therapy.

n=nose only; t=throat only; p=perineum only; o=other only, nt=nose+throat; np=nose+perineum; no=nose+other; to=throat+other; tp=throat+perineum; po=perineum+other; ntp=nose+throat+perineum; nto=nose+throat+other; tpo=throat+perineum+other; npo=nose+perineum+other; ntpo=nose+throat+perineum+other

Table 2: General characteristics of MRSA persons as measured at the time of detection of the first MRSA positive culture.

Characteristics	Health care workers n=22 (%)	Patients with MRSA eradication therapy n=68 (%)	Patients with spontaneous MRSA eradication n = 20 (%)	Spontaneous eradication vs patients with MRSA eradication therapy p-value
Sex				0.43
• male	6 (27)	41 (60)	14 (70)	
• female	16 (73)	27 (40)	6 (30)	
Age				
• median (range), yr	29 (22-53)	37.5 (1-82)	48 (1-76)	0.10 [x]
• age categorical in yr				
◦ 0 – 10 yr	0 (0)	16 (24)	2 (10)	0.30
◦ 11 – 20 yr	0 (0)	3 (4)	1 (5)	0.48
◦ 21 – 60 yr	22 (100)	40 (59)	11 (55)	0.34
◦ > 60 yr	0 (0)	9 (13)	6 (30)	0.07
MRSA colonization site:				
• number of sites colonized (median,range)	1 (1-3)	2 (1-4)	1 (1-3)	0.005
◦ nasal only	9 (41)	5 (7)	3 (15)	0.38
◦ extra nasal only	7 (32)	17 (25)	11 (55)	0.01
◦ nasal and extra-nasal	6 (27)	46 (68)	6 (30)	0.003
Hospital admission in preceding yr	-	42 (62)	16 (80)	0.13
Non intact skin	8 (38) [a]	44 (66) [b]	15 (75)	0.43
• wounds	4 (19) [a]	34 (52) [c]	14 (70)	0.15
• skin problems	8 (38) [a]	15 (22) [b]	4 (20)	0.82
Invasive device (total)	-	23 (34) [b]	11 (55)	0.10
• catheter	-	19 (29) [d]	8 (40)	
• drain	-	11 (17) [d]	4 (20)	
• tracheostoma	-	2 (3) [c]	2 (10)	
• implant	-	9 (14) [c]	6 (30)	

Table 2: Continued

Characteristics	Health care workers n=22 (%)	Patients with MRSA eradication therapy n=68 (%)	Patients with spontaneous MRSA eradication n = 20 (%)	Spontaneous eradication vs patients with MRSA eradication therapy p-value
Underlying disease (total)	2(10%) [a]	11 (16) [b]	6 (30)	0.18
• Diabetes Mellitus	1 (5%) [a]	2 (3) [b]	2 (10)	
• COPD	-	3 (5) [b]	1 (5)	
• Renal insufficiency	1 (5%) [a]	3 (5) [b]	1 (5)	
• Malignancy	-	5 (8) [b]	1 (5)	
• HIV	-	1 (2) [b]	1 (5)	
Antibiotic use in preceding month	1 (5%) [a]	20 (31) [e]	8 (42) [f]	0.38

[a] (n = 21)
[b] (n = 67)
[c] (n = 66)
[d] (n = 65)
[e] (n = 64)
[f] (n = 19)
[x] Mann-Witney U test

Figure 2 shows the outcome of MRSA positive persons after detection of MRSA. In 60 out of 88 treated persons (68%), the first MRSA eradication therapy was successful, whereas 27 persons (31%) failed to be MRSA eradicated after the first eradication course. One person was lost to follow-up during the follow-up period. Of the failure group, two individuals (7%) spontaneously cleared their MRSA without further treatment and in seven persons (30%) of this group MRSA eradication treatment was postponed because of newly arisen relative contraindications for MRSA eradication therapy. They could, therefore, not be included during the analysis. Two other persons failed to eradicate MRSA at the first attempt: one of these individuals received a new treatment regimen in another hospital and the other individual was lost to follow-up. A second attempt to eradicate MRSA was undertaken in 16 persons, and nine (56%) of those again failed on MRSA eradication therapy. One of these persons subsequently spontaneously cleared his/her MRSA and four persons (44%) did not receive a third treatment during the study period because of newly arisen relative contraindications for MRSA eradication therapy and these persons could therefore not be included in the analysis.

Of the 88 persons who received one or more MRSA eradication therapies, 71 persons (81%) became MRSA negative (intent-to-treat analysis). Three persons who received one or two MRSA eradication therapies and failed on these therapies, spontaneously cleared their MRSA, i.e. they remained MRSA culture-negative without further MRSA eradication therapy during six consecutive screens for MRSA. These three persons are not included in calculation of success rates of MRSA eradication treatment. In total, 71 persons became MRSA negative when followed up after eradication therapy. These 71 persons needed a mean of 1.5 MRSA eradication treatments per patient to remain negative during follow-up.

The success rate of the eradication regimen of first choice in case of extra-nasal sites (mupirocine and chlorhexidine body wash in combination with oral trimethoprim and rifampicin) was not significantly different compared to other regimens containing oral antibiotics.

Potential determinants of MRSA eradication therapy failure were analyzed univariately. Results are shown in Table 3. Persons who failed on the first MRSA eradication therapy were more frequently colonized at least in their throat at second measurement (74%) compared to persons who became successfully MRSA eradicated (53%) ($p=0.06$). The presence of wounds at the second measurement was significantly associated with MRSA eradication therapy failure ($p=0.05$).

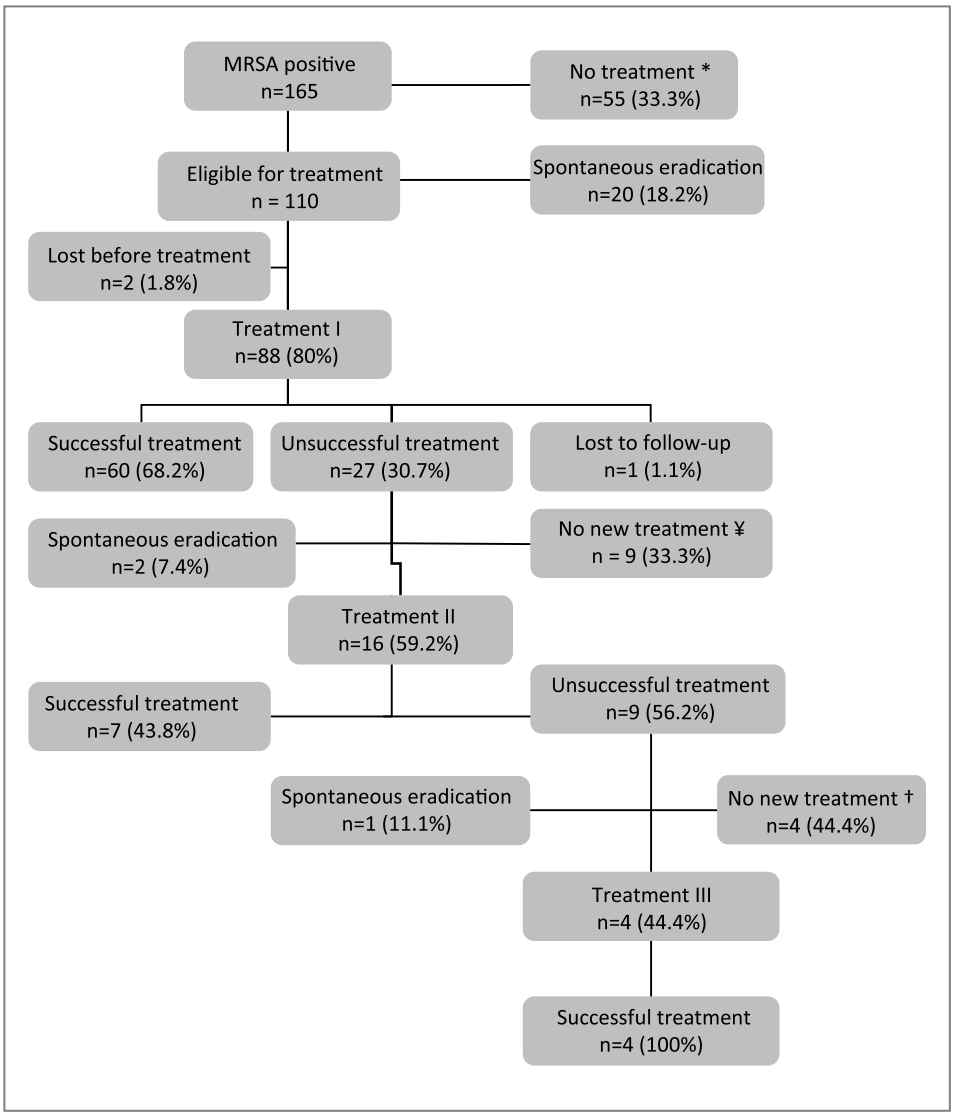

Figure 2: Flowchart demonstrating the outcome of MRSA positive persons after first detection of MRSA.

Unsuccessful treatment was defined as one or more cultures positive in one or more out of six consecutive follow-up culture sets (e.g. nose, throat, perineum and any wounds or skin lesions present).

* not included because of
1) no treatment in our hospital (n=26)
2) not eligible for treatment (relative contraindications for eradication therapy failure, lost to follow-up after first MRSA detection or high risk of non-compliance to treatment) (n=17)
3) died before treatment could be offered (n=12)

¥ no new MRSA eradication treatment because of newly arisen relative contraindications (n=7), no new treatment in our hospital (n=1) or lost to follow-up (n=1)
† no new MRSA eradication treatment because of newly arisen relative contraindications for eradication therapy failure

Table 3: Determinants of MRSA eradication therapy failure in persons receiving their first MRSA eradication therapy

Determinants	Successful treatment 1 (n=60) %	Unsuccessful treatment 1 (n=27) %	p-value	OR (95% CI)
Sex			0.36	1.53 (0.61-3.81)
• male	33 (55)	12 (44)		
• female	27 (45)	15 (56)		
Age				
• median age (range), yr	39 (1-82)	29 (1-73)	0.19 [a]	
• age categorical in yr				
○ 0 – 10 yr	12 (20)	2 (7)	0.33	
○ 11 – 20 yr	0 (0)	3 (11)	1.00	-
○ 21 – 60 yr	40 (67)	21 (78)	0.16	3.15 (0.64-15.41)
○ > 60 yr	8 (13)	1 (4)	0.83	0.75 (0.06-9.72)
MRSA colonization [□]:				
• median number of colonized sites (range)	2 (1-4)	2 (1-4)	0.10 [a]	
○ nose (n=63/87)	42 (70)	21 (78)	0.45	1.50 (0.52-4.34)
○ throat (n=51/86)	31 (53)	20 (74)	0.06	2.58 (0.95-7.02)
○ perineum (n=30/86)	18 (31)	12 (42)	0.21	1.82 (0.71-4.66)
○ other (n=30/87)[b]	21 (35)	9 (33)	0.88	0.93 (0.36-2.43)
• nasal only	10 (17)	4 (15)	1.00 [a]	
• extra nasal only	18 (30)	6 (22)	0.45	0.67 (0.23-1.93)
• nasal and extra nasal	32 (53)	17 (63)	0.40	1.49 (0.59-3.78)
Hospital admission in preceding yr				
• at baseline measurement (n=86)	27 (46)	15 (56)	0.40	1.48 (0.59-3.76)
• at second measurement (n=86)	11 (18)	1 (4)	0.10 [c]	0.18 (0.02-1.46)
Non-intact skin				
at baseline measurement (n=85)	35 (59)	16 (62)	0.40	1.10 (0.43-2.82)
at second measurement (n=84)	14 (24)	6 (23)	0.92	0.94 (0.32-2.81)
Wounds				
• at baseline measurement (n=84)	24 (41)	14 (56)	0.20	1.86 (0.72-4.78)
• at second measurement (n=83)	3 (5)	5 (20)	0.05 [c]	4.58 (1.00-20.96)
Skin problems				
• at baseline measurement (n=85)	16 (27)	7 (27)	0.99	0.99 (0.35-2.80)
• at second measurement (n=84)	7 (12)	2 (8)	0.71 [c]	0.61 (0.12-3.15)
Underlying disease				
• at baseline measurement (n=85)	9 (15)	4 (15)	1.00 [c]	
• at second measurement (n=85)	8 (14)	2 (8)	0.72 [c]	0.53 (0.11-2.69)
Antibiotic use in preceding month				
• at baseline measurement (n=82)	12 (21)	8 (33)	0.23	1.92 (0.66-5.53)
• at second measurement (n=84)	2 (3)	0 (0)	1.00 [c]	
Indwelling device				
• at baseline measurement (n=85)	15 (25)	7 (27)	0.88	1.08 (0.38-3.08)
• at second measurement (n=84)	5 (9)	3 (12)	0.69 [c]	1.47 (0.32-6.70)

At baseline measurement is defined as the time of MRSA detection

At second measurement is defined as just before the start of MRSA eradication therapy

[□] combination of more than one colonization site possible

[a] Mann-Witney U test

[b] other: sputum=4; urine=5; blood; 2; wound=16; other=3

[c] Fisher's exact test

Follow up of MRSA eradication treatment consisted of six consecutive sets of swabs from all sites. After evaluating the number of negative swabs obtained at time of therapy failure, it showed that 11 persons (31%) were tested MRSA positive again after three negative sets. To have a 90% predictive value of success, five or more culture sets seem to be needed (Figure 3).

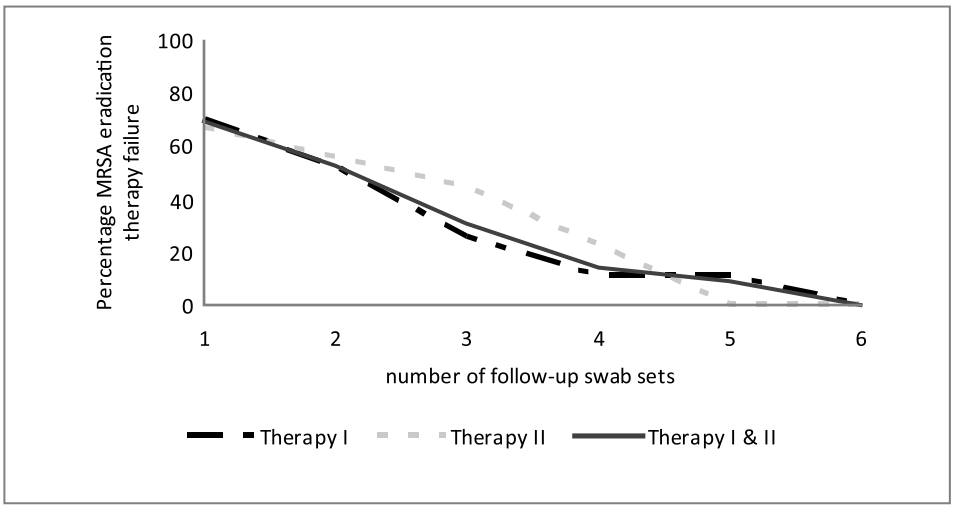

Figure 3: Number of swabs needed to evaluate success of MRSA eradication therapy as measured in 36 patients who failed on MRSA eradication therapy

Discussion and Conclusion

In this prospective observational follow-up study, we show that our MRSA eradication treatment protocol results in a high success rate (81% intention-to-treat analysis). We demonstrated that when collecting only three consecutive negative culture sets as the criteria to call someone MRSA-free again, this call would be false in approximately one third (31%) of the MRSA colonized persons. Our data show that at least five consecutive negative culture sets are needed to have a predictive value for a MRSA-free status >90%. Furthermore, MRSA colonization at least in the throat and the presence of wounds just before start of MRSA eradication therapy are associated with MRSA eradication treatment failure.

MRSA eradication therapy has been studied before and shows variable success rates [25, 26, 31-35]. A recent study by Buehlmann et al [35] showed in their intent-to-treat analysis 87% successful MRSA eradication, although they only obtained three swab sets during follow-up.

Their results are comparable to the results of our intent-to-treat analysis (81%). Seventy-nine persons (91%) received MRSA eradication therapy according to the MRSA eradication therapy regimens described in our study method section (mupirocin ointment and chlorhexidine body wash in case of nasal colonization, or a combination of topical therapy and two antibiotics in case of extra-nasal colonization with or without nasal colonization). The results of our intent-to-treat analysis might even be higher as the other six persons (7%) who had extra-nasal MRSA colonization, only received mupirocin and chlorhexidine and no oral antibiotics. On the other hand, 10 of the 165 (6%) MRSA positive persons had relative contraindications for MRSA eradication therapy during the study analysis period and were, therefore, not included in the intent-to treat analysis. Furthermore, seven (26%) of the persons who failed on their first MRSA eradication therapy and four (44%) of the persons who failed on the second MRSA eradication therapy had newly arisen relative contraindications for MRSA eradication therapy, and were therefore not treated again and thus not included in the analysis. These a priori exclusions, by their nature, increase the success rate among those that remain eligible for eradication treatment that were likely to benefit from it, and prevented exposing patients with a high a priori chance of treatment failure to become exposed to further futile medical intervention.

The mean number of MRSA eradication therapies per person in the study by Buehlman et al [35] was 2.1 compared to 1.5 mean MRSA eradication therapies in our analysis. Furthermore, our study showed that significantly more persons became MRSA negative after the first eradication treatment than in the study performed by Buehlman et al (68% vs 47%).

Several major differences between previously reported studies on MRSA eradication and our study are observed. All other studies used different definitions to ascertain the MRSA negative status of a patient other than we did. Buehlmann et al [35] defined successful MRSA eradication as no growth of MRSA in three sets of follow up surveillance cultures. Furthermore, they collected culture sets already 2-3 days after completion of MRSA eradication treatment and at relative short intervals of 2-3 days. Dupeyron et al. [36] only took nasal swabs to determine the efficacy of MRSA eradication therapy. They declared a previously MRSA positive patient MRSA-free if one week after finishing treatment with mupirocine and during the complete hospital admission period, nasal swabs remained MRSA negative. When patients became MRSA nasal carriers after several weeks, this phenomenon was considered to be a new acquisition or reacquisition and not as a failure of MRSA eradication therapy. Darouiche et al [31] used three different culture moments to ascertain the MRSA-free status. The first set of swabs was obtained during a two-week course of oral antibiotics. The second set of swabs was taken one week after completion of therapy and the third and final set was taken three or four months after completion of therapy. The second and third sets of samples were taken

from the original culture positive sites only. By our definition, Darouiche et al [31] only took two follow-up culture sets from an inadequate number of sites. Walsh et al [25] performed four surveillance cultures to ascertain the MRSA-free status of a patient after treatment with rifampicin in combination with novobiocin or trimethoprim-sulfamethoxazole. All formerly positive sites were cultured immediately after completion of therapy (24 and 28 h after) and on days 7 and 14 after therapy. As demonstrated in several studies, the antimicrobial mechanism can still be active when cultures are obtained this quickly after completion of therapy. For example, the effect of mupirocin ointment on eliminating MRSA carriage was still observed 5 weeks after therapy completion [37, 38].

Seven of the persons (26%) in our analysis who failed on their first MRSA eradication therapy (n=37) and four of the persons (44%) who failed on their second MRSA eradication therapy (n=9) had a MRSA positive swabs after three consecutive negative culture sets taken more than one week after finishing treatment. Eleven of the 36 persons (31%) who failed to eradicate MRSA would therefore be incorrectly considered MRSA eradicated if only three follow-up culture sets had been obtained. When left untreated, this group can attribute to the spreading of MRSA. Therefore, our study demonstrated that in case of five or more follow-up culture sets, the predictive value of MRSA eradication therapy success is over 90%.

As demonstrated in our study, MRSA throat carriage plays an important role in MRSA eradication therapy. However, all previous studies, except three [25, 34, 35] did not obtain throat swabs to determine and follow-up the MRSA status of the patients. As showed in several studies [35, 39-42] as well as in our analysis, the throat is a frequently colonized site where MRSA can survive in spite of MRSA eradication therapy. For this reason, it is essential to include throat swabs to monitor the MRSA status of a person.

Several potential determinants which could hamper successful MRSA eradication have been proposed in the past [25, 29, 43]. Our study analysis demonstrated that the presence of wounds before the start of MRSA eradication therapy is associated with MRSA eradication failure. For this reason, it seems preferable to have no wounds present at the time MRSA eradication therapy is started. However, Parras et al [44] observed that the presence of wounds did not influence the success rate of eradication of nasal and extra-nasal MRSA carriage.

No other potential determinants were significantly associated with MRSA eradication failure in our study. This can be explained by postponing of treatment, as much as possible, in patients with relative contraindications for MRSA eradication therapy and, therefore, leaving only novel determinants of treatment failure to be revealed.

Our study shows that patients who spontaneously cleared their MRSA were more often only MRSA positive at extra-nasal sites when compared to patients that were actively treated. On the other hand, patients with a combination of nasal and extra-nasal MRSA colonization were significantly less likely to spontaneously lose their MRSA compared to patients actively treated with MRSA eradication therapy. This finding is confirmed by the observation that patients with single-site MRSA colonization had significantly more chance of spontaneous MRSA clearance compared to patients with multi-site MRSA colonization. The reason behind the spontaneous loss of MRSA remains unclear, but on the basis of our knowledge of MSSA (methicillin-susceptible *Staphylococcus aureus*) carriage several hypotheses may be put forward [45-47]. For example, the bacterial load is likely to play an important role in the spontaneous clearance of MRSA. High bacterial *S. aureus* loads and multiple-site colonization are associated with persistent *S. aureus* carriage whereas single-site colonization and lower loads are found in patients that are only intermittent carriers [48]. Interestingly, a study by Nouwen et al [46] demonstrated that intermittent and non *S. aureus* carriers artificially inoculated with a mixture *S. aureus* strains, eliminated the inoculated strains more quickly than persistant carriers. As the MRSA bacterial load in single-site carriers is probably lower than in multi-site carriers, spontaneously losing one's MRSA can more easily be accomplished [43].

Our study has several limitations. First, our study was underpowered to assess low frequency determinants of MRSA eradication therapy outcome. In addition, we do not know whether persons who became MRSA-free according to our culture rule will remain so in the foreseeable future. In 2007 we started to follow-up individuals, from whom MRSA was eradicated, with one culture ser per 1-2 months for one year. Up until now, we have encountered recolonization in one case, although this is not confirmed with PFGE.

In conclusion, the present study analysis suggest that with our MRSA eradication treatment policy a large proportion of MRSA carriers can successfully be freed from their MRSA strain. Furthermore, we propose that five or more follow-up culture sets are needed to ascertain the MRSA status of a person. Applying this rule may be a step forward in reducing the spread of MRSA.

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Chapter 4

Prevalence, incidence and risk factors for acquisition of methicillin-resistant coagulase negative staphylococci on skin during hospital admission

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Abstract

In this prospective study, the incidence and possible risk factors for MR-CoNS skin acquisition during hospital admission were determined. Furthermore, the degree of MR-CoNS carriage and acquisition in single versus four-bed hospital rooms was assessed.

A convenience sample of 168 patients admitted to a general ward were eligible for analysis between January 2007 and August 2008. Mr-CoNS baseline skin cultures were collected on the day of admission or the first day of admission. Surveillance skin swabs were collected on 1-9 separate occasions during hospitalization.

Thirty-nine patients (24%) were MR-CoNS carriers on admission. Seventy-nine patients (63%) who were MR-CoNS negative at baseline, acquired MR-CoNS on their skin during their admission period. No significant difference was found on the probability of acquiring skin MR-CoNS between single and four-beds hospital rooms. However, patients admitted to single rooms had a significant longer admission period, which was related to the presence of a malignancy, compared to patients admitted to a four-beds room. Independent risk factors for MR-CoNS skin acquisition were the length of hospital stay and the use of antibiotics.

The incidence of MR-CoNS skin carriage increases rapidly during hospital admission and, therefore, this increased colonization pressure may lead to an increasing number of health-care-associated infections.

Introduction

Healthcare-associated, or nosocomial infections, constitute a large part of the infection rate that is observed in hospitals and are associated with increased mortality and morbidity rates among patients [1-3]. Of all patients being admitted to hospital, 5 – 15% develops a healthcare-associated infection [4] and in patients admitted at an Intensive Care Unit (ICU) this percentage can even rise to 35 – 50% [5]. Furthermore, nosocomial infections may contribute to healthcare-associated costs because of increased medical treatment and increased length of hospital stay [6-9]. Therefore, a reduction of the number of healthcare-associated infections may contribute to a substantial decrease in morbidity, mortality and admission days and this may contribute to lower healthcare-associated costs.

According to isolation guidelines of the Centers for Disease Control and Prevention (CDC) and the Dutch national Working Party of Infection Prevention (www.wip.nl), nursing in private, single bed hospital rooms is an effective method to prevent transmission of pathogens that can cause nosocomial infections [10, 11].

Coagulase-negative staphylococci (CoNS) are the most prevalent pathogens causing nosocomial infections. CoNS are opportunistic pathogens which colonize the human skin and mucous membranes [12]. *Staphylococcus epidermidis* is the most predominant CoNS associated with nosocomial CoNS infections [13]. Up to 70% of the *S. epidermidis* strains circulating in a hospital are resistant to methicillin [14] and methicillin-resistant coagulase-negative staphylococci (MR-CoNS) have been considered to have resistance genes which can, theoretically, be transferred to *S. aureus* and cause methicillin-resistant *S. aureus* (MRSA) [15, 16]. The emergence of MR-CoNS limits therapeutic options in case of infection and increases the risk of therapy failure [17, 18].

Clonal spread of CoNS within healthcare settings and hospital wards has been reported by using molecular typing methods (e.g. PFGE) as tracing technique [19-21]. Until now, little is known about the prevalence of MR-CoNS skin carriage on admission, incidence of MR-CoNS acquisition and possible risk factors which may cause transmission or acquisition and spread of MR-CoNS skin carriage.

In this prospective study we aimed to gain more insight in the incidence of and possible risk factors for MR-CoNS skin acquisition during hospital admission. Furthermore, we wanted to study the degree of MR-CoNS carriage and acquisition in single versus four-beds hospital rooms.

Patients and Methods

Study population

All patients between January 2007 and August 2008 who were expected to be admitted for at least three days at the long-stay department of Urology and Gynecology at the Erasmus University Medical Center (Rotterdam, The Netherlands), were asked to participate in this study. At this department there were 10 single and three four-beds hospital rooms.

Patients who were not admitted for the expected duration of more than three days or were in need of increased levels of care and, therefore, admitted to a single bed hospital room, were excluded from further analysis. Informed consent was obtained from all patients who agreed to participate in the study.

During the study period 871 patients who were eligible for study inclusion were admitted to the department. A convenience sample of 168 patients were eligible for analysis because they were admitted to and stayed in a single or in four-beds room during the duration of their hospitalization. Furthermore, sufficient clinical and laboratory data were available to assess the rate and acquisition of skin carriage with MR-CoNS and its associated risk factors. Through medical chart review, demographic and clinical characteristics of each patient were obtained. Gender, age, admission indication, length of admission period, operation procedure, hospitalization in the preceding year, presence of malignancy, wounds and antibiotic use during hospital admission (prophylactic and/or therapeutic) were all obtained.

Screening

To determine MR-CoNS skin carriage, skin cultures were taken from a 5x5 cm area from the forearm. MR-CoNS baseline skin cultures were collected on the day of admission (day 0) or on the next day of admission (day 1). MR-CoNS skin carriage on admission (baseline) was considered to be present when the first swab, taken on day 0 or day 1, showed MR-CoNS growth. Subsequently, surveillance skin swabs were collected on 1-9 separate occasions during the admission period; samples were preferable taken every day during the admission period. Three different carriage types could be determined. First, acquisition of MR-CoNS carriage during admission was defined as patients with a MR-CoNS negative baseline swab and one or more MR-CoNS positive surveillance swabs during hospital admission. Secondly, no acquisition of MR-CoNS carriage during admission was defined as a MR-CoNS negative baseline swab and no MR-CoNS positive surveillance swabs during hospital admission. Finally, MR-CoNS carriage on admission was defined as patients with a MR-CoNS positive baseline swab.

Microbiological methods

MR-CoNS screening swabs were submerged into a phenol-red mannitol broth (PHMB) supplemented with 5 mg/l ceftizoxin and 75 mg/l aztreonam. After 48 hours of incubation at 35°C, the PHMB was subcultured onto a BA plate. After 48 hours of incubation at 35°C, all white colonies compatible with staphylococci were tested by agglutination (Slidex Staph Plus, bioMérieux, Marcy l' Etoile, France). All agglutination negative colonies were stored at -70°C.

Statistical analysis

All analyses were performed using SPSS 15.0 for Windows (SPSS Inc, Chicago, IL, USA). Potential determinants for MR-CoNS skin carriage acquisition were first tested univariately. Chi-square test or Fisher's exact test (two-sided) was used to compare categorical variables, and the Mann-Whitney U test was used for continuous variables without a normal distribution. Statistical significant variables in the univariate analysis were further analyzed multivariately using a logistic regression analysis. Results are reported as odds ratios (OR) with 95 % confidence interval (95% CI). Kaplan-Meier curves and the log-rank test were used to compare MR-CONS skin carriage acquisition during admission in single and four-beds hospital rooms. The statistical tests were 2-tailed, a p-value of less than 0.05 was considered statistically significant.

Results

Study population

Sixty-three patients (38%) were admitted and stayed in a private, single bedroom, whereas 105 patients (62%) were admitted and stayed in a four-beds hospital room. The median age of these patients was 57.5 years (range 20 – 84 years) and 70% (n=117) were female. General characteristics of patients are shown in Table 1.

MR-CoNS skin carriage

From the 168 patients, 826 skin swabs were obtained during their hospital admission. A median of four skin swabs (range 2-10) were collected during the admission period with a median interval of one day (range 0-5 days) between the subsequent skin samples. In 33 patients (20%) two or more culture swabs were collected on the same day (range 2-4 swabs on the same day). Together, all 168 patients had a total of 1,177 admission days, therefore 0.70 skin swabs per patient per admission day were obtained. In 335/826 (41%) of all skin swabs collected culture yielded MR-CoNS. The number of MR-CoNS positive skin swabs during the hospital admission period are depicted in Figure 1.

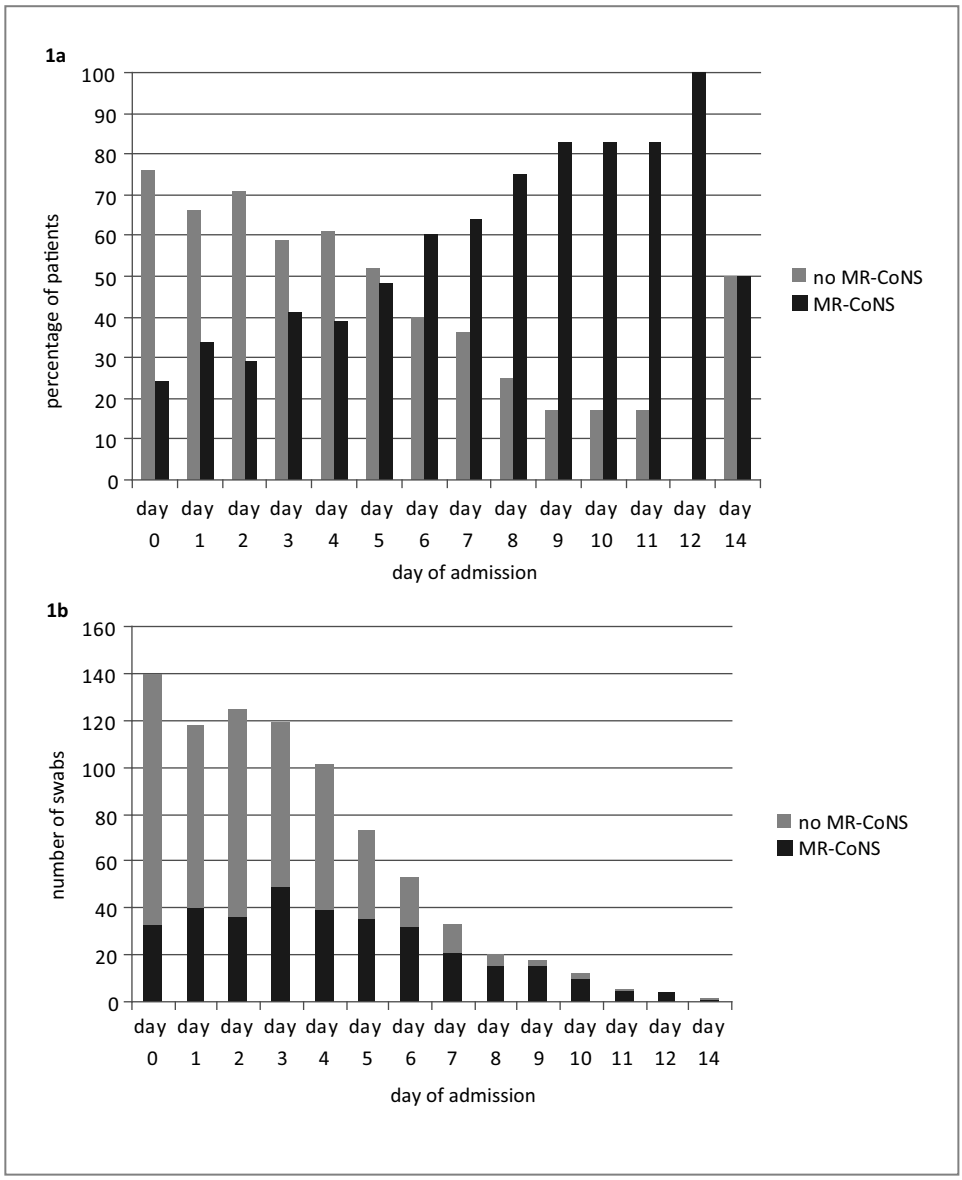

Figure 1: (**a**) Prevalence of MR-CoNS positive skin swabs per admission day. (**b**) Number of MR-CoNS positive skin swabs per admission day

Table 1: Baseline characteristics of patients enrolled in the study

	Patients (n=168) (%)
Female	117 (68)
Age, yr (median; range)	57.5 (20-84)
Hospitalization in previous yr	65 (39)
Single bed room	63 (38)
Operation wound (n=163) [a]	160 (98)
Malignancy (n=163) [a]	66 (41)
Antibiotic use during admission (n=167) [b]	73 (44)
• prophylactic	38 (23)
• therapeutic	23 (14)
• therapeutic and prophylactic	12 (7)
Admission period, days (median; range)	6 (3-31)
MR-CoNS carriage on admission (n=163) [c]	39 (24)

[a] Data could not be retrieved from medical records in five patients
[b] Data could not be retrieved from medical records in one patient
[c] Culture results from baseline MR-CoNS skin swabs were lost in five patients

MR-CoNS skin carriage acquisition

Thirty-nine patients (24%) showed positive MR-CoNS growth in the baseline skin swab and were considered MR-CoNS carriers on admission. Therefore, they were not included in the analysis of MR-CoNS skin carriage acquisition. Hospitalization in the previous year did not differ between patients with MR-CoNS positive baseline skin swabs and patients with MR-CoNS negative baseline skin swabs (29% vs 37%, p=0.90). Seventy-nine patients (63%) showed one or more MR-CoNS positive skin cultures during hospital admission after a MR-CoNS negative baseline skin swab and were, therefore, considered to have acquired MR-CoNS skin carriage during this hospital admission period.

MR-CoNS skin carriage acquisition in single and four-beds hospital rooms

General characteristics of patients admitted to single or four-beds hospital rooms are shown in Table 2. The median age of patients admitted to a single hospital room was significantly higher (p=0.04) and a significant higher number of these patients had a malignancy (p=0.03) when compared to patients admitted to a four-beds hospital room. Furthermore, the median admission period of patients admitted to a private hospital room was significantly longer than that of patients admitted to a four-beds hospital room (7 vs 6 hospital admission days; p=0.04). After multivariate analysis it was demonstrated that increased age was associated with the presence of a malignancy. Furthermore, the presence of a malignancy was associated with an increased admission period. In both single and four-beds hospital rooms, about 60% of the patients acquired MR-CoNS on their skin during their admission period. No significant

difference was found between the number of skin swabs obtained from single and four-beds hospital rooms (5 swabs; range 2-10 vs 4; range 2-10).

Figure 2 show the probability of acquiring MR-CoNS on skin during the admission period in single and four-beds hospital rooms. Median number of admission days before acquiring MR-CoNS skin carriage was 4 days (range 1-10 days) among patients in private rooms and 3 days (range 1-7 days) among patients in four-bed hospital rooms (p=0.37).

Table 2: Univariate analysis of characteristics of patients admitted to single bed versus to four-beds rooms

	Single room n=63 (%)	**four-bed room n=105 (%)**	**p-value**	**OR (95% CI)**
Female	39 (62)	78 (74)	0.09	1.78 (0.91-3.48)
Age, yr (median; range)	66 (20-84)	54 (20-84)	0.04 [a]	
Hospitalization in previous yr	24 (38)	41 (39)	0.90	1.04 (0.55-1.98)
Operation wound (n=163) [x]	58 (98)	102 (98)	1.00 [b]	
Malignancy (n=163) [x]	31 (52)	35 (34)	0.03	0.48 (0.25-0.92)
Antibiotic use during admission (n=167) [xx]	32 (52)	41 (39)	0.11	0.60 (0.32-1.13)
Admission period, days (median; range)	7 (3-31)	6 (3-30)	0.04 [a]	
MR-CoNS carriage on admission (n=165) [xxx]	17 (28)	22 (21)	0.33	0.69 (0.33-1.44)
Became MR-CoNS carrier during admission period (n=126)	27 (61)	52 (63)	0.82	1.09 (0.51-2.32)

[x] Data could not be retrieved from medical records in five patients
[xx] Data could not be retrieved from medical records in one patient
[xxx] Culture results from baseline MR-CoNS skin swabs were lost in five patients
[a] Mann-Witney U test
[b] Fisher's exact test

Risk factors for MR-CoNS skin carriage acquisition

Data obtained from all 126 MR-CoNS negative carriers on admission, were used to determine potential risk factors for MR-CoNS skin carriage acquisition. Potential risk factors were first analyzed univariately, which revealed that the presence of a malignancy (p=0.02), the use of antibiotics during hospital admission (p=0.003) and the length of hospital admission (p=0.001) were positively associated with acquisition of a MR-CoNS during admission (Table 3). After multivariate logistic regression analysis, antibiotic use (p=0.04) and the length of hospital stay (p=0.03) showed to be independent risk factors associated with MR-CoNS skin carriage acquisition (Table 3).

Table 3: Univariate analysis of risk factors for MR-CoNS skin carriage acquisition during hospital admission

	MR-CoNS acquired n=79 (%)	No MR-CoNS acquired n=47 (%)	p-value	OR (95% CI)	Adjusted OR (95% CI) [c]
Female	55 (70)	39 (83)	0.10	0.47 (0.19-1.16)	
Age, yr (median; range)	57 (24-84)	54 (20-81)	0.18 [a]		
Hospitalization in previous yr	27 (34)	20 (43)	0.35	0.70 (0.33-1.47)	
Single bed room	17 (36)	27 (34)	0.82	0.92 (0.43-1.95)	
Operation wound (n=123) [x]	76 (97)	45 (100)	0.53 [b]		
Malignancy (n=124) [xx]	40 (51)	13 (29)	0.02	2.53 (1.16-5.51)	
Antibiotic use during admission	40 (51)	11 (23)	0.003	3.36 (1.50-7.52)	2.41 (1.04-5.59) *
• no antibiotic use	39 (49)	36 (77)	0.03		
• prophylactic antibiotic use	20 (25)	7 (15)	0.05	2.64 (1.00-6.98)	
• therapeutic antibiotic use	13 (16)	3 (8)	0.04	4.00 (1.05-15.19)	
• therapeutic and prophylactic	7 (9)	1 (2)	0.09	6.46 (0.76-55.12)	
Admission period, days (median; range)	6 (3-31)	5 (3-13)	0.001 [a]		1.23 (1.02-1.47) *

[x] Data could not be retrieved from medical records in three patients
[xx] Data could not be retrieved from medical records in two patients
[a] Mann-Witney U test
[b] Fisher's exact test
[c] Multivariate adjusted OR after correction for malignancy
* p-value < 0.05

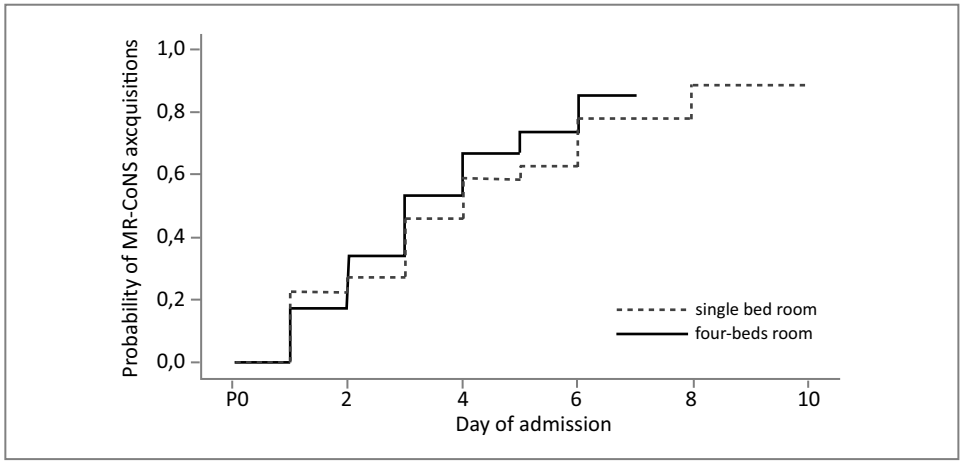

Figure 2: Kaplan Meijer analysis of MR-CoNS skin carriage acquisition in patients admitted to single bed versus four-beds rooms

Discussion and Conclusion

In this prospective study we demonstrate that approximately two-thirds (63%) of patients admitted to hospital will acquire MR-CoNS on their skin during their hospital stay. Independent risk factors for MR-CoNS skin acquisition were use of antibiotics during admission and a longer hospital stay. MR-CoNS acquisition develops within one week of admission in the majority of cases. No significant difference in MR-CoNS skin carriage acquisition was observed between patients admitted in single versus four-beds hospital rooms. However, patients admitted to a single room had a significant longer admission period when compared to patients admitted to a four-beds hospital room (7 vs 6 admission days), a finding that was associated with the presence of a malignancy.

Some limitations should be addressed in this present study. First, this study was based on a convenience sample of patients that were admitted to and stayed in either single or in four-beds rooms. Also, only patients with a complete dataset on clinical and laboratory variables was included in the analysis. However, the baseline characteristics of all potentially eligible patients (data not shown) were similar to those of the cohort included in this analysis. Furthermore, patients were not equally divided between single and four-beds hospital rooms (38% vs 68% respectively). Patients admitted to single rooms were significantly older and had more malignancies, which resulted in a longer admission period than patients in four-beds

hospital rooms. However, admission to single rooms itself was not associated with increased acquisition of MR-CoNS on patient skin during hospital stay. The prevalence of MR-CoNS skin on and during hospital admission found in this study may be underestimated as routine skin culture techniques sample only a fraction of the skin's complex flora, potentially missing small subpopulations of the skin flora.

To our knowledge this is the first prospective study studying the prevalence of MR-CoNS skin carriage on the day of admission and incidence during hospital admission and defining possible risk factors for MR-CoNS skin carriage acquisition during hospital admission.

Our study shows that 23% of patients were MR-CoNS skin carriers on admission which was not associated with hospitalization in the preceding year; 28% in private rooms and 21% in four-beds hospital rooms. Comparison of our result with other studies is difficult because of major differences in study design [22-27]. Kernodle et al [23] studied the prevalence of MR-CoNS carriage in patients admitted for elective cardiac surgery or coronary angioplasty. In 74% of the patients, cultured from the nares, right subclavian or left inguinal area, MR-CoNS was obtained from one or more of the swabs. Significantly less MR-CoNS colonization on admission was demonstrated in a study by Ohno et al [26]. Five-hundred-fifty-seven children admitted to a pediatric surgical ward, 84% of the patients were admitted from their home; 26% were transferred from another ward or hospital, were swabbed to determine MR-CoNS carriage. Throat and stool swabs were collected within 48 hours after admission and MR-CoNS carriage on hospital admission was observed in only 2.5% of the infants. The reason of this tenfold decrease compared to our study, is probably related to a differences in study population, and, less so, due to different culture methods used.

Furthermore, our study demonstrated that 63% of our study population became MR-CoNS skin carriers during hospital admission. In a study among skilled nursing facility patients, screening CoNS surveillance nasal cultures were obtained in 126 patients at baseline and after a follow-up period of 15 months. Twenty-one percent of these patients (n=26) were CoNS colonized at baseline and 59% of the patients (n=66) was CoNS colonized at the time of follow-up. In this study, the swabs obtained at the moment of follow-up were not obtained from the same patient population which was cultured at baseline as the average length of stay in the skilled nursing facility was three months [24]. Nyström et al [25] conducted a study in two neonatal units, looking at the colonization with CoNS during the first two weeks of life in 10 infants from each unit. On day four 80% of the patients in both units were colonized and by the 10th day in both units 90% of the infants were CoNS colonized.

The risk of acquisition of microbiological pathogens is influenced by the colonization status of other patients and hospital personnel (also called ‘‘colonization pressure’’) as described by Bonten et al [28]. This influence of colonization pressure has also been demonstrated for methicillin-resistant *S. aureus* (MRSA) [29], Enterobacteriaceae [30] and vancomycin-resistant enterococci [31].

Continuous changes in the prevalence of colonization with pathogens within hospital settings may be the result of different mechanisms: dynamics of admission and discharge of colonized and noncolonized patients; or selection of resistant bacteria due to antibiotic pressure; and potential cross-transmission through colonized health-care workers or the environment [32-34]. Two studies performed by Tammelin et al [33, 35] demonstrated that approximately 25-45% of the hospital staff in an operating theatre dispersed MR-CoNS and were, therefore, a potential source of cross-transmission to patients. Unfortunately, no surveillance skin swabs from hospital staff or air samples were collected during our study period.

It has been demonstrated by several studies that (methicillin-resistant) CoNS clones can spread within a hospital [19, 21, 36-40] and can even have geographic dissemination [41]. Therefore, it might be plausible that the increasing MR-CoNS skin prevalence during hospital admission in our study was caused by only a limited number of MR-CoNS clones circulating in the hospital. By multivariate analysis we demonstrated that the use of antibiotics during hospital admission and a prolonged median admission period, were both independent risk factors for MR-CoNS skin acquisition. The use of antibiotics during hospital admission has previously been demonstrated to have a significant correlation with acquiring nosocomial MRSA colonization [42]. In addition, Bonten et al [28] demonstrated that the acquisition of VRE in patients admitted to ICU was positively associated, among other risk factors, with the use of antibiotics during hospital admission. On the other hand, Nyström et al [25] did not observe that use of antibiotics was associated with CoNS acquisition in newborns during admission on neonatal units.

Our study demonstrated that with each hospital admission day the risk of acquiring MR-CoNS skin carriage increases with 23%. Previous hospital admission is known to be a significant risk factor of MRSA colonization as Jernigan et al [42] demonstrated that a previous hospital admission of at least five days is a significant risk factor to become MRSA colonized. Not only the risk of acquiring colonization, but also the risk of an CoNS infection is increased with a prolonged hospital stay [20] as colonization precedes infection.

According to isolation guidelines, it has been advised to nurse patients in single hospital rooms, to prevent direct or indirect transmission of pathogens [43]. Our study did not demonstrate

that patients admitted to four-beds hospital room were more at risk of acquiring MR-CoNS skin carriage, than patients admitted in single hospital rooms. However, our study study was not designed to properly test this hypothesis. Other studies have determined the colonization rate of pathogens between single and multi-patient hospital rooms. Mulin et al [44] conducted a study looking at the colonization rate of *Acinobacter baumannii* on a surgical ICU before and after transforming four-beds hospital rooms into single rooms. After the reconstruction, a significant decrease in colonization with *Acinobacter baumannii (*28.1% before and 5.0% after the rebuilding) was observed in patients admitted to single rooms. Not only a significant decrease in colonization, but also a reduction in nosocomial infections has been observed after rebuilding multi-patient hospital rooms into single rooms [45]. However, in both cases the authors used retrospectively collected data for the control group. Therefore, the need remains to study the potential barrier effect of private room nursing on the spread and acquisition of MR-CoNS and other hospital pathogens.

In conclusion, the incidence of MR-CoNS skin carriage increases rapidly during hospital admission period. Apart from the length of stay, the use of antibiotics is the major independent risk factor for MR-CoNS skin acquisition. This increased incidence, and therefore increased colonization pressure, may lead to an increasing number of healthcare-associated infections.

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PART II

Methicillin Susceptible Staphylococci in HIV Infected Persons

Chapter 5

Prevalence and determinants of *Staphylococcus aureus* and *Streptococcus pneumoniae* carriage in HIV infected persons compared to healthy controls

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Abstract

To assess the prevalence and possible (HIV related) determinants of colonization with *Staphylococcus aureus* and the *Streptococcus pneumoniae* in HIV positive adult patients (n=248) as compared to healthy controls (n=239), a cross-sectional case-control study was performed.
From each participant two nasal swabs and one nasopharyngeal swab were obtained to determine the *S. aureus* and *S. pneumoniae* carrier state.

In contrast to earlier non-controlled studies, no significant differences were found in carrier rates of *S. aureus* and *S. pneumoniae* between HIV positive cases and healthy controls. Persistent carrier rates for *S. aureus* were comparable for both HIV infected and healthy controls (19% HIV cases, 18% controls, p=0.67). Increasing CD4 cell counts were shown to be significantly associated with persistent *S. aureus* carriage (p=0.02). Carriage with *S. pneumoniae* was rare in both groups (2% in both groups, p=0.95). No interference between carriage with both bacteria and no other potential determinants for carriage were demonstrated.

Introduction

Staphylococcus aureus is an important microbial agent known to cause a wide range of clinically important infections that can vary from mild to life-threatening [1].

Earlier non-controlled studies demonstrated high *S. aureus* carriage rates among human immunodeficiency virus (HIV) infected patients with a concordant high risk of developing *S. aureus* infections [1-8]. The mechanism(s) underlying these high *S. aureus* carriage and infection rates still remain to be elucidated. However, a study by Nguyen et al [9] demonstrated that HIV infected patients with a low CD4 cell count were at increased risk of becoming infected with *S. aureus*.

Low CD4 cell counts seem to play an important role in *Streptococcus pneumoniae* carriage as well [10]. Bacterial pneumonia caused by *S. pneumoniae* is a frequent cause of morbidity and mortality in HIV infected persons. The risk of invasive pneumococcal disease is 60 times higher in HIV positive individuals, than in HIV negative individuals [11]. Whether adult HIV infected patients have an increased colonization rate with *S. pneumoniae* compared to healthy adult volunteers, remains unclear [10, 12].

In this cross-sectional, case-controlled study we aimed to identify independent determinants of *S. aureus* and *S. pneumoniae* carriage in an outpatient population of HIV infected individuals as compared to matched healthy controls. Within the HIV infected patients, we specifically wanted to test if CD4 cell levels and antiretroviral therapy are associated with *S. aureus* and *S. pneumoniae* carriage.

Patients and Methods

Study population

All HIV infected patients visiting the Infectious Diseases outpatient clinic of the Erasmus University Medical Center, Rotterdam, The Netherlands between March 1st 2007 and July 1st 2007 were invited to participate in this study. Patients could be included in the study when they were 18 years or older and did not have a life expectancy less than one month. In total 248 patients agreed to participate.

To assess independent determinants of *S. aureus* and *S. pneumoniae* carriage in an outpatient population of HIV infected patients, we performed a cross-sectional case controlled study. A “case” was defined as a HIV positive patient. To compose a control group, we addressed

employees of the Erasmus University Medical Center Rotterdam and other healthy volunteers outside the hospital and aimed to match cases and controls by age and sex. The HIV status of the control group was not determined by lab test, thus the control group consisted of persons assumed to be HIV negative. Control persons could be included in the study when they were 18 years or older and healthy. In total 239 control persons agreed to participate.

The protocol for this study was approved by the Committee for Ethics in Research (METC) at the Erasmus University Medical Center Rotterdam, The Netherlands and all patients and controls gave their written informed consent.

Data collection

Data of HIV infected patients were recorded by chart review, including demographic data (age, sex and birth country), hospitalization that occurred in the preceding year, HIV infection related data (recent CD4-cell count, *Pneumocystis jirovecii* prophylaxis with trimethoprim-sulfamethoxazole, highly active antiretroviral therapy (HAART), recent HIV RNA viral load) and other factors that may contribute to *S. aureus* and *S. pneumoniae* carriage (diabetes mellitus, co-infection with Hepatitis B and chronic obstructive pulmonary disease (COPD)). At the first visit a questionnaire was completed including questions concerning drug and alcohol use, smoking habits (actively as well as passively), skin diseases, vaccination status and antimicrobial therapy in the preceding month.

Two nasal swabs and one nasopharyngeal swab were taken from each participating patient or control person. The patients had their first nasal swab and the nasopharyngeal swab taken during their regular outpatient clinic visit. The second nasal swab was taken at least one week later. When patients were unable to come to the outpatient clinic for their second nasal swab, a package was send to their address with a nasal swab and an instruction manual so they could perform it themselves.

Microbiological procedures

Nasal swab cultures were performed according to a standard operating procedure [5]. Nasal swabs were obtained using dry sterile cotton-wool swabs (Transwab, Medical Wire & Equipment Co. Ltd., Corsham, United Kingdom). The left and right anterior nares were swabbed and the swab was immediately placed in Stuart's transport medium and kept at room temperature for a maximum of 6 hours or at 4°C for a maximum of 24 hours before inoculation. Swabs were then cultured quantitatively on phenol-red mannitol salt agar (PHMA) and phenol red mannitol salt broth (PHMB).

When PHMB also showed staphylococcal growth after 72 hours of incubation at 37°C, the broth was then sub-cultured on Colombia agar plates containing 5% sheep blood. *S. aureus* identification was based on colony morphology on the Colombia agar plates, a catalase test and a latex agglutination test (Staphaurex Plus ®, Murex, Dartford, United Kingdom). All *S. aureus* isolates were stored at -70°C in glycerol containing liquid media.

The posterior wall of the nasopharynx was touched transnasally using a flexible swab. The swab was immediately placed in Stuart's transport medium and kept at room temperature for a maximum of 6 hours or at 4°C for a maximum of 24 hours before inoculation. Swabs were directly plated on a Colombia blood agar plate with 5% sheep blood. Identification and determination of *S. pneumoniae* was based upon morphology, Gram's stain, catalase and an optochin disk tests. All *S. pneumoniae* isolates were stored at -70°C glycerol containing liquid media.

S. aureus nasal carriage was defined according to an earlier validated culture rule [5]. This rule distinguishes between persistent (both nasal swabs positive with *S. aureus*) and either intermittent or non-carriers (one or no swabs positive with *S. aureus*, respectively). Intermittent and non-carriers have the same clinical outcomes, therefore the non- and intermittent carriers were combined to contrast with the persistent carrier group.

Persons were classified as carriers of *S. pneumoniae* when the nasopharyngeal swab was positive with *S. pneumoniae*. Persons were classified as non-carriers when the nasopharyngeal swab was negative with *S. pneumoniae*. No distinction was made between persistent or intermittent carriage.

Statistical analysis

Proportions were compared by the Chi-square test (Fisher's exact test in case of small numbers) and continuous data with Mann-Whitney U test. To identify independent determinants of *S. aureus* and/or *S. pneumoniae* carriage, variables were tested univariately. All statistical tests were two-tailed and a significance level of 0.05 was used.

Results

Between March 2007 and July 2007, 248 HIV infected adult patients and 239 healthy controls were included in this study. The general characteristics of both populations are shown in Table 1. The median age of the HIV infected patients was 44 years (range 20-80 years) and 75% were male. The median age of the control group was 38 years (range 19-63 years) and 67% were

male. The control group comprised of 98% Caucasians, while in the HIV infected group only 55% were Caucasian.

Table 1: General characteristics of HIV infected patients (n = 248) and healthy controls (n = 239)

	Case n=248 (%)	**Control n=239 (%)**	**p-value**
Gender			0.08
• male	185 (75)	161 (67)	
Age			
• mean , yr	44.2	38.8	
• median, yr [range]	44 [20-80]	38 [19-63]	0.000 [a]
Birth country			
• European	136 (55)	234 (98)	0.000
• Not European			
◦ sub-Saharan Africa	53 (21)	-	
◦ Arabic	11 (4)	3 (2)	
◦ Asia	16 (6)	1 (0.4)	
◦ Middle-/South America	32 (13)	1 (0.4)	
Number of people living in same household median (range)	2 [1-15]	2 [1–9]	0.000 [a]
Children under 18 yr living in the same home	48 (19)	80 (34)	0.000
Hospital admission in the past year	44 (18)	10 (4)	0.000
Current active smoking	114 (46)	46 (19)	0.000
Current passive smoking	86 (35)	41 (17)	0.000
Drugs			
• former intra-venous drug addict	17 (7)	-	0.000
• current use of drugs	44 (18)	3 (1)	0.000
Alcohol use			0.15
• < 21 units p/wk	150 (61)	145 (61)	0.35
• > 21 units p/wk	23 (10)	7 (3)	0.004
Skin problems	103 (42)	37 (16)	0.000
Diabetes Mellitus	16 (7)	8 (3)	0.11
COPD	13 (5)	15 (6)	0.62
Co-infection Hepatitis B	17 (7)	N.T. [b]	
Antibiotic use in preceding month	12 (5)	6 (3)	0.17
Vaccination			
• *H. influenzae* (n=486)	106 (43)	69 (29)	0.001
• *S. pneumoniae* (n=585)	2 (0.8)	1 (0.4)	0.58 [c]
• other (n=457)	98 (45)	78 (33)	0.009

Table 5.1: Continued

	Case n=248 (%)	Control n=239 (%)	p-value
CD4 cell (10^6/L)			
• median [range]	410 [40 – 1380]	500 – 1500 [d]	
• < 200	32 (13)		
• > 200	216 (87)		
HIV-RNA copies/ µl			
• median	50 [50 ->1*105]	N.T. [b]	
Viral load			
• undetectable	175 (71)	-	
HAART medication	191 (77)	0	
PJP prophylaxis	25 (10)	0	

[a] Mann-Whitney U test
[b] N.T. = not tested
[c] Fisher's exact test
[d] Reference value, no lab tests were performed

In the HIV infected group, CD4 cell counts ranged from 40-1380*10^6/L (median 410 *10^6/L). A CD4 cell count < 200*10^6/L was found in 13% of the patients. HIV-RNA plasma concentrations of the HIV patients ranged from ≤ 50-100.000 copies/µl. One hundred seventy-five patients (71%) had an undetectable viral load (HIV-RNA ≤ 50 copies/µL) and 191 patients (77%) were on HAART. Only 26 patients (10%) used PJP prophylaxis.

Table 2 shows the *S. aureus* and *S. pneumoniae* carriage rates. Of the 248 patients that were included, one nasal swab was obtained at the first investigation moment. In 191 patients (77%) a second nasal swab was done at least one week later. In 12 cases (5%), who had the first swab positive for *S. aureus*, it was impossible to obtain a second nasal swab and therefore they were excluded from further analysis. Sixty-seven (27%) of the first nasal swabs were positive for *S. aureus*, compared to 63 (25%) of the second cultures. From 35 controls (15%) we were not able to obtain a second nasal swab, and therefore they were excluded. Eighty-six controls (36%) had the first nasal swab positive with *S. aureus* and 59 (25%) had a second positive nasal swab. According to the culture rule, 19% of cases (n= 48) versus 18% of controls (n=44) were persistent carriers of *S. aureus* carriage (p=0.67).

Within the HIV positive group, the median CD4 cell counts in the persistent carriers were significantly higher than of the non- or intermittent *S. aureus* carriers (p=0.02). None of the other HIV related factors, such as undetectable viral load (p=0.69), PJP prophylaxis (p=0.31) and use of HAART (p=0.43) were associated with persistent *S. aureus* carriage (Table 3a). Furthermore, in the HIV infected as well as in the control group, no determinants were found to be significantly associated with persistent *S. aureus* carriage (Table 3a & 3b).

Table 2: *S. aureus* and *S. pneumoniae* carriage

	Case n=248 (%)	**Control n=239 (%)**	**p-value**
Staphylococcus aureus data			
• non-intermittent carriage	188 (76)	160 (67)	0.67
• persistent carriage	48 (19)	44 (18)	
• missing	12 (5)	35 (15)	
Streptococcus pneumoniae data			
• carriage	4 (2)	4 (2)	0.95
• no carriage	244 (98)	234 (98)	
• missing	-	1 (0.4)	

S. pneumoniae was cultured in four (2%) of the 248 patients as well as in four (2%) out of 239 persons in the control group (p=0.95). In the patient group four (2%) out of 191 second nasal swabs versus two (1%) in the control group were also positive for *S. pneumoniae* (p=0.65).
As for the *S. aureus* cultures we intended to look at risk factors that could be associated with *S. pneumoniae* carriage. Because of the very small number of carriers with *S. pneumoniae* no further statistical analysis was performed.

We analyzed the first nasal swab and nasopharyngeal swab for co-colonization of the two bacteria. In the HIV infected population, the four *S. pneumoniae* positive patients did not carry *S aureus* in their nasal cavity (p=0.58). In the healthy control group, two out of the four positive *S. pneumoniae* carriers were *S. aureus* carriers (p=0.56). Therefore, no interference could be demonstrated between *S. aureus* carriage and *S. pneumoniae* carriage.

Table 3a: Determinants of persistent nasal carriage of *S. aureus* in HIV patients (n=236)

	Persistent carrier (n=47) n (%)	Non-/intermittent carrier (n=189) n (%)	p-value	OR (95% CI)
Male	40 (85)	139 (74)	0.10	2.06 (0.87 – 4.89)
Age median, yr [range]	45 [21-60]	44 [20-80]	0.96 [a]	
European	30 (64)	104 (55)	0.28	1.44 (0.75 - 2.79)
Number of people living in same household • median (range)	2 [1-12]	2 [1-15]	0.44 [a]	
Children under 18 yr living in the same home	7 (15)	38 (20)	0.42	0.70 (0.29 - 1.67)
Hospital admission preceding yr	5 (11)	35 (19)	0.20	0.52 (0.19 - 1.42)
Current active smoking	18 (38)	92 (49)	0.20	0.65 (0.34 - 1.26)
Current passive smoking	13 (28)	68 (36)	0.28	0.68 (0.34 - 1.38)
Alcohol use	37 (79)	130 (69)	0.18	1.68 (0.78 - 3.60)
Skin problems	20 (43)	77 (41)	0.82	1.08 (0.56 - 2.06)
Diabetes Mellitus	1 (2)	14 (7)	0.32 [b]	0.72 (0.04 - 2.12)
COPD	1 (2)	12 (6)	0.47 [b]	0.32 (0.04 - 2.53)
Hepatitis B	5 (11)	11 (6)	0.33 [b]	1.93 (0.64 - 5.84)
Antibiotics	3 (6)	9 (5)	0.71 [b]	1.36 (0.35 - 5.25)
Vaccination				
• *H. influenzae* (n = 235)	15 (32)	86 (46)	0.09	0.56 (0.28 - 1.09)
• *S. pneumoniae* (n = 234)	0 (0)	1 (0.4)	1.00 [b]	
• other (n = 210)	20 (48)	75 (45)	0.73	1.13 (0.57 - 2.22)
CD4 cell count (* 10^6/L , median)	460	400	0.02 [a]	
CD4 > 200 * 10^6/L	42 (89)	162 (86)	0.51	1.40 (0.51 - 3.86)
Undetectable viral load	34 (72)	131 (69)	0.69	1.16 (0.57 - 2.36)
PJP prophylaxis	3 (7)	22 (12)	0.31	0.53 (0.15 - 1.84)
Use of HAART	34 (72)	147 (78)	0.43	0.75 (0.36 - 1.54)

[a] Mann-Whitney U test
[b] Fisher's exact test

Table 3b: Determinants of persistent nasal carriage of *S. auerus* in healthy controls (n=204)

	Persistent carrier (n=44) n (%)	Non-/intermittent carrier (n=160) n (%)	p-value	OR (95% CI)
Male	29 (66)	105 (65)	0.99	1.04 (0.52 – 2.10)
Age median [range]	41.5 [21-56]	37.0 [19-63]	0.65 [a]	
European	43 (98)	157 (98)	0.86	0.82 (0.08 - 8.10)
Number of people living in same household				
• median, yr [range]	2 [1-5]	2 [1-9]	0.61 [a]	
Children under 18 yr living in the same home	16 (36)	50 (31)	0.52	1.26 (0.63 - 2.53)
Hospital admission preceding yr	1 (2)	7 (4)	0.53	0.51 (0.06 - 4.25)
Current active smoking	10 (23)	33 (21)	0.76	1.13 (0.51 - 2.53)
Current passive smoking	9 (21)	28 (18)	0.65	1.21 (0.52 - 2.81)
Alcohol use	33 (75)	96 (60)	0.07	2.00 (0.94 - 4.24)
Skin problems	9 (21)	21 (13)	0.22	1.70 (0.72 - 4.04)
Diabetes Mellitus	2 (5)	6 (4)	0.68 [b]	1.22 (0.24 - 6.28)
COPD	2 (5)	8 (5)	1.00 [b]	0.91 (0.19 - 4.42)
Antibiotics	1(2)	4 (3)	1.00 [b]	0.91 (0.10 - 8.33)
Vaccination				
• *H. influenzae* (n = 204)	13 (31)	49 (30)	0.89	0.95 (0.46 - 1.97)
• *S. pneumoniae* (n = 204)	0 (0)	1 (0.6)	1.00 [b]	
• other (n=203)	13 (30)	52 (33)	0.69	0.86 (0.42 - 1.79)

[a] Mann-Whitney U test
[b] Fisher's exact test

Discussion and Conclusion

Many studies have been performed in the field of *S. aureus* and *S. pneumoniae* carriage in HIV infected persons. To our knowledge this is the first case-control study in the HAART-era looking at persistent carriage and possible determinants of *S. aureus* and *S. pneumoniae* in HIV infected patients versus healthy controls. The prevalence of persistent nasal *S. aureus* carriage (19% versus 18%) and nasopharyngeal *S. pneumoniae* carriage (2% versus 2%) did not differ between the HIV infected persons and healthy controls. These results are in contrast with previous studies, showing that HIV infected patients have higher rates of *S. aureus* and *S. pneumoniae* carriage and that they are at a higher risk of infections with these pathogens [4, 9, 13-25].

Table 4 demonstrates the different studies that are performed in the field of *S. aureus* carriage in HIV infected patients. Eight of these studies used a control group [13-15, 19, 20, 23-25], but only three of these studies were performed in the HAART-era [19, 20, 24]. None of these three studies are comparable to our study, as the control groups used in these studies did not consist of healthy volunteers as the controls were in our study. Persistent *S. aureus* carriage was only assessed in three studies in the HAART-era [18, 19, 22] and the prevalence varied between 26.8% and 39.4%. As the study by Miller et al [19] was performed in a group of current and former drug users, we cannot compare our results with this study as only 13% of our population was a current or former (intravenous) drug user.

In the uncontrolled study performed by Melles et al [18], the prevalence of *S. aureus* persistent carriage was nearly 30%, which is 10% higher than in our study, for which there does not seem to be an obvious explanation. Study design (patient recruitment and culture techniques) and study population (sex, mean age, CD4 cell count, PJP prophylaxis and use of HAART) of the two studies were similar, the only differences being the controlled design of the current study. PJP prophylaxis [18, 22] and use of HAART [18] have been demonstrated to be protective against (persistent) *S. aureus* carriage. Although not statistically significant, our study also seems to show that these two determinants are protective against persistent *S. aureus* carriage. The studies performed by Melles et al [18] and Padoveze et al [22] showed to have a lower percentage of HIV infected patients with an undetectable viral load (62% and 45% respectively) as compared to our study (71%).
In our study, an increased median CD4 cell count was associated with persistent *S. aureus* carriage. The reason behind this unexpected result might be associated with the use of PJP prophylaxes. PJP prophylaxis is only offered to patients who have CD4 cell counts under 200 * 10^6/L. As shown by Melles et al., people tend to be protected against persistent carriage with *S. aureus* when PCP prophylaxis is used.

Table 4: *S. aureus* carriage studies performed in HIV infected

Reference number	year	country	study population	study exclusion criteria
23	88	USA	Admitted AIDS patients	Yes [a]
15	88-89	UK	MSM	No
14	91	USA	MSM	Yes [b]
13	92	Kenia	Hospitalized patients	No
25	92	Germany	Hospitalized and out-patients	No
9	99	USA	Outpatients	No
24	99	Austria	Hospitalized patients	Yes [e]
20	99-00	USA	Current and former drug users	No
19	99-00	USA	Current and former drug users	No
16	01	USA	Former drugs users	No
22	00-03	Brazil	Outpatients	Yes [i]
18	04-05	Netherlands	Outpatients	No

reference number	*S. aureus* carriage HIV infected	number of swabs	persistent *S. aureus* carriage	antibiotic use < mth	CD4 <200	HAART use	HIV viral load undetectable
23	55%	1	-	n.s. [aaa]	N.R.	Zidovudine n.s.	N.R.
15	45%	1	-	N.R.	N.R.	-	N.R.
14	32%	1	-	N.R.	N.R.	Zidovudine n.s.	N.T.
13	27%	1	-	N.R.	N.R.	-	N.R.
25	44.1%	N.R.[cc]	12% [ccc]	N.R.	n.s. [cccc]	-	N.R.
9	34%	3 [d]	12.9% [d]	N.R.	n.s. [dd]	-	N.R.
24	42.6%	1	-	0.02 [ee]	n.s.	n.s.	n.s.
20	29%	1	-	n.s. [f]	n.s.	n.s.	N.R.
19	29%	1	Data not shown [g]	N.R.	n.s.	n.s.	N.R.
16	49.3%	1		N.R	n.s	N.R.	N.R.
22	58% [ii]	3 [iii]	39.4% [iii]	n.s [iiii]	n.s. [iiiii]	n.s [iiiii]	n.s. [iiiii]
18	-	2	26.8%	N.R.	N.R.	0.04	n.s.

[a] exclusion criteria: i.v. drug abuse; insulin-dependent diabetes mellitus; hemodialysis; injections for treatment of allergies.
[aa] pentamidine inhalation instead of co-trimoxazole
[aaa] antistaphylococcal antibiotic use 2 weeks before admission
[b] exclusion criteria: i.v. drug abuse (within 1 week); insulin-dependent diabetes mellitus; dialysis in case of chronic renal failure.
[c] mean CD4 cell count (range)
[cc] hospitalized patients: the first swab was obtained within 24 hours after admission and once weekly thereafter. Outpatients: swabs were obtained when patients reported to the outpatient department during the study period of 8 months.
[ccc] persistent carriage = 80% of the swabs positive with *S. aureus*
[cccc] p-value based on *S. aureus* colonization
[d] persistent carriage = two or more positive *S. aureus* cultures separated by one or no negative *S. aureus* culture.
[dd] p-value based on total group of nasal carriers (n=69)
[e] exclusion criteria: history of i.v. drug abuse; nursing home residency; underlying clinical conditions (insulin-dependent diabetes mellitus, continuous ambulatory peritoneal dialysis or hemodialysis; lymphedema; and patients who had undergone organ transplantation.

number of patients included	gender (male)	age mean	HAART use	CD4 median	CD4<200 copies/mL	PCP prohpylaxis	HIV undetectable
64	97%	38.9	Pre-HAART	N.R.	N.R.	27% [aa]	N.R.
47	100%	N.R.	Pre-HAART	N.R.	N.R.	N.R.	N.R.
90	100%	N.R.	Pre-HAART	N.R.	N.R.	N.R.	N.R.
264	N.R.	N.R.	Pre-HAART	N.R.	N.R.	N.R.	N.R.
136	87%	35.7	Pre-HAART	0-416 [c]	N.R.	N.R	N.R.
201	N.R.	N.R.	Pre-HAART	N.R.	N.R.	51%	N.R.
47	85%	40.2	42.6%	173	57.4%	N.R.	47%
193	N.R.	N.R.	24%	231	45%	20%	N.R.
131	N.R.	N.R.	+/- 33%	252	N.R.	N.R.	N.R.
75	65%	42	84%	N.R.	64%	N.R.	41% [h]
111	62%	36.9	74% [ii]	455-484[ii]	9% [ii]	9.9% [ii]	45% [ii]
507	71%	41.7	75%	410 [j]	N.R.	11%	62%

PCP prophylaxis	skin lesions	control group	*S. aureus* carriage control group	p-value (case vs controle)
N.R.	N.R.	Admitted to hospital with other disease(n=64)	28%	<0.005
N.R.	N.R.	HIV negative MSM (n=56)	27%	<0.05
N.T.	N.T.	HIV negative MSM (n=39)	19%	0.05<p<0.01
N.R.	N.R.	HIV negative patients (n=290)	17%	0.008
N.R.	N.R.	Healthy hospital staff (n=47)	23%	<0.05 [cccc]
		hospitalized pt with chronic disease (n=30)	31%	n.s.[cccc]
0.008 [dd]	0.04 [dd]	-	-	-
N.R.	n.s.	HIV negative patients at infectious disease department (n=123)	24%	0.03
n.s.		HIV negative current and former drug users (n=307)	21%	0.04
n.s.	N.R.	HIV negative current and former drug users (n=151)	18%	0.03 [gg]
N.R.	N.R.	-	-	-
0.05 [iiiii]	N.R.	-	-	-
0.04	n.s.	-	-	-

[ee] antistaphylococcal antibiotic use 2 weeks before admission
[f] antibiotic use in previous two weeks
[g] Persistent carriage = a minimum of two nasal cultures positive with *S. aureus* during a 1-year follow-up. Percentage of persistent carriage in HIV infected is not mentioned. 36/96(38%) of the total group were persistent carriers, but the only predictor of persistent *S. aurus* carriage was HIV-seropositive status (p=0.002).
[gg] p-value based on first nasal swab
[h] HIV undetectable = HIV viral load < 400 copies/mL
[i] exlusion criteria: hospitalization in the previous two years; *S. aureus* infection at baseline.
[ii] percentage or (median) number based on first nasal swab (n=111)
[iii] persistent carriage = two or three nasal cultures positive with *S. aureus* (n=99)
[iiii] antibiotic use in previous 6 months
[iiiii] p-value based on first nasal swab (n=111)
[j] mean CD4 cell count
MSM men having sex with men
N.R. not reported
n.s. not significant

An explanation for not finding a difference in persistent carriage, between cases and controls and the previous studies performed, may be that the current HIV cohort had a relatively high CD4 cell count and that 71% of the patients had an undetectable viral load. This improved immunological status of the HIV infected patients in our study population may have resulted in the fact that prevalence of persistent *S. aureus* carriage in HIV infected is similar to that in a healthy population and therefore it may be plausible that no further determinants of carriage have been observed.

Studies on carriage of *S. pneumoniae* in HIV positive patients show conflicting results. One study [21] found a higher carriage rate in HIV positive people, while others [10, 12] found no difference in carriage rates between HIV positive or negative patients. The HIV infected patients in this study showed low rates of *S. pneumoniae* carriage probably because of high percentages (90%, n=172) of successful HAART. Nicoletti et al. [21] demonstrated that over more than one-year use of the same HAART regimen significantly reduced the risk on *S. pneumoniae* carriage.
The overall low carriage rate of *S. pneumoniae* in both HIV positive as in HIV negative controls may be explained by different culture methods. In this study we used non-selective blood agar plates instead of blood agar plates containing 2.5 µg/mL gentamicin. The gentamicin plates are suggested to be selective in isolating *S. pneumoniae* [26, 27].

In a recent study by Bogaert et al [28] a negative correlation was reported between *S. aureus* and *S. pneumoniae*. In the present study we found no association between the presence of both bacteria, therefore no bacterial interference could be demonstrated. However, the low number of *S. pneumoniae* carriers in our study makes that no firm conclusions can be drawn from our data.

This case-controlled study has some limitations. We aimed to match every patient to a healthy control within the same age category and with the same gender. However, there were more male patients (75%) than male controls (67%) and the median age of the control group was significantly lower. The general characteristics of the HIV infected patients and healthy control group were not concordant in many cases. These differences are probably not relevant for the results of this study as none of these characteristics showed to be significantly associated with persistent *S. aureus* carriage in our study.

In summary, we found that the prevalence of *S. aureus* and *S. pneumoniae* carriage among HIV infected patients is similar to a healthy population. This is in contrast to earlier non-controlled observational studies. An increasing median CD4 cell count is significantly associated with persistent *S.aureus* carriage. No further determinants of carriage for either and no evidence

for bacterial interference between the two bacteria could be demonstrated in this case-controlled study. The increased immunological status of the HIV infected persons has induced that the carriage rates of *S. aureus* and *S. pneumoniae* does not differ anymore from non-HIV infected persons. Therefore we conclude that carriage with *S. aureus* or *S. pneumoniae* is not the reason why HIV positive patients more often have infections with *S. aureus* and have more invasive pneumococcal pneumonias.

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Chapter 6

Prevalence of carriage and antibiotic resistance of *Staphylococcus aureus* in HIV infected persons in rural Zambia

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Abstract

The prevalence of *S. aureus* nasal carriage in HIV infected adult persons in rural Zambia and antibiotic resistance of *S. aureus*, were assessed.

During a 2 months period 129 HIV positive adult patients were included in this study. A questionnaire was filled-out with potential determinants of *S. aureus* carriage. Two nasal swabs were obtained to determine *S. aureus* carrier state: persistent carriage (both cultures positive), non- or intermittent carriage (none or one culture positive). All swabs were sent for culture and resistance testing according to standard operating procedures

Forty-one (32%) patients were persistent *S. aureus* carriers. The presence of boils or furuncles was significantly associated with persistent *S. aureus* carriage. Twenty-one percent of *S. aureus* strains were MRSA, 21% were resistant to gentamicin, 85% to trimethoprim-sulfamethoxazole, 38% to erythromycin, 73% to tetracycline, 96% to penicillin, 23% to ceftriaxone and 3% were ciprofloxacin resistant. Eighty-eight percent of the *S. aureus* strains were resistant to 3 or more antibiotics.

Due to the high prevalence of *S. aureus* nasal carriage and high level of antibiotic resistance, implementing diagnostic microbiologic facilities are needed. These facilities can have an impact on improving antibiotic treatment of (*S. aureus)* infections in developing countries.

Introduction

Human immunodeficiency virus (HIV) infection, with or without progression to acquired immunodeficiency syndrome (AIDS), has been associated with increased rates of colonization with *S. aureus* resulting in an increased risk of developing an *S. aureus* infection [1]. The mechanism that leads to this increased colonization rate with *S. aureus* remains unclear but it is known that *S. aureus* is an important pathogen which can cause a wide range of clinically important infections that can vary from mild to life-threatening [2]. In the past, *S. aureus* infections in HIV positive patients have been attributed to low CD4 cell counts [3].

In sub-Saharan Africa, where the prevalence of HIV-1 is high, the burden of disease caused by staphylococci is significant, also among HIV uninfected individuals [4-6]. Studies on *S. aureus* carriage and infection in HIV infected patients in sub-Saharan Africa have been predominantly aimed at children [7, 8]. Antimicrobial resistance increases worldwide and therefore it will not spare the developing countries [9]. Data regarding endemic antimicrobial resistance are scarce in many parts of the world, especially from areas where over-the-counter antibiotic usage is common. Therefore, data concerning *S. aureus* carriage, infection rates and antibiotic susceptibility profiles in an adult sub-Saharan HIV infected population are lacking.

With this study, we set out to assess the prevalence and possible determinants of *S. aureus* carriage, as well as the prevalence of antibiotic resistance in *S. aureus* to evaluate the appropriateness of national treatment guidelines of *S. aureus* infections in a rural Zambian outpatient population of adult HIV infected patients.

Patients and Methods

Study population and data collection

All HIV infected patients visiting the HIV outpatient department of Macha Mission Hospital, Choma, Zambia, between October 4th 2007 and November 22th 2007 were invited to participate in the study. Patients could be enrolled when they were 18 years or older and had a life expectancy of more than one month.

The protocol for this study was approved by the Committee for Ethics in Research at the Erasmus University Medical Center Rotterdam, The Netherlands and the University of Zambia Research Ethics Committee (UNZA REC), Lusaka, Zambia (REC 008-08-07). All patients gave their written informed consent prior to enrolment.

Data of HIV infected patients were recorded by chart review. The medical records were reviewed for the following data: year of birth; gender; hospital admissions in the preceding year; HIV infection related data (the most recent CD4 cell count, the use of antiretroviral therapy (ART) and *Pneumocystis jirovecii* pneumonia prophylaxis (PJP) with trimethoprim-sulfamethoxazole) and other factors that may contribute to *S. aureus* carriage such as skin problems (furunculosis/boils, rash, eczema); current respiratory tract infections (upper or lower); the most recent ALT (alanine aminotransferase) and history of antimicrobial treatment in the preceding month. A short questionnaire was performed when patients visited the outpatient department. This questionnaire included questions concerning alcohol use, smoking habits and household compositions (how many people does the household consists of; how many people are sleeping in the same room; are there children under 18 years present in the household).

Two nasal swabs were taken from each participating patient. The patients had their first nasal swab obtained during their regular outpatient clinic visit. The second nasal swab was taken approximately four weeks later during their next outpatient clinic visit.

Microbiological procedures

Nasal swab cultures were performed according to a standard operating procedure [10]. Nasal swabs were obtained using dry sterile cotton-wool swabs (Transwab, Medical Wire & Equipment Co. Ltd., Corsham, United Kingdom). The left and right anterior nares were swabbed and the swabs were stored in the refrigerator at 4°C before inoculation within 4 hours. Swabs were then cultured in phenol red mannitol salt broth (PHMB). Only when PHMB showed staphylococcal growth after 5 days of incubation at 37°C, the PHMB was sub-cultured on agar plates specially designed to identify *S. aureus* (BBL™ CHROMagar™ Staph aureus). A catalase test and a latex agglutination test (Staphaurex Plus ^{R}, Murex, Dartford, United Kingdom) were performed for confirmation.

In Zambia, susceptibility testing of all isolates was performed for gentamicin, penicillin, ciprofloxacin, erythromycin, tetracycline, sulphamethoxazole-trimethoprim and ceftriaxone (Oxoid, Hampshire, England) by disk-diffusion on Mueller-Hinton agar plates. Methicillin-resistant *S. aureus* was detected using an oxacillin 1 µg (Oxoid, Hampshire, England) disk on Mueller-Hinton agar plates.

All *S. aureus* isolates were stored in a transport medium (choc-agar) at 4°C. At the Erasmus University Medical Center Rotterdam, all isolates were to be confirmed as *S. aureus* using Staphaurex Plus and the AccuProbe hybridization test (Gen Probe Inc., San Diego, CA). Antimicrobial susceptibility testing was repeated using Vitek2 (bioMerieux-Vitek,

Hazelwood,Mo.) and cefoxitin disc diffusion. Methicillin resistance was confirmed by *mec*A PCR [11]. No PFGE was performed to genotype the *S. aureus* strains.

All tests were performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines [12].

Definitions

Staphylococcus aureus nasal carriage was defined according to an earlier validated culture rule [10]. This rule distinguishes between persistent (both nasal swabs positive with *S. aureus*) and either intermittent or non-carriers (one or no swabs positive with *S. aureus*, respectively). Intermittent and non-carriers have the same clinical outcomes, therefore the non- and intermittent carriers were combined to contrast with the persistent carrier group.

Statistical analysis

Proportions were compared by the Chi-square test (Fisher's exact test in case of small numbers) and continuous data with Mann-Whitney U test. To identify independent determinants of *S. aureus* carriage, variables were tested univariately. All statistical tests were two-tailed and a significance level of 0.05 was used.

Results

Between October 4th 2007 and November 22nd 2007, 129 HIV positive patients were included in the study. General characteristics are shown in Table 1. The median age of the study participants was 39 years (range 20-74 years) and 63% were female. Sixty-five percent of the patients lived with four or more persons in one house and over 88% had children under 18 years sleeping in the same room. Only eight patients (6%) were exposed to cigarette smoke; 15 patients (12%) were using alcohol; 50 (39%) had at least one hospital admission in the preceding year; 61 (47%) reported skin problems (current or in the preceding year); 22 (17%) had a current respiratory tract infection; the most recent ALT was elevated in 16 patients (12%); and 36 patients (28%) had a history of antibiotic treatment in the month prior to the nasal swabs.

The CD4 cell counts ranged from 45-1321 cells/mm^3 (median 249 cell/mm^3). A CD4 cell count < 200 cell/mm^3 was recorded in 37% of the patients. A majority (61%) of patients was using anti-retroviral therapy (ART) and PJP prophylaxis was used by 88% (n=114).

Table 1: General characteristics of HIV positive patients in rural Zambia participating in the study

	N = 129 (%)
Female	81 (63)
Age (yr)	
• mean	39.8
• median (range)	39 (20 – 74)
Children sleeping in same room	114 (88)
> 4 persons sleeping in same room	85 (66)
Alcohol use	15 (12)
Smoking	8 (6)
Hospital admission preceding year	50 (39)
RTI [a] preceding month	22 (17)
Skin problems	61 (47)
Elevated ALT [b]	16 (12)
On ART [c]	79 (61)
• AZT/3TC/EFV	27 (23)
• AZT/3TC/NVP	11 (14)
• d4T/3TC/EFV	18 (23)
• d4T/3TC/NVP	13 (17)
• other	10 (13)
On PJP prophylaxis [d]	114 (88)
PJP prophylaxis resistant *S. aureus* strain [e]	62 (85)
Antibiotic use in the preceding month	36 (28)
$CD4^+$ cell counts (cell/mm^3)	
median $CD4^+$ count	249 (45 – 1321)
• $CD4^+$ <200	47 (36)
• $CD4^+$ 200 - 500	59 (46)
• $CD4^+$ >500	15 (12)
Staphylococcus aureus data	
• non- or intermittent carriage	72 (56)
• persistent carriage	41 (32)
• missing	16 (12)

[a] RTI = respiratory tract infection
[b] ALT = alanine aminotransferase
[c] ART = anti-retroviral therapy; AZT=zidovudine; 3TC=lamivudine; EFV=efavirenz; d4T=stavudine
[d] PJP = *Pneumocystis jirovecii* pneumonia prophylaxis with trimethoprim-sulfamethoxazole
[e] number of *S. aureus* strains n = 73

Of the 129 patients that were included, one nasal swab was obtained at study enrollment. In 101 of the 129 patients there was a second nasal swab done at least one week later. In 16 patients (12%) who had a first positive swab, it was impossible to obtain a second nasal

swab within a month and therefore they were excluded from further analysis. Sixty-two (48%) positive *S. aureus* cultures were obtained at the first swab moment and 57 (42%) positive *S. aureus* cultures obtained the second time. According to the culture rule 36% (n=41) of the patients were persistent *S. aureus* carriers.

Determinants that could be associated with persistent *S. aureus* carriage were analyzed and are shown in Table 2. After univariate analysis only the presence of furuncles or boils (p=0.02, OR 12.95, 95% CI 1.46-115.20) showed a positive association with persistent *S. aureus* carriage. More specific HIV-related determinants were also analyzed through univariate analysis. None of the HIV-related factors, such as the use of ART (p=0.34), increasing CD4 cell count (p=0.16) or PJP-prophylaxis (p=0.75) were associated with persistent *S. aureus* carriage.

Table 2: Determinants of persistent *S. aureus* nasal carriage in HIV patients

Characteristic	Persistent carrier (n=41)	Non-/intermittent carrier (n=72)	p-value	OR (95% CI)
	n (%)	n (%)		
Sex (female)	25 (61)	45 (63)	0.87	0.94 (0.43 – 2.06)
Children sleeping in same room	38 (93)	61 (85)	0.22	2.28 (0.60 – 8.72)
Hospital admission preceding year	13 (38)	29 (44)	0.58	0.79 (0.23 – 1.84)
RTI preceding month	7 (18)	12 (17)	0.91	1.06 (0.38 – 2.95)
Skin problems	22 (54)	31 (43)	0.28	1.53 (0.71 – 3.31)
• boils	6 (15)	1 (1)	0.02 [a]	12.95 (1.46 – 115.20)
On ART	28 (68)	42 (59)	0.34	1.49 (0.66 – 3.34)
On PJP prophylaxis	35 (92)	65 (90)	0.75	1.26 (0.31 – 5.16)
AB use preceding month	11 (28)	21 (29)	0.85	0.92 (0.60 – 1.85)
$CD4^+$ cell counts < 200 (cell/mm^3)	17 (44)	23 (34)	0.34	1.48 (0.66 – 3.32)

[a] Fisher's exact test (two sided)

Antimicrobial susceptibility was determined from each *S. aureus* strain. In 88 % of the patients carrying a *S. aureus* strain, the isolate was resistant to three or more classes of antibiotics. The results of the susceptibility testing are shown in Table 3.

Although not statistically significant, we observed that all MRSA positive patients (n=15), compared to MSSA (methicillin-susceptible *Staphylococcus aureus*) (n=56) were using trimethoprim-sulfamethoxazole prophylaxis (p=0.19).

Eighty-five percent of the *S. aureus* strains were resistant to trimethoprim-sulfamethoxazole. The use of trimethoprim-sulfamethoxazole was significantly associated with a trimethoprim-sulfamethoxazole resistant *S. aureus* strain (p=0.001, OR 18.67 95% CI 3.41-102.05).

Table 3: Antibiotic resistance patterns of *S. aureus* strains

strain nr.	nr of identical strains	P	SXT	TE	E	CN	OX	CRO	CIP
1	3	S	S	S	S	S	S	S	S
2	2	R	S	S	S	S	S	S	S
3	9	R	R	S	S	S	S	S	S
4	1	R	S	S	R	S	S	S	S
5	4	R	S	R	S	S	S	S	S
6	20	R	R	R	S	S	S	S	S
7	1	R	R	R	S	S	S	R	S
8	5	R	R	S	R	S	S	S	S
9	10	R	R	R	R	S	S	S	S
10	1	R	R	R	S	R	S	S	S
11	2	R	R	R	R	S	S	S	R
12	1	R	S	R	R	S	R	R	S
13	5	R	R	R	S	R	R	R	S
14	9	R	R	R	R	R	R	R	S
Total resistance (%)	73	70 (96)	62 (85)	53 (73)	28 (38)	15 (21)	15 (21)	16 (23)	2 (3)

R = resistant; S = susceptible
P= penicillin; SXT= trimethoprim-sulfamethoxazole; TE= tetracycline; E= erythromycin; CN= gentamicin; OX= oxacillin; CRO= ceftriaxone; CIP= ciprofloxacin

Discussion and Conclusion

Our study shows a high rate of persistent *Staphylococcus aureus* carriage (32%) in an outpatient HIV positive adult cohort in rural Zambia. The frequent use (28%) of antimicrobials up to a month prior to the nasal cultures may have hampered the detection of organisms susceptible to commonly used antimicrobials. For this reason the study may even underestimate the prevalence of persistent *S. aureus* nasal colonization.

Socio-economic factors such as housing, crowding, poor hygiene and access to healthcare are major determinants of *S. aureus* colonization [1]. The rural environment in which this study was performed may have attributed to the high colonization rate with *S. aureus* observed. Over 50% of the patients slept with more than four persons in one room. This crowded environment supports the spreading of (antibiotic-resistant) pathogens among household contacts. The high percentage of MRSA positive strains (21%) can be explained by the transmission of MRSA into the community and it may cause a further spread and an ongoing problem for community-acquired MRSA.

The presence of furuncles or boils was significantly associated with persistant *S. aureus* nasal carriage, which has been described in other studies. No further (HIV-related) determinants which could attribute to *S. aureus* carriage were demonstrated within this study, probably due to the relatively small population studied. Previous studies concerning *S. aureus* carriage in HIV infected patients have linked CD4 cell depletion to persistent carriage [3, 7]. Thirty-seven percent of the patients in this study had a CD4 cell count under $200*10^6$ cells/L, but no significant association with persistent *S. aureus* carriage was found. It is possible that from some patients the CD4 cell count was not up to date because in some cases CD4 cell counts were tested 6 months before the nasal swabs were obtained. Therefore this might be an explanation for not demonstrating a significant relation with persistent *S. aureus* carriage and CD4 cell counts.

The role of PJP prophylaxis on *S. aureus* nasal carriage remains unclear. A recent study in a HIV positive adult cohort showed that receiving ART and PJP prophylaxis were negatively associated with persistent *S. aureus* carriage [13]. No evidence of this result was found within our study because even under high percentages of trimethoprim-sulfamethoxazole usage (92% of the patients with CD4 < $200*10^6$ cells/L and in 89% of the patients with a CD4 count > $200*10^6$ cells/L) a high rate of persistent *S. aureus* carriage was found. Zar et al [14] as well found that HIV infected children on trimethoprim-sulfamethoxazole prophylaxis had significant higher rates of *S. aureus* carriage in their nasopharynx compared to HIV infected children not on prophylaxis [14].

PJP prophylaxis with trimethoprim-sulfamethoxazole seems to have another effect on *S. aureus* carriage. One study showed that MRSA carriage was significantly associated with trimethoprim-sulfamethoxazole prophylaxis [7]. Even though no significant result was found in our study, it showed that all MRSA positive swabs (n=15) belonged to patients who were using trimethoprim-sulfamethoxazole prophylaxis, therefore all MRSA isolates were trimethoprim-sulfamethoxazole resistant.

Trimethoprim-sulfamethoxazole prophylaxis is an important intervention to reduce morbidity and mortality of PJP. However, long-term monitoring of the effect on other bacterial pathogens and their resistance patterns seems necessary as it may have effect on therapeutic outcomes in settings where trimethoprim-sulfamethoxazole is also often used.

The high rates of antibiotic resistance of *S. aureus* in a rural Zambia HIV infected adult population confirms data found in the few previous studies that were done in sub-Saharan Africa, although these two studies were performed in HIV infected children [7, 8].
In many healthcare centers in sub-Saharan Africa the first line antibiotics prescribed are ampicillin, chloramphenicol, erythromycin, tetracycline, penicillin, trimethoprim-sulfamethoxazole and ceftriaxone [15, 16]. It has been demonstrated that *S. aureus* strains

that are isolated in rural Africa, are generally penicillin resistant and often resistant to other antibiotics as well [17]. With local surveillance data on causative micro-organisms and antibiotic resistance lacking, antibiotic treatment guidelines are based on national and/or global (WHO) guidelines [15, 16]. This, in combination with the sometimes life-threatening infections that require immediate treatment, means that antibiotic prescription is largely guesswork and that resistance will only reveal itself by the occurrence of treatment failures. As demonstrated in this study, 88% of the strains isolated from *S. aureus* carriers were resistant to 3 or more of the first line antibiotics prescribed and, therefore, treatment options as well as treatment success will be limited [18].

A clonal strain of *S. aureus* could not be revealed, because no PFGE data was available. Because of the high percentage of persistent *S. aureus* carriage and the high number of MRSA and other antibiotic-resistant strains, a clonal strain cannot be ruled out.
We do not have epidemiological or clinical data to evaluate the extent in which the persistent carriage rate of *S. aureus* and the resistance patterns found in this rural hospital reflects endemic antimicrobial resistance within the whole of Zambia. A study performed in the north of Zambia demonstrated a high percentage of *S. aureus* strains as a causative agent of community-acquired pneumonias and a high number of antibiotic resistant strains were observed [19].

Our data suggest that a high colonization rate with *S. aureus* and a widespread antimicrobial resistance might exist in Zambia. Further research is needed to provide national policy makers with information to update antibiotic treatment guidelines in view of resistance development.

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DISCUSSION

Chapter 7
General discussion

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General Discussion

The aims of the studies described in this thesis were to explore the clinical epidemiology of methicillin-resistant and susceptible staphylococci carriage, transmission and eradication. During the last decades, MRSA has become the most prevalent antibiotic-resistant pathogen in hospitals in many parts of the world and an increasing number of reports describe the increasing prevalence in various populations in the community [1-3]. Furthermore, coagulase-negative staphylococci (CoNS) have long been regarded as apathogenic, but their important role as nosocomial pathogens has been recognized and studied in recent years [4, 5] and the emergence of methicillin-resistant CoNS has been limiting therapeutic options in case of infection and increases the risk of therapy failure [4, 6]. Finally, it has been demonstrated that metchillin-susceptible *S. aureus* is a major pathogenic causing increased rates of infections. HIV-infected patients are thought to have an increased rate of *S. aureus* colonization.

In **Chapter 7** the main findings of the studies performed in **Part I** and **Part II** of this thesis are discussed and future perspectives are presented.

Part I Methicillin-resistant staphylococci

The contribution of MRSA transmission in household contacts has so far not been studied extensively and because of lack of data and well-calculated scenarios, no evidence based policy for this reservoir has been developed. For this reason, being a household member of a MRSA carrier has not yet been established as a risk group for MRSA by the Dutch "Search-and-Destroy" policy. In **Chapter 2** we described the frequency and risk factors for MRSA (methicillin-resistant *Staphylococcus aureus*) transmission to household contacts of a MRSA positive index person. We aimed to perform a prospective observational study to determine the rate of transmission and risk factors associated with MRSA transmission from index persons to their household contacts. In order to do so, we screened household contacts from MRSA positive persons for MRSA, to determine whether transmission from index persons to household contacts had occurred. In addition, potential determinants of index person related MRSA transmission risk factors were obtained. Our study showed that MRSA transmission to household contacts occurred in nearly half of the index persons (47%) and two-third of the exposed household contacts (67%) became MRSA positive. The spread of MRSA among household contacts has been previously reported, but the described MRSA transmission rates to household contacts are variable [7-12]. As the lager part of these studies used different study and culture methods, direct comparison of study results was not feasible [8, 9, 11, 12]. The major, and perhaps most important difference is that these studies did not obtain

throat swabs to determine MRSA throat colonization in the index person. The importance of swabbing the throat to determine MRSA or MSSA (methicillin-susceptible *S. aureus*) carriage has been previously described [13-16]. Our study demonstrated that in the population studied, 42 out of 62 (68%) persons were MRSA colonized at least in the throat and that this throat colonization induces nearly a fourfold increase on the risk of MRSA transmission to household contacts.

Furthermore, risk factors for MRSA transmission were related to both MRSA index persons and household contacts. The study described in **Chapter 2** showed that MRSA transmission depended on the exposure time of MRSA to household contacts at home. This result is confirmed by a study reporting an increased transmission rate to household contacts from MRSA positive patients returning to their homes than to residential care homes after hospital discharge [7]. In addition, our study demonstrated that patients with eczema had an increased risk and patients with wounds had a decreased risk for transmitting MRSA to their household contacts. The most likely explanation for these results is that eczema sites, in contrast to wounds, usually are not covered by skin bandages. This may induce dispersion of MRSA by skin particles and thus may cause contamination of the environment and facilitate transmission to household contacts. Furthermore, our study confirmed other studies showing that the median age of index persons with transmission was significantly lower than the median age of those without transmission [7, 10]. Finally, two household contact related risk factors for MRSA acquisition from an index person were established in this study. First, partners of MRSA index persons were more at risk to acquire MRSA colonization than other household contact of an index person. This may be explained by the sharing of bed linen and increased intensive bodily contact, which is a known risk factor for MRSA carriage acquisition [17]. Secondly, transmission of MRSA to household contacts was significantly associated with an increased number of household contacts. Crowding has been shown before to be a risk factor for transmission of MRSA [7, 10].

Although the R_0 in our study was smaller than one, it seems plausible that MRSA transmission can cause small outbreaks in the direct community of an index person (e.g. in schools or sport clubs). In the United States several outbreaks of CA-MRSA (community-associated MRSA) have been described within prisons or football teams [18, 19]. Both studies described a highly conserved, community-associated MRSA clone (USA-300) which caused abscesses and that was indistinguishable from isolates from various other regions of the United States. Our study, on the other hand, demonstrated that no predominant PFGE type was transmitted more frequently compared to other PFGE types.

Carriage of MRSA precedes endogenous MRSA infections. In addition, as demonstrated in **Chapter 2**, MRSA carriage plays an important role in transmission of MRSA to household contacts and therefore potential dissemination within healthcare facilities and into the community [20-23]. To prevent further spread of MRSA and prevent endogenous MRSA infections, it seems essential to eradicate MRSA colonization. A recent systematic review on eradication of MRSA carriage showed varying success rates of different MRSA eradication therapies [24]. This review demonstrated that mupirocin was highly effective in short-time eradication of MRSA. In case of extra-nasal MRSA colonization or other factors that are associated with eradication treatment failure (e.g., skin lesions or mupirocin-resistant strains), systematic eradication treatment (e.g., rifampicin in combination with another oral antibiotic), in addition to mupirocin nasal ointment, was the treatment of choice. After eradication therapy, no consensus exists regarding the number, sites and time period of cultures that should be obtained to reliably assess the MRSA status of a previously positive individual. The Dutch national policy by the Working Party on Infection Prevention (WIP) suggests that a minimum of three follow-up culture sets should be obtained to declare a previous MRSA carrier as negative. However, this culture rule was not based on solid experimental evidence but on expert opinion [25].

The aims of the study described in **Chapter 3** were to assess the success rate of MRSA eradication therapy by using our MRSA eradication therapy protocol; to analyze determinants predicting MRSA eradication therapy outcome; and to assess the minimum number of follow-up screening moments after completion of MRSA eradication therapy needed to determine the effectiveness of MRSA eradication therapy. To do so, a prospective observational study was conducted with newly acquired MRSA positive healthcare workers (HCWs; n=22) or patients (n=88) who were admitted or treated and completed the follow-up period in our hospital during a two year period. This study demonstrated that 23% of the patients (n=20) spontaneous cleared their MRSA. These patients with spontaneous MRSA eradication were significantly more MRSA colonized at a single site and had significantly more extra-nasal MRSA colonization compared to patients that were actively treated with MRSA eradication therapy. The reason behind spontaneous eradication of MRSA remains unclear, but on the basis of our knowledge about MSSA carriage, several hypotheses could be put forward [26-28]. One of the presumed determinants of *S. aureus* carriage, is bacterial interference, as a negative correlation in co-colonization of *S. aureus* and *S. pneumoniae* in healthy children has been observed, which suggests a natural competition between colonization of bacteria [29]. We, on the other hand were not able to demonstrate this negative correlation between *S. aureus* and *S. pneumoniae* carriage in a HIV infected population as described in **Chapter 5.** Furthermore, as the MRSA bacterial load in single site carriers is probably lower than in multi-site carriers, spontaneously eradication of MRSA could more easily be accomplished [30, 31].

Our eradication treatment protocol resulted in a high success rate (81% intention-to-treat) of MRSA eradication. To accomplish this success rate, a mean of 1.5 MRSA eradication therapies was offered to our study population. A recently published study in 62 consecutive hospitalized patients with MRSA colonization or infection showed a high success rate (87% intent-to-treat analysis) of MRSA eradication, which is in agreement with our findings [32]. A major difference in study methods between this previous study (among others) and our study as described in **Chapter 3**, is the number of MRSA culture sets obtained to determine the success of the MRSA eradication therapy offered [32-35]. When the MRSA-free status of an individual would be determined on three culture sets, 31% of the individuals would be considered MRSA-negative incorrectly as these persons showed MRSA growth in one of the subsequent culture sets. Our study demonstrated that five or more culture sets were needed to have a at least a 90% predictive value of MRSA eradication therapy success. Finally, our study showed that the presence of wounds just before the start of MRSA eradication therapy, was significantly associated with MRSA eradication therapy failure. In case of MSSA, patients with *S. aureus* skin infections and skin diseases have been shown to be have higher *S. aureus* nasal carriage rates [36].

In addition, our study revealed, although not statistically significant, that MRSA throat colonization plays an important role in failure of MRSA eradication therapy. Although MRSA throat colonization was a risk factor for MRSA eradication therapy failure, it increases the risk of MRSA transmission to household contacts (see **Chapter 2**). Therefore, it is essential to start MRSA eradication therapy in case of throat colonization and to monitor the MRSA colonization status by obtaining throat swabs. Finally, in **Chapter 2** we described that a prolonged exposure time to a MRSA index person at home, increased the risk of MRSA transmission to household contacts. On the other hand, in **Chapter 3** we observed that by a 'wait-and-see' option, a significant amount of patients spontaneous lost MRSA without MRSA eradication therapy. These 'wait-and-see' patients expose their household contacts to MRSA for a longer period of time, which promotes the risk of MRSA transmission to these household contacts. Therefore, the 'wait-and-see' option should only be offered to those patients who have a significant chance to spontaneous lose their MRSA. Patients with a single site MRSA colonization (although not in the throat) are significantly more prone to lose the MRSA spontaneously and patients with wounds present just before the start of MRSA eradication therapy, have an increased risk on MRSA eradication therapy failure. Therefore, we suggest that only these two groups should be offered this 'wait-and-see' option. Furthermore, household contacts of MRSA index persons should be simultaneously cultured in case of MRSA eradication therapy but also in case of the 'wait-and-see' option.

MR-CoNS together with (methicillin-resistant) *S. aureus,* are both opportunistic pathogens which are colonizing the human skin and mucous membranes and are the most prevalent pathogens causing healthcare-associated infections [5, 37, 38]. It has been demonstrated by several studies that (methicillin-resistant) CoNS clones can spread within a hospital [39-45] and can even have geographic dissemination [46]. Despite the importance of MR-CoNS as a cause of healthcare-associated infections, limited data is available on the incidence and risk of acquisition and transmission of MR-CoNS skin carriage during hospital admission. With the prospective study described in **Chapter 4,** we aimed to gain insight in the incidence and possible risk factors for MR-CoNS skin carriage acquisition during hospital admission. Furthermore, we wanted to study the degree of MR-CoNS carriage and acquisition in single and four-beds hospital rooms. To do so, skin swabs were obtained to determine MR-CoNS skin carriage on and during hospital admission from a convenience sample of 168 patients admitted to a general ward (38% admitted in single rooms; 62% admitted in four-beds rooms). Our study demonstrated that 23% of the patients carried MR-CoNS on admission and 63% of the patients acquired MR-CoNS skin carriage during their hospital admission. Comparison of these result to other studies is difficult because of major differences in study design [47-52]. The risk for acquisition of microbiological pathogens is influenced by the colonization status of other patients also called ‘‘colonization pressure’’ [53-56]. Furthermore, cross-transmission of MR-CoNS through colonized healthcare workers or the environment has been demonstrated in the past [57, 58] even as the study described in **Chapter 2** demonstrated that MRSA is transmitted to household contacts. Independent risk factors for MR-CoNS skin carriage acquisition were use of antibiotics during admission and a longer hospital admission period. In the past, the use of antibiotics during hospital admission has been demonstrated to have a significant correlation with acquisition of nosocomial MRSA colonization [59]. In addition, Bonten et al [53] demonstrated that the acquisition of vancomycin-resistant enterococci in patients admitted to ICU was positively associated, among other risk factors, with the use of antibiotics during hospital admission. These effects are probably due to selection of resistant bacteria due to antibiotic pressure. Colonization with resistant bacteria may remain undetectable within a largely susceptible microflora until, because of the selective growth advantage provided by antibiotics, bacterial overgrowth of the resistant pathogen occurs. Furthermore, not only the risk of acquiring colonization, but also the risk of an CoNS infection is increased with a prolonged hospital stay [60] as colonization precedes infection. According to isolation guidelines, it has been advised to nurse patients in single hospital rooms to prevent direct or indirect transmission of pathogens [61]. No significant difference in MR-CoNS skin carriage acquisition was observed between patients admitted in single or four-beds hospital rooms. However, our study was not designed to properly test this hypothesis. It has been described that hospitalization in single person rooms is associated with a decreased admission period [62]. Therefore, as MR-CoNS acquisition is independently associated with

a prolonged hospital admission, single person hospital rooms may reduce the risk of MR-CoNS skin acquisition. Other studies have determined the colonization rate of pathogens between single and multi-patient hospital rooms which demonstrated a significant decrease of colonization when patients were admitted to private rooms [63, 64]. However, these studies used retrospectively collected data for the control groups. Finally our study demonstrated that patients admitted to a single hospital room had a significant longer admission period than patients admitted to a four-bed hospital room (7 vs 6 admission days), a finding that was associated with the presence of a malignancy.

Part II Methicillin-susceptible staphylococci in HIV infected persons

In the past, multiple studies showed that human immunodeficiency virus (HIV) infected patients are more frequently colonized with *S. aureus* and therefore have a concordant high risk of developing *S. aureus* infections [22, 26, 30, 38, 65-68]. Furthermore, bacterial pneumonias caused by *S. pneumonia* are a frequent cause of morbidity and mortality in HIV infected persons. The risk of invasive pneumococcal disease is 60 times higher in HIV positive individuals, than in HIV negative individuals [69]. Whether adult HIV infected patients have an increased colonization rate with *S. pneumoniae* compared to healthy adults, has been debated [70, 71].

In the past, it has been demonstrated that persistent *S. aureus* carriers are more prone on developing infections than non-or intermittent *S. aureus* nasal carriers [22]. An earlier validated culture rule was used to distinguish between persistent and non-or intermittent *S. aureus* nasal carriage [30]. In **Chapter 5** we described the results of the first ever case-control study in the HAART-era looking at persistent carriage and possible determinants of *S. aureus* and *S. pneumoniae* in HIV infected patients versus healthy controls.

The prevalence of persistent nasal *S. aureus* carriage (19% versus 18%) and nasopharyngeal *S. pneumoniae* carriage (2% versus 2%) did not differ between the HIV infected persons (n=248) and healthy controls (n=239). These results were in contrast with previous studies [22, 72-85]. As six of the 12 previous studies performed in the field of *S. aureus* carriage in HIV infected were performed in the pre-HAART era, direct comparison of these studies to our results is not of any relevance [72-74, 80, 83, 85]. Only three of the remaining studies performed in the HAART-era used a control group, although none of these control groups comprised of healthy individuals as in our study [78, 79, 84]. In the most recent uncontrolled study conducted in the Netherlands [77], the prevalence of persistent *S. aureus* carriage was nearly 30% , which is 10% higher than in our study. There seems no obvious explanation for this as study design and

study population of these two studies were similar, the only differences being the controlled design of the study described in **Chapter 5**.

Our study revealed that an increasing median CD4 cell count was significantly associated with persistent *S.aureus* carriage. We related this effect to the use of PJP (*Pneumocystis jirovicii* pneumonia) prophylaxes. When CD4 cell counts drop below 200 * 10^6 cells/L, PJP prophylaxis is prescribed. The most commonly used prophylaxis is trimethoprim-sulfamethoxazole, which is an antibiotic that is usually effective against *S. aureus* infections. Although this study did not demonstrate the effect, the beneficial effect of PJP prophylaxis on persistent *S. aureus* carriage has been observed in the past [77].

In **Chapter 5** we further described the low prevalence of *S. pneumoniae* carriage in both HIV infected patients (2%) and healthy controls (2%). A previous study on *S. pneumoniae* carriage demonstrated that over more than one-year use of the same HAART regimen significantly reduced the risk of *S. pneumoniae* carriage [81]. As the HIV infected patients in this study showed low rates of *S. pneumoniae* carriage, this is probably due to high percentages (90%, n=172) of successful HAART. Furthermore, we used non-selective blood agar plates instead of blood agar plates containing 2.5 µg/mL gentamicin which are suggested to selectively isolate *S. pneumoniae* [86, 87].

An explanation for not finding a difference in persistent *S. aureus* carriage and *S. pneumoniae* carriage between cases and controls may be that the current HIV cohort had a relatively high CD4 cell count and that 71% of the patients had an undetectable viral load. This improved immunological status of the HIV infected patients in our study may have resulted in the fact that prevalence of carriage of both bacteria in HIV infected patients is similar to that of a healthy population. No further determinants of carriage for either, and no evidence for bacterial interference between the two bacteria could be demonstrated in this case-controlled study.

In sub-Saharan Africa, where the prevalence of HIV is high, the burden of disease caused by staphylococci is significant, also among HIV uninfected individuals [88-90]. In **Chapter 6** we assessed the prevalence and determinants of *S. aureus* carriage, as well as the prevalence of antibiotic-resistant *S. aureus* strains in a rural Zambian outpatient population of adult HIV infected patients.

According to the culture rule mentioned above [30], 36% of the patients (n=129) were persistent *S. aureus* carriers. Compared to the persistent carriage rate as described in **Chapter 5,** this percentage is doubled. Several study population differences might explain this

difference in persistent *S. aureus* carriage. As previously described, persistent nasal carriage is negatively associated with the use of HAART [77]. In Zambia, significantly less patients received anti-retroviral therapy than the HIV infected patients analyzed in the Netherlands (61% vs 77% respectively). Furthermore, the median CD4 cell count was significantly lower in the Zambian patients than in the Dutch patients (249 * 10^6 cells/L vs 410 * 10^6 cells/L). Although an increasing median CD4 cell count in the Dutch HIV infected cohort was associated with persistent *S. aureus* carriage, we related this effect to the use of PJP prophylaxis by trimethoprim-sulfamethoxazole as this is an effective antibiotic in *S. aureus* infections. In the Zambian cohort, 88% of the HIV infected patients received PJP prophylaxis. Therefore, it could be assumed that less patients became persistent carriers. Unfortunately, 85% of the *S. aureus* strains from the Zambian patients were resistant to trimethoprim-sulfamethoxazole. In addition, the use of trimethoprim-sulfamethoxazole was significantly associated with a resistant *S. aureus* strain. Therefore, the beneficial effect of PJP prophylaxis on persistent *S. aureus* carriage was undone. This same effect has been confirmed in a study among HIV infected children on trimethoprim-sulfamethoxazole prophylaxis in South-Africa. This study showed that children on trimethoprim-sulfamethoxazole prophylaxis had significant higher rates of *S. aureus* carriage in their nasopharynx compared to HIV infected children not on prophylaxis [91].

The rural environment in which this study was performed may also have attributed to the high *S. aureus* colonization rate observed. Socio-economic factors such as housing, crowding, poor hygiene and access to healthcare are major determinants of *S. aureus* colonization [22]. As demonstrated in **Chapter 2** transmission of MRSA (and therefore also MSSA) to household contacts occured frequently and is related to an increased number of household contacts. As 65% of the Zambian patients lived together with four or more people in the same house, this increased the risk of *S. aureus* transmission to household contacts. The high percentage of MRSA positive strains (21%) might be explained by the transmission of MRSA into the community and it can cause a further spread and an ongoing problem for community-acquired MRSA.

The study in **Chapter 6** further described the extent of antibiotic resistance in *S. aureus* strains obtained from HIV infected patients in rural Zambia. Twenty-one percent of these strains showed to be methicillin-resistant and 88% of the isolated strains were resistant to three or more of the first line antibiotics prescribed. The high rates of antibiotic resistance of *S. aureus* in this rural Zambia HIV infected out-patient adult population confirmed data found in the few previous studies that were done in sub-Saharan Africa, although these two studies were performed in HIV infected children [92, 93].

General conclusions and future perspectives

Intrafamilial transmission of MRSA has raised important issues, such as whether household screening should be routinely performed and carriers to be detected. Failure to identify MRSA positive household contacts may cause MRSA recolonization of the index patient and contributes to the MRSA burden. Unknown recolonization of index persons can also reintroduce MRSA into the hospital. Furthermore, we demonstrated that when collecting three consecutive culture sets, 31% of the MRSA colonized individuals would be incorrectly considered as MRSA-free. Our study showed that it is essential to have five or more consecutive culture sets to reliable ascertain the MRSA status of an individual after MRSA eradication therapy. We suggest including this and the swabbing and simultaneously eradicating MRSA in household contacts, in prevention guidelines for MRSA as this may reduce further spread of MRSA strains into the community and in healthcare settings. Therefore, we recommend to change the guidelines from the Dutch national policy by the Working Party on Infection prevention (WIP) to obtain at least five MRSA follow-up culture sets instead of three. In addition, we suggest that in case of MRSA colonization at least in the throat no 'wait-and-see' option should not be used but MRSA eradication therapy should be offered, as MRSA carriage at least in the throat promotes MRSA transmission to household contacts. On the other hand when patients have a single site MRSA colonization or when wounds are present just before the start of MRSA eradication therapy, the 'wait-and-see' option can be offered. This is because single site MRSA carriers have a significantly better chance to spontaneously become MRSA-free and because the presence of wounds just before the start of MRSA eradication therapy is significantly associated with MRSA eradication therapy failure.

The incidence of MR-CoNS skin carriage increases rapidly during hospital admission and this increases the colonization pressure which may lead to an increased number of MR-CoNS healthcare-associated infections. Therefore, new prevention measures should be determined to reduce MR-CoNS skin carriage during hospital admission. Furthermore, knowledge of prevalence and degree of the resistance in the commensal flora on admission and during hospitalization may contribute to an optimal choice of empirical therapy in the event of a healthcare-associated infection. Finally, the need remains to study the potential barrier effect of single room nursing on the spread and acquisition of MR-CoNS and other hospital pathogens.

The increased immunological status of the HIV infected persons, due to the success of HAART, has induced that the carriage rates of *S. aureus* and *S. pneumoniae* is indifferent of that of non-HIV infected persons in the Netherlands. It seems essential to preserve this increased immuniological status as patients in countries where HAART has not been introduced for

a long period, still have an increased *S. aureus* carriage rate. Our data suggest that a high colonization rate with *S. aureus* and a widespread antimicrobial resistance exists in Zambia, therefore, further research is needed to provide national policy makers with information to update antibiotic treatment guidelines in view of resistance development.

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Chapter 8

Summery
Nederlandse samenvatting

Summary

A general introduction on methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci (MR-CoNS) and methicillin-susceptible *Staphylococcus aureus* (MSSA) carriage in HIV infected patients is described in **Chapter 1**.

In **Part I** we described two prospective observational studies concerning MRSA transmission to household contacts and the success of MRSA eradication therapy. Furthermore a prospective study on the acquisition of MR-CoNS skin carriage during hospital admission was described.

In **Chapter 2** we described the frequency and risk factors for MRSA transmission to household contacts of a MRSA positive index person. Our study demonstrated that MRSA transmission to household contacts occurred in nearly half of the index persons (47%) and two-third of the exposed household contacts became MRSA positive. Risk factors for MRSA transmission were present in both MRSA index persons and household contacts. MRSA carriage at least in the throat, the duration of MRSA exposure time at home, eczema and younger age were all significant risk factors associated with transmission to household contacts. Household contact related risk factors for MRSA acquisition were significantly associated with being a partner of the index person and having an increased number of household contacts.

In **Chapter 3** we showed that our eradication treatment protocol results in a high success rate (81% intention-to-treat analysis) of MRSA eradication. We demonstrated that when collecting only three consecutive culture sets as advised by the Dutch guidelines, 31% of the MRSA colonized person would be incorrectly considered as MRSA eradicated. We demonstrated that it is essential to have five or more consecutive culture sets to reliable assess the MRSA status of a person after MRSA eradication therapy. Furthermore, MRSA colonization at least in the throat and the presence of wounds before start of eradication therapy were associated with eradication treatment failure.

Chapter 4 described a prospective study in which we demonstrated that 23% of the patients carried MR-CoNS on their skin on admission and 63% of the patients acquired MR-CoNS skin carriage during their hospital admission. Independent risk factors for MR-CoNS skin carriage acquisition were use of antibiotics and with each admission day the risk of MR-CoNS skin carriage acquisition was 23%. With this study, although a plausible risk factor, we were unable to show a decrease in the number of patients acquiring MR-CoNS skin carriage during admission in single hospital rooms compared to patients admitted to four-bed hospital rooms.

In **Part II**, two studies in the field of persistent *S. aureus* carriage in HIV-infected persons were described.

The study described in **Chapter 5** is the first ever case-control study in the HAART-era looking at persistent carriage and possible determinants of *Staphylococcus aureus* and *Streptococcus pneumoniae* in HIV-infected patients versus healthy controls. The prevalence of persistent nasal *S. aureus* carriage (19% versus 18%) and nasopharyngeal *S. pneumoniae* carriage (2% versus 2%) did not differ between the HIV-infected persons and healthy controls. Furthermore, our study demonstrated that an increased median CD4 cell count increases the risk on persistent *S. aureus* carriage. This effect might be explained by the use of trimethoprim-sulfamethoxazole in case of CD4 cell counts under $200*10^6$cells/L. Trimethoprim-sulfamethoxazole is an antibiotic which is effective against *S. aureus.* No further determinants for *S. aureus* or *S. pneumoniae* carriage could be revealed, probably due to the increased immunological status of the HIV-infected patients studied.

The study described in **Chapter 6** showed a high rate of persistent carriage (32%) of *S. aureus* in an outpatient HIV positive adult cohort in rural Zambia. MRSA was detected in 21% of the strains isolated from *S. aureus* carriers and over 88% of the strains were resistant to 3 or more of the first line antibiotics prescribed. Therefore, treatment options as well as treatment success of *S. aureus* infections will be limited.

Chapter 7 provides a general discussion based on main findings and future perspectives are described.

Nederlandse Samenvatting

In **Hoofdstuk 1** wordt een algemene introductie gegeven over methicilline-resistente *Staphylococcus aureus* (MRSA), methicilline-resistente coagulase-negatieve staphylococcen (MR-CoNS) en methicilline-gevoelige *Staphylococcus aureus* (MSSA) dragerschap in HIV geïnfecteerde patiënten.

In **Deel I** beschrijven we twee prospectieve observationele onderzoeken welke gaan over transmissie van MRSA naar de huisgenoten van een MRSA index persoon en over het succes van MRSA eradicatie therapie. Verder wordt er in **Deel I** een prospectieve studie beschreven welke gaat over het verkrijgen van MR–CoNS dragerschap op de huid gedurende een ziekenhuis opname.

In **Hoofdstuk 2** beschrijven wij de frequentie van en risicofactoren voor MRSA transmissie van een MRSA index patiënt naar zijn of haar huisgenoten. Onze studie laat zien dat bijna de helft van de index personen (47%) MRSA verspreidt naar zijn of haar huisgenoten en dat tweederde (67%) van deze aan MRSA blootgestelde huisgenoten ook MRSA drager wordt. Risicofactoren voor MRSA transmissie zijn zowel aan de index persoon als mede aan de huisgenoten van de index persoon gerelateerd. MRSA dragerschap op zijn minst in de keel, de duur van blootstelling aan MRSA in de thuissituatie, eczeem en een jonge leeftijd van de MRSA index persoon zijn allen significant geassocieerd met het risico op MRSA transmissie naar huisgenoten. Partners van een index persoon en huishoudens met een groter aantal personen hebben een significant grotere kans om ook MRSA drager te worden.

In **Hoofdstuk 3** tonen wij aan dat het MRSA eradicatie protocol dat wij gebruiken zeer succesvol is (81% in de intention-to-treat analyse). Verder laat deze studie zien dat wanneer er drie opeenvolgende kweek sets worden afgenomen, zoals geadviseerd wordt in de Nederlandse richtlijnen, 31% van de personen die een MRSA eradicatie behandeling krijgt onterecht als MRSA negatief wordt beschouwd. Indien er vijf opeenvolgende kweek sets worden afgenomen kan met meer dan 90% zekerheid het succes van de MRSA eradicatie behandeling worden vastgesteld. MRSA dragerschap op zijn minst in de keel en de aanwezigheid van wonden voordat een MRSA eradicatie behandeling wordt gestart, zijn risicofactoren voor het falen van een potentiële MRSA eradicatie behandeling.

Hoofdstuk 4 beschrijft een prospectieve studie waarin wij hebben aangetoond dat 23% van de patiënten die worden opgenomen op een algemene afdeling in het ziekenhuis, MR-CoNS op de huid draagt. Gedurende hun opname heeft 63% van de MR-CoNS negative patiënten bij binnenkomst, één of meerdere MR-CoNS positieve huidkweken. Onafhankelijke risicofactoren

voor het verkrijgen van MR-CoNS huid dragerschap gedurende een ziekenhuisopname zijn het gebruik van antibiotica en de duur van ziekenhuis opname. Iedere opnamedag vergroot de kans op het krijgen van MR-CoNS huid dragerschap met 23%. Ondanks dat het een plausibele risicofactor zou kunnen zijn, hebben wij met deze studie niet kunnen aantonen dat patiënten die opgenomen liggen op een één-persoonskamer minder kans lopen op het krijgen van MR-CoNS dragerschap dan patiënten die op een vier-persoons kamer liggen.

In **Deel II** worden twee studies beschreven die persisterend *S. aureus* dragerschap bij HIV geïnfecteerde patiënten betreffen.

De studie die in **Hoofdstuk 5 w**ordt beschreven, is de eerste case-controle studie in het HAART tijdperk die kijkt naar (persisterend) dragerschap en risicofactoren voor dragerschap van *Staphylococcus aureus* en *Streptococcus pneumoniae* bij HIV positieve patiënten en gezonde vrijwilligers. De prevalentie van persisterend *S. aureus* neusdragerschap (19% versus 18%) en *S. pneumoniae* nasopharynx dragerschap (2% versus 2%), verschilde niet tussen HIV geïnfecteerde patiënten en gezonde vrijwilligers. Verder laat deze studie zien dat een hoger mediaan CD4 cel aantal, de kans op persisterend *S. aureus* dragerschap vergroot. Dit effect kan mogelijk verklaard worden door het gebruik van trimethoprim-sulfamethoxazole bij patiënten met een CD4 cel aantal kleiner dan $200*10^6$ cellen/L . Trimethoprim-sulfamethoxazole is een antibiotica welke vaak goed gevoelig is tegen *S. aureus*. Middels deze studie zijn geen andere risicofactoren voor *S. aureus e*n *S. pneumoniae* dragerschap aangetoond, mogelijk als gevolg van de sterk verbeterde immuunstatus van de HIV geïnfecteerde patiënten in deze studie populatie.

In **Hoofdstuk 6** tonen wij aan dat er een hoog percentage (32%) persisterend *S. aureus* dragerschap is in een poliklinische HIV positieve volwassen populatie in ruraal Zambia. Van de *S. aureus* kweken die in ruraal Zambia werden gevonden was 21% resistent voor methicilline en 88% van de kweken was resistent tegen 3 of meer van de eerstelijns antibiotica die wordt voorgeschreven in het geval van een *S. aureus* infectie. Als gevolg hiervan zullen minder behandel opties voor *S. aureus* infecties overblijven, evenals dat het succes van deze behandelingen zal verminderen.

Hoofdstuk 7 geeft een algemene discussie over primaire uitkomstmaten en geeft een aantal aanbevelingen voor de toekomst.

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Curriculum Vitae

Femke Petronella Neeltje Mollema werd geboren op 9 oktober 1981 te Terneuzen. Na het afronden van de HAVO in 1998 aan het Zeldenrust-Steelant College te Terneuzen, heeft zij in 2000 haar VWO-examen aan hetzelfde college behaald. In dat jaar begon zij met haar studie Geneeskunde aan de Erasmus Universiteit te Rotterdam. Tijdens haar studie Geneeskunde heeft zij één jaar Psychologie aan de Erasmus Universiteit Rotterdam gestudeerd en behaalde van deze studie haar propedeuse. In 2006 heeft zij een extra-curriculair onderzoek gedaan naar de prevalentie van invasieve pneumococcen pneumoniën bij HIV geïnfecteerde patiënten (afdeling Inwendige Geneeskunde – sectie Infectieziekten, Erasmus MC Rotterdam). Dit onderzoek was de voorbode van haar afstudeeronderzoek in 2007 naar *S. aureus* en *S. pneumoniae* dragerschap in HIV geïnfecteerde patiënten (afdelingen Inwendige Geneeskunde – sectie Infectieziekten en Medische Microbiologie en Infectieziekten, Erasmus MC Rotterdam). Na afronding van dit onderzoek heeft zij in het Macha Mission Hospital (MIAM, Zambia) enkele maanden onderzoek gedaan naar *S. aureus* dragerschap en antibiotica resistentie bij een rurale HIV positieve patiënten populatie. Na terugkomst is zij begonnen aan haar promotieonderzoek binnen de afdeling Medische Microbiologie en Infectieziekten van het Erasmus MC te Rotterdam onder begeleiding van Prof.dr. H.A. Verbrugh (promotor), dr. J.L. Nouwen en dr. M.C. Vos (copromotors).
In oktober 2008 is zij gestart met de klinische fase van haar studie, welke zij in augustus 2010 hoopt af te ronden.

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PhD Portfolio

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Mar 2008 : HIV symposium on HIV infections
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International scientific presentations

Oct 2008 : 48th Interscience Conference on Antimicrobial Agents and Chemotherapy/ Infectious Diseases Society of America. Washington DC, USA (oral presentations and poster presentations)
Sep 2008 : 13th International Symposium on Staphylococci and Staphylococcal Infections. Cairns, Australia (poster presentations)

National scientific presentations

May 2009 : Infection control practitioners meeting Rotterdam-Rijnmond (oral presentation)
Dec 2008 : Oral presentation for scientific research day Medical Microbiology and Infectious Diseases on research progress
Jan 2008 : Scientific research days Internal Medicine Erasmus University Medical Center. Antwerp, Belgium (poster presentation)