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MRSA colonization

MRSA colonization

Aspects on epidemiology and treatment

Anna-Karin Lindgren



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DOCTORAL DISSERTATION

By due permission of the Faculty of Medicine, Lund University, Sweden.
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Faculty opponent

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Abstract		
<p>Methicillin-resistant <i>Staphylococcus aureus</i>, MRSA, has become a common pathogen within healthcare facilities and the community and is a major challenge to the treatment of <i>S. aureus</i> infections. In Sweden the prevalence is still low with less than 1 % MRSA among <i>S. aureus</i> in blood cultures in 2014, but an increasing proportion of cases in the community is noted. In 2011 a novel <i>mecA</i> gene homologue, <i>mecC</i>, was reported.</p> <p>In this thesis different aspects of MRSA colonization is studied in order to create a greater understanding on how patients with MRSA should best be dealt with and treated in healthcare. The overall objective is preventing the spread of MRSA.</p> <p><i>The duration of MRSA colonization and factors influencing the duration of MRSA colonization.</i></p> <p>The median duration of MRSA colonization was 5.9 months. Having household contacts with MRSA, young age, <i>spa</i>-type t002 and colonization in 2 or more locations, was significantly associated with a longer duration of colonization. Having a clinical infection treated with antibiotics was significantly associated with a shorter time of carriage. Eradication treatment was associated with a shorter carriage time. These results may have implications for the management of patients with MRSA carriage. The study indicates that MRSA carriage can be defined as 'negative' in a follow-up program and thereby be freed from control measures in health care. The results also shows the importance of performing contact tracing among household members in MRSA follow-up programs.</p> <p><i>The epidemiology of different MRSA spa-types in Skåne County</i></p> <p>There was a change in the epidemiology of MRSA in Skåne County during the study period with a ten-fold increase from 2000 to 2010. There was a strong association between MRSA acquisition and either a non-Swedish origin or travelling and staying abroad. Different <i>spa</i> types were seen in patients who had travelled to or originated from different regions of the world. The PVL-positive strains with <i>spa</i>-types t008, t019 or t044 caused significantly more skin infections compared to the other <i>spa</i>-types. In our study, 35% of the screened household contacts were positive for MRSA with none of the <i>spa</i>-types being significantly more prone to spread.</p> <p>Our results supports screening for MRSA in patients treated in health-care settings abroad and taking cultures from patients with skin infections contracted outside Sweden. It also supports continued contact tracing of household contacts. Knowledge of the <i>spa</i>-type may give valuable clues in the process of contact tracing</p> <p><i>Eradication of MRSA colonization in the throat</i></p> <p>MRSA colonization in the throat is possible to eradicate. Our study shows that topical treatment is not sufficient to eradicate MRSA carriage in the throat and that addition of systemic treatment substantially increases the success rate.</p> <p><i>The epidemiology of mecC MRSA in Skåne County</i></p> <p><i>MecC</i> MRSA mainly affects older patients with underlying diseases or with an existing skin lesion. The <i>mecC</i> MRSA in our region appears to have a domestic origin. Data indicates that it could be a poor colonizer with few secondary cases and few people being colonized for a long time</p> <p>It is important and worthwhile with interventions to prevent the spread of MRSA. The interventions have to be continuously evaluated in relation to the prevalence of MRSA in the community. General measures with basic hygiene precautions and environmental cleaning, is and will be of even more importance in limiting spread of MRSA in health care.</p>		
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MRSA colonization

Aspects on epidemiology and treatment

Anna-Karin Lindgren



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List of publications

- I. Duration of methicillin-resistant *Staphylococcus aureus* colonization after diagnosis: a four year experience from southern Sweden.
Larsson AK, Gustafsson E, Nilsson AC, Odenholt I, Ringberg H, Melander E. Scand J Infect Dis. 2011 Jul;43(6-7):456-62. Epub 2011 Mar 2 2011
With permission of Tandfonline
- II. Epidemiology of MRSA in southern Sweden: strong relation to foreign country of origin, health care abroad and foreign travel
A.-K. Larsson, E. Gustafsson, H. Johansson, I. Odenholt ,A.-C. Petersson & E. Melander Eur J Clin Microbiol Infect Dis
2014 Jan;33(1):61-8
With permission of Springer
- III. Eradication of MRSA colonization in the throat-a randomized trial comparing topical treatment alone or in combination with two oral antibiotics
A.-K. Lindgren, Anna C. Nilsson, Per Åkesson, E. Gustafsson, E. Melander
Manuscript
- IV. Methicillin-resistant *Staphylococcus aureus* with *mecC*: a description of 45 human cases in southern Sweden.
Lindgren AK, Gustafsson E, Petersson AC, Melander E.
Eur J Clin Microbiol Infect Dis. 2016 Jun;35(6):971-5
With permission of Springer

The author's maiden name was Larsson

Abbreviations

BURP	Based Upon Repeat Pattern
CA-MRSA	Community Associated MRSA
CoNS	Coagulase Negative <i>Staphylococci</i>
HA-MRSA	Healthcare Associated MRSA
LA-MRSA	Livestock Associated MRSA
MLST	Multilocus Sequence Typing
MRSA	Methicillin-Resistant <i>S.aureus</i>
MSSA	Methicillin-Sensitive <i>S.aureus</i>
NGS	Next Generation Sequencing
PBP2a	Penicillin-Binding Protein 2a
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
PVL	Panton-Valentine Leucocidin
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SCC <i>mec</i>	Staphylococcal Cassette Chromosome <i>mec</i>
<i>Spa</i>	<i>S. aureus</i> Protein A
<i>Spa-CC</i>	<i>S. aureus</i> Protein A-Clonal Complex
VISA	Vancomycin-Intermediate <i>S. aureus</i>
VRSA	Vancomycin-Resistant <i>S. aureus</i>

Introduction

Staphylococcus aureus (*S. aureus*) is one of our most common and potent pathogens and it contributes significantly to morbidity and mortality in the society. It is a ubiquitous commensal gram-positive bacterium and is the major cause of skin and soft tissue infections. It can cause serious and sometimes life-threatening infections like deep abscesses, endocarditis, osteomyelitis and pneumonia and is one of the most prevalent pathogens in bloodstream infections. *S. aureus* is of major concern in the healthcare environment and is one of the main causes of nosocomial infections like surgical site infections, pneumonia and sepsis (1, 2). It is also one of the prominent pathogens in biofilm-related infections of indwelling medical devices (3). Effective treatment is a major clinical challenge.

S. aureus is a grampositive, facultative anaerobic coccus. The bacterium was first described in 1880 by a Scottish surgeon, Sir Alexander Ogston. When opening the abscess of one of his patients, and examining it under a microscope he detected bacteria growing in clusters. He named them staphylé after the greek word for a bunch of grapes (4). The name aureus refers to the fact that colonies formed on solid rich media have a golden color in opposition to the pale, translucent, white colonies formed by CoNS (Coagulase negative *Staphylococci*) (5).

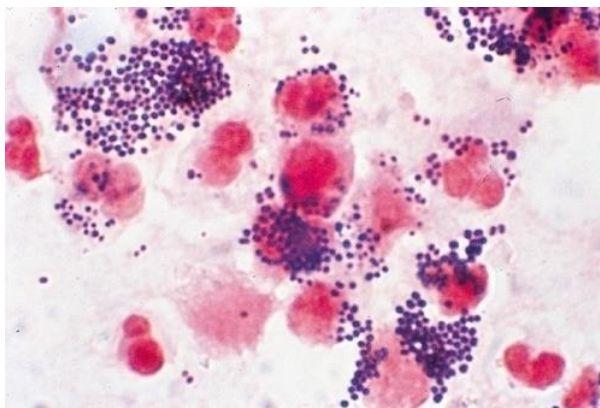


Figure 1. *Staphylococcus* in an abdominal infection. Picture from Ann-Cathrine Petersson

Virulence

Evidence for *S. aureus* virulence was shown in 1941 when the mortality rate associated with *S. aureus* bacteremia in the pre antibiotic era was 82% (6). The virulence of *S. aureus* is multifactorial and caused by the combined action of several virulence determinants. It produces a vast number of virulence factors allowing the bacterium to adhere to surfaces/tissues, to avoid or invade the immune system, and to cause harmful toxic effects to the host (7-9). The factors can be divided into cell-surface-associated (adherence) and secreted (exotoxins) factors. Examples of adhesive factors are *spa* (*S. aureus* protein A) that binds to immunoglobulins and coagulase that activates prothrombin. Examples of secreted factors are the enzyme catalase that inactivates free hydrogen peroxide and the toxin PVL (Panton-Valentine Leucocidin) that stimulates and lyses neutrophils and macrophages (10).

Staphylococcus aureus colonization

S. aureus is a pathogen that is responsible for a multitude of hospital and community-acquired infections. In contrast to its invasive infectious potential it also forms part of the human microbiome. About 20 % of the human population is persistent carriers of *S. aureus* in the nasal vestibule (11). The remainder of the population is intermittently colonized and most individuals will be exposed to the organism transiently throughout their lifetime (12). The infection rate is higher in carriers of *S. aureus* than in non-carriers and invasive disease is often caused by the strain carried by the patient (13), (14). The ability of *S. aureus* to be an efficient colonizer is the result of a complex interplay between host factors and bacterial factors (12),(15). Factors that are known to influence carriage of *S. aureus* are genetic factors, bacterial interference, the host immunity and environmental factors, fig. 2 (12). There is a specific relationship between *S. aureus* and the human host and persistent carriers that are artificially colonized with a mixed culture will specifically re-acquire their autologous strain (11). The frequency of carriage differs between geographic location, age, gender, ethnicity and body niche. Children have higher rates of persistent carriage than adults. (14). Horizontal transmission from the mother is probably the major source for *S. aureus* carriage in newborns (16). The nares, throat and perineum are the most prevalent sites for carriage in the adult population (14), (15). Other sites of the skin and the intestine are also frequently colonized (14), (17).

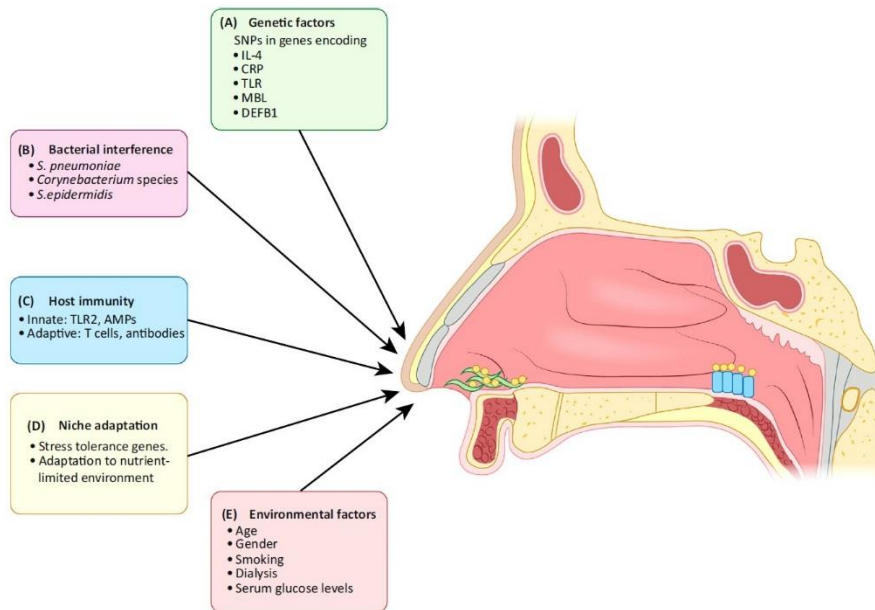


Figure 2. .Host and Bacterial Influences on Nasal carriage.

This figure depicts the nasal cavity and nasal epithelium. *Staphylococcus aureus* (yellow circle) adheres to the squamous epithelium of the anterior nares (green cells) as well as the cells of the inner nasal cavity (blue). The evolved relationship between *S.aureus* and the host has led to several specific and complex interactions that are known to influence nasal carriage. (A) Genetic factors. Single nucleotide polymorphisms (SNPs) in several genes that encode factors involved in local immunity correlate with persistent carrier status. (B) Bacterial interference. The presence of other bacterial species in the nasopharyngeal cavity can exert an antagonistic influence on *S. aureus* nasal carriage. (C) Host immunity. Impairment in TLR2 expression and the production of AMPs by keratinocytes can cause a reduction in colonization. T cell-mediated adaptive immunity facilitates clearance of *S.aureus* from the nose in murine models. Distinct patterns of humoral adaptive responses are observed between *S.aureus* nasal carriers and noncarriers. (D) Niche adaptation. *S. aureus* utilizes specific mechanism to adapt to the nasal niche. Genes involved in stress tolerance are important for colonization *in vivo*. (E) Environmental factors. Correlations between age, gender, smoking, serum glucose level, dialysis use and persistent carriage has been recorded. The figure is published in Mulcahy et al (12) and reprinted with the permission from the author and the journal.

Antibiotic treatment of *S. aureus* infections

S. aureus cause a wide range of infections and the choice of treatment should assess the location and the severity of the infection. After the introduction of penicillin in 1941 this was the drug of choice treating *S. aureus* infections (18). However, only four years after the introduction of penicillin, penicillin-resistant *S. aureus* were

identified (19). This resistance was shown to be mediated by the enzyme penicillinase which inactivates the penicillin molecule by hydrolyzing the β -lactam ring. In 1959 methicillin, a new kind of penicillin, was introduced. It belongs to the group isoxazolyl-penicillins that are stable to the penicillinase enzyme. Today there is a wide range of antibiotics available for the treatment of staphylococcal infections but the first choice is still the isoxazolyl-penicillins. Other antibiotics, such as vancomycin and clindamycin are predominantly used for treatment of resistant *S. aureus* or in case of allergy (20).

Methicillin-resistant *staphylococci* (MRSA)

In 1961, within two years after the introduction of methicillin resistance was observed (21). Since then the methicillin-resistant *S. aureus* (MRSA) has rapidly emerged and has become a major problem worldwide.

Mechanism of resistance for MRSA

Methicillin resistance in *S. aureus* is conferred by the acquisition of an SCC (staphylococcal cassette chromosome) *mec* element, which carries the *mecA* gene encoding a penicillin-binding protein homologue (PBP2a) with reduced affinity for Beta-lactam antibiotics. The PBP2a confers resistance to all clinically in use β -lactam antibiotics, the penicillins, cephalosporins, carbapenems and monobactams. The only exceptions are the cephalosporines like ceftobiprole and ceftaroline which have an ability to bind to PBP2a and hence bypass the resistance mechanism (22). In 2011 a novel *mecA* gene homologue, *mecC*, was reported in isolates from both humans and dairy cattle (23). Similarly to *mecA*, it is located within a SCC*mec* element.

Typing of MRSA

Understanding how MRSA spreads within health care institutions and communities is critical in developing containment strategies. Molecular typing has become a powerful tool in this regard, providing key insights regarding the evolution of *S. aureus* (24).

The most important techniques used to investigate the molecular epidemiology of *S. aureus* are pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *S. aureus* protein A (*spa*) typing and SCC*mec* typing (only for MRSA), (24), (25). Next generation sequencing (NGS) will probably be the method of choice in the near future.

PFGE

PFGE is one of the first used molecular methods for typing of MRSA. The advantage of PFGE is its high discriminatory power but there is no standardized interpretation making it difficult to use for national and international comparison.

MLST

MLST is based on the sequence analysis of fragments of seven *S. aureus* housekeeping genes. The DNA sequences are compared to those of previously identified alleles at each locus on the MLST online database (<http://www.mlst.net>). When five of the seven genes are identical the strains are clustered into a CC (Clonal Complex). A disadvantage of the method is that it is expensive and time consuming.

spa-typing

Typing of the *spa* locus is today the most used method in Sweden for local and national MRSA surveillance and detection of outbreaks. The method determines the sequence variation of the polymorphic region X of the *S. aureus* protein A (*spa*) and provides a discriminatory power between those of PFGE and MLST. The advantage is that it is less expensive and less time-consuming. A universal nomenclature and public access to the *spa* typing data are assured by the SeqNet.org initiative which curates the central *spa* server (<http://spaserver.ridom.de>) it can be used for national and international comparison. Cluster analysing into *spa*-clonal complexes (*spa*-CC) using repeat pattern algorithms (BURP) has good concordance with similar grouping schemes from MLST and PFGE, (25).

SCCmec

The cause of resistance to methicillin and all other β -lactam antibiotics is the *mecA* gene, which is situated on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*). Seven major variants of SCC*mec*, type I to VII, are distinguished. The structure of SCC*mec* can be determined with a number of PCR-based methods and may define the seven major SCC*mec* types and their subtypes. A universal nomenclature using MLST and SCC*mec* typing to define unique MRSA clones has been accepted (24).

Next generation sequencing (NGS)

NGS is probably the future method of choice for local, national and international typing as it has an ultimate discriminatory power and genome wide single nucleotide resolution. It is still expensive and requires heavy computer resources and well-trained bioinformaticians, but will most likely be the method of choice in the near future (26).

Classification of MRSA

Traditionally distinctions between HA-MRSA (hospital associated) and CA-MRSA (community associated) are made with classifying HA-MRSA as belonging to SCC*mec* I, II, III and IV and being resistant to many drugs in comparison with CA-MRSA being classified as belonging to SCC*mec* IV and being less resistant to other antibiotics but on the other hand having an increased virulence, possibly related to the PVL-toxin. The term also refers to the epidemiologic definition whether MRSA is acquired in the community or in healthcare. However, epidemiologic case classifications and strain characteristics has become less closely linked over time. For example, several reports have documented transmission in healthcare settings of MRSA strains initially described in community settings (24), (27). In Sweden the classification of hospital- or community acquired MRSA are defined only by epidemiological information.

Additive antibiotic resistance in MRSA

MRSA strains with concomitant resistance to many commonly used antibiotic groups like aminoglycosides, macrolides, clindamycin, fluoroquinolones, and tetracyclines have emerged (28). Multidrug-resistant strains of MRSA are rapidly evolving and resistance to all available antibiotics has been described (28), (29). In most cases glycopeptide antibiotics, usually vancomycin, are used as first-line antibiotics for treating serious MRSA infections. For long there were no reports of vancomycin resistant *S. aureus* strains, but in 1996 Japanese researchers identified strains with reduced susceptibility to vancomycin (VISA), and has since been found in hospitals elsewhere in Asia, as well as in the United Kingdom, France, and the U.S, (30). These bacterial strains present a thickening of the cell wall, which is believed to reduce the ability of vancomycin to diffuse into the division septum of the cell required for effective vancomycin treatment (31). In 2002 the first VRSA

was discovered in a patient in The United States (32). This resistance is caused by the resistance gene *van-A* which encodes an enzyme that produces an alternative peptidoglycan to which vancomycin will not bind. Fortunately VRSA strains are to date rarely found. Resistance to newer antimicrobial-agents such as linezolid and daptomycin have been reported (33), (28) but is still uncommon. High-level resistance to ceftaroline has already been described (34).

Epidemiology of MRSA

Since MRSA was first described in 1961 a number of clones of MRSA have spread widely throughout the world (35). Until the 1990s colonization or infection with MRSA used to be related to the elderly with healthcare contact and healthcare-associated risk factors (HA-MRSA) However, community-associated cases (CA-MRSA) have become more frequent in the 1990s and 2000s, and mostly children, young adults and previously healthy individuals without any apparent risk factors are affected (36). CA-MRSA clones have been spreading rapidly in the community and has also infiltrated healthcare in many regions worldwide. In 2003 livestock-associated MRSA (LA-MRSA) was detected for the first time and it is prevalent in certain high-risk groups of workers in direct contact with live animals (37).

The prevalence of MRSA show considerable geographical variation with low prevalences in the Netherlands and the Scandinavian countries and high prevalences in southern Europe (fig.3). In some countries, like the UK, great efforts to decrease infections due to MRSA has been successful with a decrease in bloodstream infections from 44% in 2003 to 11% in 2014 (38). Nonetheless in 2014 more than one third of the European countries had a prevalence above 20%. In Sweden the prevalence is still low with less than 1 % MRSA among *S. aureus* in blood cultures in 2014 (38). Most likely the differences in prevalence is due to differences in infection control procedures as well as differences in the use of antibiotics.

It is difficult to find comparable data from countries outside Europe but reports suggest that the prevalence of MRSA is high in most parts of the world. A review from 2011 (32) where the culture site is not stated describes a prevalence of hospital-acquired meticillin-resistant *Staphylococcus aureus* above 50 % in The United States and parts of South America and a prevalence above 25 % in Asia and Africa. Cumulative data from The United States 1998–2005 shows a percentage of 49 % MRSA among *S. aureus* in blood cultures in inpatients and 41% in outpatients (39).

Between 2005 and 2011 fewer invasive MRSA infections occurred in the United States (40).

Comparable data for the the prevalence of colonization with MRSA in the community and the incidence of MRSA infections in the community is hard to find. According to CDC 2% of the population in the United States carry MRSA (41). A cross-sectional study in nine European countries showed a low prevalence of colonization with MRSA in the nose (0 % in Sweden and 0.4 % in France and the UK) (42). In the United States rates of visits to hospital outpatient departments due to skin and soft tissue infections was tripled between 1993 and 2005 (27). This was probably due to the simultaneous rise of MRSA infections in the community. Moreover, there was a fourfold increase in hospitalizations due to skin and soft tissue infections caused by *S. aureus* 1999-2005. This suggests that community acquired MRSA has been added to the total volume of severe skin and soft tissue infections caused by MSSA.

In outpatients with *S. aureus* infections, MRSA accounted for 6% in the Ligurian region in Italy, 14% in Germany, 18% in France and 30% in Greece (43).

In Sweden an increased proportion of cases in the community is noted with an incidence of 20/100000 in 2011 rising to 39/100000 in 2015 (44). This includes clinical cases as well as cases found by screening and contact tracing.

The number of MRSA cases in Skåne County is increasing every year, (fig. 4) with an incidence of 9.1 MRSA-carriers per 100,000 inhabitants in 2003 rising to 56/100000 in 2015 (44). The percentage of MRSA of *S.aureus* in cultures from wounds was 1% in 2011 and increased to 2 % in 2015. The percentage of MRSA in blood cultures has for long been around 1 % and has not increased.

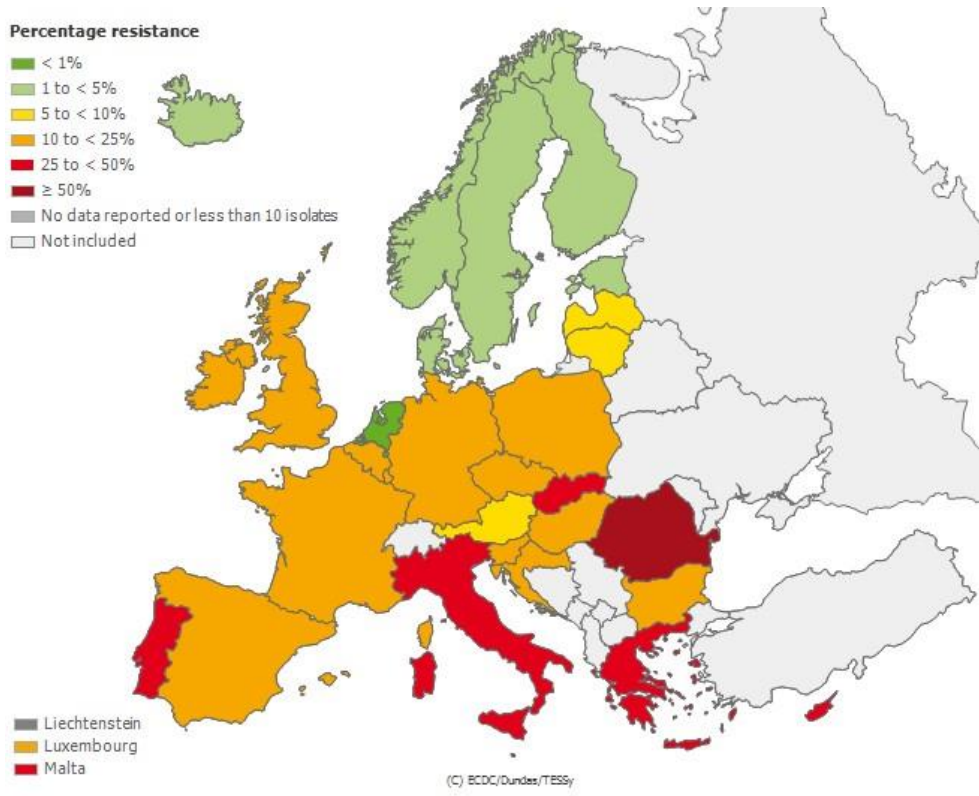


Figure 3. Proportion of Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolates in Participating Countries in 2014 .Printed with permission from European Centre for Disease Control (ECDC)

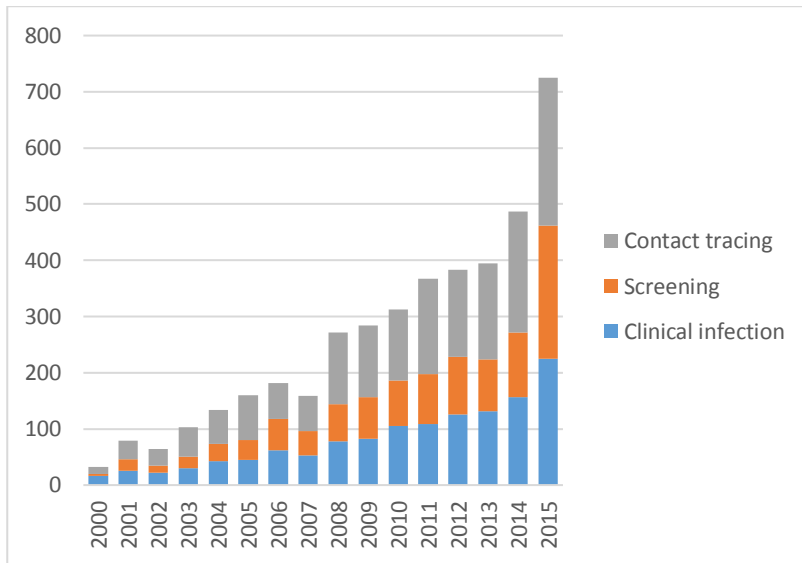


Figure 4. The number of MRSA cases and the reason for culturing in Skåne County 2000-2015

MRSA colonization

Most of the patients with infections due to MRSA are colonized prior to infection (14), and carriage of MRSA is associated with a higher risk of infection than carriage of MSSA (14). Patients with eczema, wounds or other skin injuries are more susceptible for being infected with *S. aureus*. Riskfactors for colonization with MRSA in the healthcare setting are co-morbidities, longer hospitalization, previous surgery and greater exposure to antibiotics (especially quinolones) compared to MSSA-colonized patients (45). Other risk factors are high age, urinary catheter and intrahospital transfer (46). Risk factors for the development of CA-MRSA infection include close contact with other people with CA-MRSA, e.g. having a family member from a country with a high prevalence of CA-MRSA, living in crowded facilities, poor hygiene, sharing of personal items and performing contact sports. So far, the most important risk factor for CA-MRSA infections in many European countries is travel to countries with a higher prevalence of CA-MRSA (43). In a Danish study the patients with CA-MRSA shared the same risk factors as the patients with CA-MSSA apart from a non-Danish origin in the MRSA patients (47). It has been described that the risk for CA-MRSA increases with the number of antimicrobial prescriptions and that quinolones poses the highest risk (48).

Few available studies reports the natural history of MRSA colonization and the studies are heterogeneous (49). The duration of colonization with MRSA varies widely among studies (50-56). Most studies deal with healthcare-associated MRSA and the duration of carriage after hospital stay. The impact of household contacts carrying MRSA has not been studied in these reports. Some studies have shown that skin lesions are associated with a prolonged carriage. A recent American study, showed a duration of colonization of only 21 median days after community-onset MRSA skin and soft tissue infection (57). However, samples were not taken from colonization locations at inclusion and follow-up samples did not include the throat. Treatment with clindamycin was associated with shorter duration of colonization, which was not the case with treatment with other antibiotics.

MRSA infections and treatment

MRSA cause the same type of infections as MSSA. The most common manifestations of MRSA infections are skin and soft-tissue infections. Some times it causes serious and potential life-threatening infections like septicemia, deep abscesses, endocarditis, osteomyelitis, and pneumonia. In the healthcare environment it causes nosocomial infections like surgical site infections, pneumonia and sepsis. It is also a pathogen in biofilm-related infections. Colonization with MRSA is associated with an increased risk of infection compared to colonization by MSSA (58-62). The reasons for this is unclear. Several explanations have been proposed, such as differences in virulence and that severely ill patients are more prone to acquire MRSA than MSSA because of prolonged hospital stay, greater antimicrobial use and more invasive procedures (62). Infections caused by MRSA are associated with a higher mortality and morbidity compared to corresponding infections caused by methicillin-susceptible *S. aureus* (MSSA) (63). This may be explained by delay in correct antibiotic treatment and the necessity to use less effective antibiotics. MRSA is as well associated with higher healthcare costs (64, 65). For the treatment of outpatients with less severe infections, primarily skin and soft tissue infections, oral agents such as clindamycin, trimethoprim sulfamethoxazole and fusidic acid can be used, but increasing levels of resistance to these antibiotics among MRSA is of concern. For the treatment of serious MRSA infections vancomycin has been the "gold standard". However the emergence of strains with reduced susceptibility to vancomycin, poor clinical outcomes and increased nephrotoxicity with high-dose vancomycin therapy is a concern (22). For pneumonia caused by MRSA linezolid is recommended. Daptomycin may be used in septicemia and endocarditis. MRSA remains to be one of the difficult-to-treat pathogens despite the introduction of several new anti-infective agents, as many of

the newer agents are associated with dose-limiting adverse events, emerging resistance and high drug costs (22).

Eradication of MRSA

Eradication of MRSA carriage may serve two purposes, prevention of infection in individual carriers and prevention of transmission to other individuals.

Preventing transmission of MRSA is important since MRSA infections are associated with considerable mortality and excess hospital costs (32), (63). Several eradication strategies have been proposed but the optimal treatment schedule has not yet been defined (66). Topical, intranasal, treatment, with mupirocin is the most studied and is shown effective for nasal colonization (66). In an observational study from the Netherlands 60 % of MRSA carriers were successfully decolonized after the first eradication attempt (67). Carriage of MRSA in the throat was associated with failure (68).

In Sweden, eradication treatment is not given routinely, only on an individual basis. Reasons for treatment are for example having regular healthcare contacts, having recurrent MRSA infections or working in healthcare. Since 2003, a non-evidence based treatment schedule with nasal mupirocin application for 1 week together with chlorhexidine washes and a hygiene protocol is used in Sweden. For extra nasal carriage (the throat and the perineum) 2 systemic antibiotics has been prescribed for 2 weeks. In a retrospective review of all cases treated between 2003-2006 it was found that 68 % of the patients with nasal carriage and 69 % of the patients with extra nasal carriage cleared their MRSA (non-published local data). The follow-up was 1 year.

MRSA with *MecC*

A novel *mecA* gene homologue, *mecC*, was reported 2011 in isolates from both humans and dairy cattle (23). It is located within a novel SCC*mec* element and given the designation type XI SCC*mec* (69). *MecC* MRSA is reported from 13 European countries and is isolated from 14 different host species (69). In humans, *mecC* MRSA have been isolated in a range of infections, predominantly skin and soft tissue infections, but also in severe infections, such as sepsis (69).

In Denmark, the prevalence of *mecC* MRSA among all MRSA in humans was 1.9 % in 2010, and increased to 2.8% in 2011 (70). In the UK and Germany, only a few isolates have been reported (71, 72). The detected isolates of *MecC* MRSA have

belonged to two genetic lineages (MLST clonal complexes) only, CC130 and CC2361. The most commonly described *spa* type is t843.

In Sweden, the first *mecC* MRSA was isolated in 2003 from a hedgehog but was not described as *mecC* until 2012 (73). Since then, it has been isolated from dairy cattle, cats, and humans (76 reported cases during the period 2011–2015), (74, 75).

So far, little is known about the epidemiology of *mecC* MRSA in humans.

In 2012, *mecC* MRSA was included as a notifiable disease in Sweden and has since then, been handled in the same way as *mecA* MRSA regarding follow-up and contact tracing among household and healthcare contacts. Active screening for *mecC* MRSA is not yet included in the Skåne county screening program after healthcare abroad.



Figure 5. A Swedish hedgehog

Measures to prevent spread of MRSA

In Sweden, MRSA is seen as a threat to public health and is therefore regulated by the Swedish Communicable Diseases Act. Since year 2000, MRSA has been a mandatory notifiable disease. All detected cases have to be reported and registered, and contact tracing among healthcare- and household contacts has to be performed for each new case.

General measures with basic hygiene precautions and environmental cleansing, is important in preventing spread of MRSA. Additionally, it is recommended that patients and medical staff who have recently been hospitalized or employed at a hospital or a nursing home outside the Nordic countries or in a hospital or nursing home in Sweden known to have had an outbreak of MRSA are screened for MRSA. Preemptive isolation is performed in hospitalized patients until the culture result is ready.

Aims of the thesis

General aim

To study different aspects of carriage of MRSA in order to create greater understanding of how patients with MRSA should best be dealt with and treated in healthcare with the overall objective of limiting the spread of MRSA.

Specific aim

- To study the duration of MRSA colonization and to identify factors influencing the duration of MRSA colonization.
- To study the epidemiology of different MRSA *spa*-types in Skåne County, and to investigate if some *spa*-types are more prone to spread and cause infections.
- To investigate if topical treatment with mupirocin is sufficient to eradicate carriage of MRSA in the throat or if an addition of systemic antibiotics is needed.
- To study the epidemiology of *mecC* MRSA in Skåne County

Patients and Methods

Background

In year 2001 Skåne County in southern Sweden had a population of 1.1 million inhabitants with 13 % born outside Sweden. In 2015 the population had increased to 1.3 million inhabitants with 20% of born abroad. Skåne County has both rural and urban areas. The Regional Centre for Communicable Disease Control in Skåne has registered all known cases of MRSA since 1999. Guidelines for the management of carriers were set up in 2001, and from 2003 a long-term follow-up started. All carriers were assigned a medical doctor at the departments of infectious diseases. An assigned nurse followed the patients with repeated cultures from the nares, throat, perineum and possible skin lesions with good compliance. Examination of risk factors and contact tracing among household contacts and possible healthcare contacts was performed. MRSA carriers were followed as long as cultures were positive for MRSA and further until 1 year with consecutive negative cultures with 3 to 4 cultures during the first 2 months and a final culture 1 year after the first negative culture. Deregistration was decided on when an examination showed no sign of skin defect and when all household contacts were negative for MRSA. All isolates identified since 2000 have been *spa* typed.

Eradication treatment has not routinely been given to MRSA carriers in our county. It is prescribed to healthcare workers with MRSA colonization and on an individual basis to patients with recurrent MRSA infections, sometimes if the patients have a lot of healthcare contacts and sometimes if the carriage is prolonged. The treatments used are topical intranasal mupirocin and chlorhexidine washings for nasal carriers. For carriage in other locations topical treatment is combined with systemic treatment with two antibiotics (mainly rifampicin in combination with clindamycin or fusidic acid) for 2 weeks. All carriers in the household are treated at the same time.

From 2012 and onwards, all clinical cefoxitin resistant isolates negative for the *mecA* gene have been routinely tested for the presence of the *mecC* gene. The patients with *mecC* MRSA have been notified, registered and followed up in the same way as patients with *mecA* MRSA and contact tracing has been performed.

Patients

Paper I

All cases with an MRSA-positive culture in Skåne County during the period 2003–2006 (n= 578) were eligible for the study. Data were collected from the database at the Regional Centre for Communicable Disease Control and the results of cultures were collected from the 3 clinical microbiology laboratories in the county. Clinical data were retrieved from the medical records of the patients at the infectious diseases departments. Age, gender and *spa*-type were registered for each patient. Culture results, location/s for MRSA carriage, possible household or healthcare contacts and whether it was a clinical infection or an asymptomatic carriage were recorded. In the case of a clinical infection, antibiotic treatment was recorded. Some of the patients had received treatment in an attempt to eradicate MRSA, either with topical intranasal mupirocin and chlorhexidine washings or combined with systemic treatment with 2 antibiotics (mainly rifampicin in combination with clindamycin or fusidic acid) for 2 weeks. It was also noted whether the patients had any risk factors in terms of a chronic skin lesion or any kind of a skin disease. One person at the Regional Centre for Communicable Disease Control evaluated if the cases were community- or healthcare-acquired. It was recorded whether the MRSA was found by contact tracing (household or healthcare contacts), screening (due to healthcare contacts abroad or in a Swedish institution with known spread of MRSA) or clinical investigation. Transient carriers (defined as having only 1 positive culture from the nose and/or the throat and a following negative culture within 1 week) and patients who had more than 1 year between the first positive culture and the first negative culture and who had had no cultures performed between these time points, were excluded.

Paper II

All individuals with an MRSA-positive culture in Skåne County during the period 2000–2010 (n=1,807) were eligible for the study. Of these, 1,020 were index cases and the remaining 787 cases were found by contact tracing. Epidemiological data were collected from the database at the Regional Centre for Communicable Disease Control in Skåne and the culture results were collected from the three clinical microbiology laboratories in the county. The Swedish Population Registration provided information about household contacts and the country of origin of family and family members. Age, *spa*-type and the presence or absence of the PVL encoding genes were registered for each MRSA case together with culture results. For the index cases, the number of household contacts and whether or not they were positive for MRSA was registered together with the country of origin of the family. It was noted if the case was infected or colonized at the time of detection and whether the cases were community or healthcare-acquired. The latter assessment

was based on reported epidemiological data. Individuals who were hospitalized or who had stayed in a nursing home within the previous 6 months were defined as having “healthcare-acquired” MRSA. The same applied to medical staff working in hospitals or nursing homes. Cases with no such contacts were defined as having a “community-acquired” MRSA. Cases with recent immigration, recent travel to or recent health care in a foreign country (≤ 6 months), were defined having an MRSA “acquired abroad”.

Paper III

This study was conducted as an open randomized multi center study in six centers in Sweden (Malmö, Lund, Helsingborg and Kristianstad in Skåne County together with Örebro and Stockholm) between 2011 and 2015.

All cases with MRSA were reported to the Regional Centre for Communicable Disease Control and their MRSA colonization was followed up at the infectious disease departments. Thus all patients who meet the inclusion criteria were offered to participate in the study. To be included the patients had to be colonized with MRSA in the throat, with or without colonization in other locations. The patients should have had at least 2 positive cultures and having been positive for at least 3 months. The MRSA isolate should be sensitive to rifampicin and clindamycin or trimethoprim-sulfamethoxazole. The patients should be 5 years or older. The criteria for exclusion were having an allergy to any of the study drugs, being treated with immunosuppressive drugs or pregnancy. Patients having an ongoing infection with MRSA or having skin lesions or eczema needing treatment were also excluded. A written informed consent was given before inclusion.

All MRSA positive household contacts were treated at the same time, either within the study, or if not meeting the inclusion criteria according to local guidelines

Randomization and treatments

The patients were randomized into two different groups using the program SAS proc plan (www.sas.com). If there were 2 or more patients in the same household they were randomized into the same group. Group I received nasal application with mupirocin for 5 days together with oral rifampicin and clindamycin. In case of resistance or contraindication to clindamycin, they received trimethoprim-sulfamethoxazol for 7 days. Group II only received nasal mupirocin. Both groups received chlorhexidine washings and followed a protocol regarding personal hygiene with instructions on such as changing and washing linens and towels.

Cultures, follow up

Cultures were taken from the nares, throat, perineum and from any existing skin lesions before treatment, 2 weeks, 2 months and 6 months after completed treatment. All household members were cultured before treatment and after 6 months.

Paper IV

A total of 45 patients with an isolate positive for *mecC* MRSA detected at the Clinical Microbiology Laboratory in Skåne between 2005 and 2014 were included in the study. Twenty six isolates had been saved because of being cefoxitin-resistant but *mecA* negative. These isolates were retrospectively tested positive for *mecC*. During 2012 to 2014 19 *mecC* MRSA isolates were identified through routine testing of clinical *S. aureus* isolates.

The patients detected between 2012 and 2014 were included in the follow-up programme with repeated cultures from the nares, throat, perineum and possible skin lesions for as long as they were colonized and 6 months thereafter. Contact tracing of household members and healthcare contacts of these patients was performed.

Culture results, resistance patterns, the PVL-genes and *spa*-types were collected from the Clinical Microbiology Laboratory. In all patients epidemiological data were received from the patient's medical files. For patients detected between 2012 and 2014 additional data was collected from the database at the Regional Centre for Communicable Disease Control.

Microbiological methods

Detection of MRSA in clinical samples

Colonies were presumptively identified as *S. aureus* by colony morphology on blood agar and/or by giving a coloured reaction on *S. aureus* selective plates (76). Coagulase-positive colonies were tested for isoxacillin/cefoxitin susceptibility by the disk diffusion method according to the instructions of the Nordic Committee on Antimicrobial Susceptibility Testing (www.nordiccast.org).

Detection of MRSA in contact-tracing and screening samples

Enrichment broths to detect staphylococci were used on all samples from patients in whom MRSA was actively searched for, i.e. patients designated 'screening' or 'contact tracing' but not on samples from patients where MRSA was not initially suspected, i.e. patients designated 'clinical infection' (76). The samples were cultured in the broth overnight, followed by real-time PCR detecting *S. aureus* (*nuc* and *gltB*) and *mecA* gene detection by real-time PCR. Broths containing *S. aureus* and *mecA* were cultured onto plates and processed further. Oxacillin/cefoxitin-resistant isolates of *S. aureus* strains were verified as meticillin-resistant by a triplex real-time PCR targeting *nuc*, *gltB* and *mecA* PCR.

Detection of mecC MRSA

Prior to 2012, *mecA*-negative strains were reported as non-MRSA with reduced susceptibility to isoxazolyl penicillins of unknown course. The strains were frozen at -80°C for future analysis. In late 2011, the *mecA*-negative isolates were tested for *mecC* using an in house real-time PCR with melting point analysis using forward primer (5'- CAT CAC CAG GTT CAA CCC A -3') according to García-Álvarez et al. (23) and a new reverse primer (5'- CGC CTT GGC CAT ATC CTG -3'). A *mecC* positive isolate obtained from Dr. Anders Rhod Larsen, SSI Copenhagen, Denmark, was used as the positive control. Since 2012, all isolates with reduced susceptibility to cefoxitin that lacked *mecA* have been tested for *mecC*.

MIC

Minimum inhibitory concentration (MIC) determination for oxacillin was performed using the Etest® (bioMérieux, France), according to the manufacturer's instructions.

spa-typing

Molecular characterization was performed on one of the initial MRSA isolates collected from each specific patient. For this, *spa*-typing (sequencing of the polymorphic X-region of the *S. aureus* protein A gene) was used as described elsewhere (77). The ridom staphtype® software (Ridom GmbH, Würzburg, Germany) was used for sequence analysis and assignment of *spa* types (79).

PVL genes

The PVL genes lukS-PV and lukF-PV were detected by PCR as described elsewhere (80)

Statistical analysis

Paper I

The data were analyzed with SPSS software (version 15; IBM SPSS, Chicago, IL, USA). The duration of MRSA colonization was analyzed by Kaplan–Meier estimates, and since data were not normally distributed the median time was used. Determinants for the duration were analyzed by univariate and multivariate Cox regression analysis. It was modelled with the chance of becoming negative for MRSA as the event, thus hazard ratios (HR) of > 1 indicate a shorter carriage time. A p-value of < 0.05 was considered significant. Since the patients started topical or systemic eradication treatment at different times after the detection of MRSA, these were included as time-dependent variables. Proportional hazard assumption was

checked by investigating the Kaplan–Meier curves and checking for intersection for the significant variables.

Paper II

The data were analysed using SPSS software (version 18; IBM SPSS, Chicago, IL, USA). In the analysis of differences between *spa* types regarding the ability to cause infection and spread to household contacts statistical calculations were performed with logistic regression using the program R (version 2.14.1, R Development Core Team) (<https://www.r-project.org/>). In post-hoc calculations the multcomp library, version 1.2-10, by Hothorn et al.(81) was used. In the analysis of the ability to cause infections *spa*-types with less than 10 cases and the heterogeneous group “other” were excluded.

Paper III

The power calculation estimated a treatment effect of 69 % for systemic treatment and 49% for the topical treatment. To reach a power of 0.83 with a significance of 0.05, 115 patients in each arm should be included. The data was analyzed with SPSS software (version 22; IBM SPSS, Chicago, IL, USA). As household members were randomized together the comparison of the treatment results between the two groups was done on household level. For comparison between the two groups Fisher exact test was used. A p-value of <0.05 was considered significant.

Paper IV

Paper 4 is a descriptive study and no statistic calculations was performed.

Results and discussion

The duration of MRSA colonization (paper I)

Between January 2003 and December 2006, 578 MRSA cases were notified in Skåne County. Of these 43 cases were excluded. Twenty three were considered to be transient carriers and 20 cases had had more than 1 year between the first positive culture and the first negative culture and no cultures performed in the lag time. The remaining 535 cases were included in the study. Of these, 150 cases were included as censored cases due to fact that they had not completed the follow-up schedule (29 died, 45 moved before follow-up, 30 had not yet completed the follow-up schedule and 46 patients were still carriers of MRSA).

Duration of colonization

The median duration of MRSA colonization was 5.9 months. There was a great variation, and 43 % cleared the MRSA colonization in less than 2 months (fig. 6).

The duration of MRSA carriage varies between different studies (50-57). Most studies have shown longer durations of carriage compared to our study, and the existence of household contacts or *spa*-type has not been taken in account. In a study by Marshall and Muhlemann (52) comprising 116 patients followed intermittently after hospital discharge, the median duration for carriage was 7.4 months. Scanvic et al. (55) performed a prospective study of 78 patients who were readmitted to hospital and showed a median duration of MRSA carriage of 8.5 months. In a cohort of 135 patients who had been hospitalized, Vriens et al. (56) found a median carriage time of 14 months. Sanford et al. (54) found a half-life of MRSA carriage of more than 40 months in a cohort of 102 hospitalized carriers. In a French study by Lucet et al. (50) an estimated time to clearance of MRSA of 9.4 months was found in a group that was screened for MRSA before being discharged from hospital to home healthcare. In a study by Robicsek et al. (53) including 1564 patients readmitted to hospital, 48.8% were still colonized after 1 year. However, in the study of Robicsek a reduction to a 50% rate of colonization in less than a month was noted. In accordance with the latter results, we noted that 43% of our patients became MRSA-negative in less than 2 months. In a recent American study, the duration of colonization was only 21 median days after community-onset MRSA skin and soft tissue infection (57). However, samples were not taken from colonization locations

at inclusion and follow-up samples did not include the throat. For participants which confirmed MRSA colonization at the time of their first study visit (2 weeks after enrollment) the median time to MRSA clearance was 140 days.

The difference between the results in our study and those of other studies may be due to that our study cohort included all known MRSA cases in the county of which a third of the cases consisted of clinical infections with MRSA. The remaining two thirds were asymptomatic carriers detected by screening or contact tracing. The study population was a mix of ages with a median age of 28 years and consisted mostly of previous healthy individuals. 64% of the cases were community-associated. Most other studies have only included patients with healthcare associated MRSA, where the patients were older, had more underlying conditions and had been hospitalized. Another difference is that our patients were followed at regular intervals with repeated cultures from at least 3 body sites and the presence of household contacts was evaluated. In other studies the patients were screened only at readmission to the hospital and a negative screening results might have been detected more rapidly in these patients if they had been cultured at regularly intervals. Also the prevalence of MRSA in our country is low and thus the risk of becoming recolonized is small. Most of the other studies have been performed in high prevalence countries.

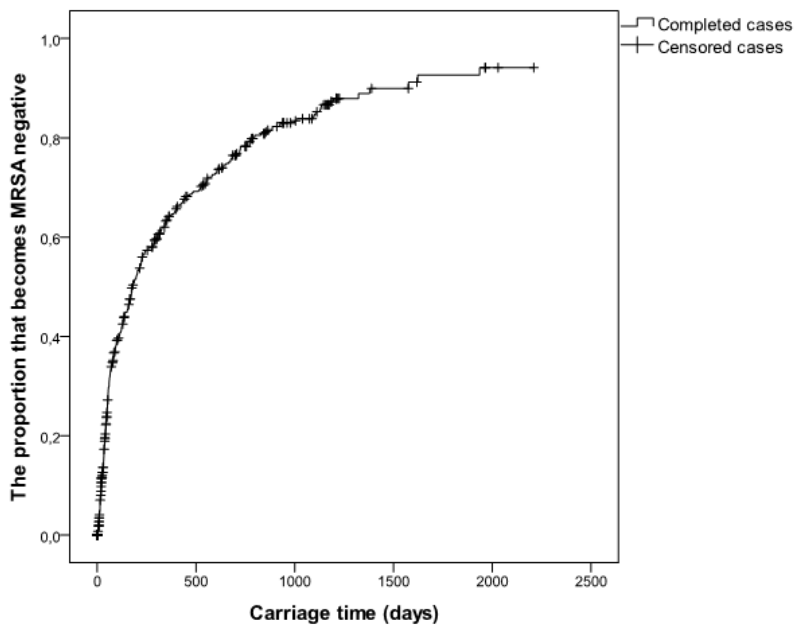


Figure 6. The duration of colonization with MRSA

Factors influencing the duration of MRSA colonization. (Table 1)

In accordance with other studies, no difference was noted in the duration of carriage between men and women in our study cohort (52,53,55).

The young MRSA carriers (0-17 years) were colonized for a significantly longer time than the older carriers with a median time of carriage of almost one year. This is in accordance with studies on colonization with MSSA, where a higher persistent carriage is seen in children compared to adults (82). The pattern of carriage changes between the ages of 10 and 20 years in a majority of cases (14, 83). In a study by Datta et al (84) a high carriage rate of *S. aureus* in infants (57%) and children aged 8-13 y (45.1-65.5%) was noted. In contrary to our study Cluzet et al (57) found that older age was associated with later clearance of MRSA colonization in patients with community-onset MRSA skin and soft tissue infection.

Johansson et al (85) has shown the importance of culturing household contacts for MRSA, and Bogaert et al. (86) found large households to be positively associated with an increase of *S. aureus* nasal carriage. In the study of Lucet et al.(50), transmission to 20% of the household contacts was seen. This is in agreement with our results where we found a strong correlation between long duration of colonization and other persons in the household carrying MRSA. The carriers having household contacts positive for MRSA were colonized for a median time of 296 days in contrary to those without household contacts who were carriers for only 94 days. This is probably due to recolonization from the household members.

Patients with a clinical infection treated with antibiotics carried MRSA for 64 median days which was shorter compared to those with a clinical infection without treatment or asymptomatic carriers found by screening or tracing. This is in agreement with the study of Cluzet et al in which treatment with clindamycin was associated with shorter duration of colonization (57). An explanation may be that many of these patients had a primary skin infection and received treatment before becoming colonized in the other locations. Forty-five per cent of the patients that received antibiotics for a skin infection were only positive for MRSA in the culture from the skin lesion. These successful results indicates that antibiotic treatment could be considered for a clinical infection with MRSA not only to treat the infection but to prevent colonization.

Patients who received topical or topical plus systemic eradication treatment were colonized for a shorter time, indicating that it is possible to achieve decolonization of MRSA carriers. Eradication of MRSA colonization is further studied and discussed in paper III.

In the univariate analysis patients with chronic skin lesions and skin disease had a significantly longer duration of colonization compared to patients without skin lesions and skin disease. It did not remain significant in the multivariate analysis but

as other studies have shown it to be associated with longer duration (52, 55) it should be interpreted with caution.

A previous study by Harbarth et al. (87) showed that carriage in ≥ 2 locations was associated with persistent MRSA carriage. This is in accordance with our results, which showed that if MRSA was found in ≥ 2 locations the patients carried MRSA for a longer time. Carriage of MRSA ≥ 2 locations probably indicates a higher MRSA load and hence it is more difficult to clear the colonization.

To our knowledge carriage time for MRSA has not previously been studied in relation to *spa*-type. The MRSA isolates in our study belonged to 83 different *spa* -types. The 5 most common types represented 47 % of all *spa*-types (t044, t002, t008, t131 and t355). We found that t002 was associated with a longer duration of colonization with a median time of colonization of 554 days.

These results may have implications for the management of patients with MRSA carriage. The study indicates that MRSA carriers can be defined as 'negative' in a follow-up program and thereby be freed from follow-up and control measures in health care. The results also shows the importance of performing contact tracing among household members in MRSA follow-up programs.

Table 1.

Duration and factors influencing the time of colonization with MRSA. Note The Cox regression analysis is modelled with the chance of becoming negative for MRSA as event, thus, hazard ratios (HR) > 1 indicates shorter carriage time. a time dependant variable

Variable	n (censored)	Time (mediandays) Kaplan Meier (95% CI)	Univariate Cox regression HR(95% CI)	P	Multivariate Cox regression HR(95% CI)	P
Overall	535 (150)	179 (143-215)		0.23		
Female	280 (80)	210 (154-266)	0.88 (0.72-1.08)			
Men (reference)	255 (70)	161 (108-214)	1.00			
Age (years)						
0-17	190 (56)	344 (250-438)	0.65 (0.49-0.87)	<0.001	0.66 (0.49-0.90)	0.023
18-50	227 (53)	124 (81-167)	1.14 (0.87-1.49)		0.85 (0.64-1.13)	
>50 (reference)	118 (41)	168 (74-262)	1.00		1.00	
Household contacts with MRSA						
Yes	292 (76)	258 (176-340)	0.61 (0.50-0.72)	<0.001	0.75 (0.60-0.94)	0.011
No (reference)	243 (74)	94 (55-133)	1.00		1.00	
Clinical infection						
Yes: antibiotic treatment	125 (19)	64 (37-91)	2.06 (1.63-2.59)	<0.001	2.17 (1.66-2.83)	<0.001
Yes: no antibiotic treatment	70 (28)	221 (19-423)	0.94 (0.68-1.30)		1.07 (0.76-1.50)	
No (reference)	340 (103)	258 (191-325)	1.00		1.00	
Source of MRSA acquisition						
Community associated	344 (84)	173 (136-210)	0.99 (0.65-1.49)	0.72		
Healthcare associated	154 (54)	210 (152-268)	0.72 (0.70-1.68)			
Unknown	37 (12)	224 (11-437)	1.00			
Riskfactor-chronic skinlesion						
Yes	103 (36)	172 (137-207)	0.68 (0.52-0.88)	0.004	1.27 (0.95-1.69)	0.105
No (reference)	432 (114)	239 (85-393)	1.00		1.00	
Spa-type						
<i>t044</i>	79 (13)	160 (81-239)	1.24 (0.94-1.64)	<0.001	1.10 (0.82-1.48)	0.014
<i>t002</i>	79 (35)	554 (0-1220)	0.54 (0.39-0.76)		0.57 (0.40-0.80)	
<i>t008</i>	43 (12)	135 (53-217)	1.08 (0.74-1.57)		0.90 (0.61-1.33)	
<i>t131</i>	20 (2)	183 (0-374)	1.47 (0.91-2.39)		1.12 (0.66-1.90)	
<i>t355</i>	20 (3)	46 (30-62)	2.20 (1.34-3.61)		1.38 (0.82-2.33)	
Other (reference)	294 (85)	198 (147-249)	1.00		1.00	
No. of locations						
1(reference)	198 (44)	52 (46-58)	1.00	<0.001	1.00	<0.001
2	130 (32)	224 (130-318)	0.38 (0.29-0.49)		0.35 (0.26-0.45)	
3	142 (45)	404 (302-506)	0.29 (0.22-0.37)		0.28 (0.21-0.37)	
4	65 (29)	521 (409-632)	0.25 (0.17-0.36)		0.18 (0.12-0.27)	
Eradication treatment a						
Topical	67 (15)		1.45 (1.02-2.07)	0.039	1.70 (1.16-2.49)	0.006
Systemic	137 (26)		3.16 (2.51-3.98)	<0.001	3.45 (2.69 -4.43)	<0.001
No	331 (109)					

Epidemiology of MRSA in Skåne County 2000-2010 (Paper II)

The prevalence of MRSA in Skåne County is very low from an international perspective but between 2000 and 2010 there was a 10-fold increase in the number of detected MRSA cases with 31 cases detected in 2000 and 315 in 2010. Contrary to many other countries, the MRSA patients in our study were younger (median age 30 years) and more often had community-acquired infection (Fig.7) (43). During the years that were studied, only a few minor healthcare-acquired MRSA outbreaks occurred in Skåne County. The MRSA cases contracted abroad were mostly healthcare-acquired, but the number of cases with MRSA acquired in the community abroad increased over time (Fig.7), probably reflecting the worldwide emerging epidemic of MRSA in the community (25). In contrast to other countries the majority of our MRSA cases were asymptomatic carriers detected by contact tracing or screening. Only 36 % had an MRSA infection at the time of diagnosis, with another 10 % developing infection after the initial MRSA diagnosis. Less than 1 % had severe infections with positive bloodcultures.

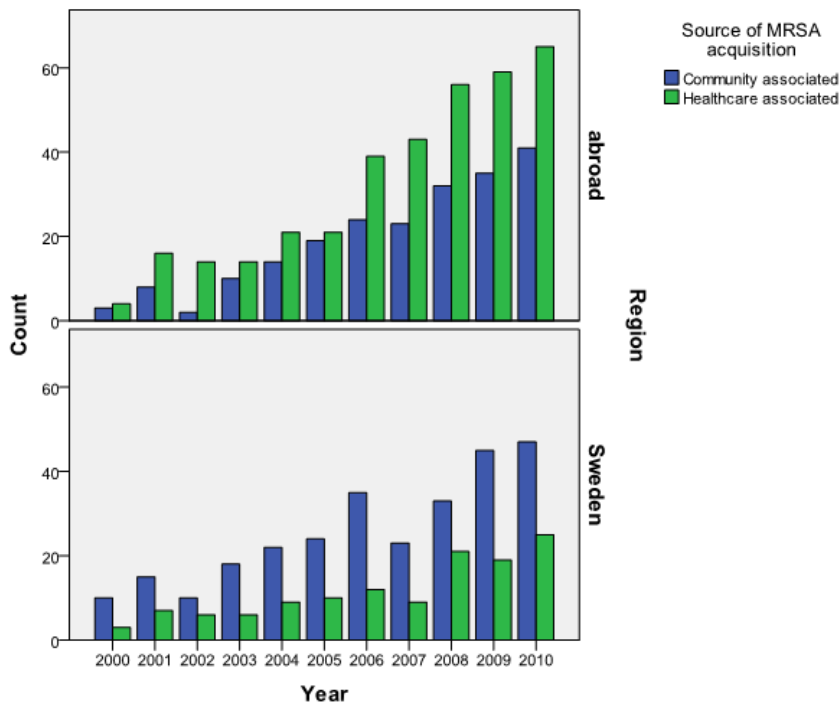


Figure. 7. Number of MRSA cases that acquired their MRSA in Sweden and abroad in the years 2000 – 2010 and number community- and healthcare-acquired

Spa-types - change over time

A wide spectrum of MRSA strains with 233 different *spa* types was seen. There was a change in epidemiology over time, with some strains present at the beginning of the study period (*spa* types t044, t002, t032 and t015) and others emerging and increasing over time during the last years of the study (t355, t437, t127, t223 and t019). *Spa*-type t008 was first seen in 2003 and was the predominant type in 2010. This *spa*-type rapidly became the community associated clone in the US (88) and represented 40 % of the community-associated MRSA in Europe in a study published 2012 (89). This reflects a dynamic spread of MRSA strains across the globe (90)

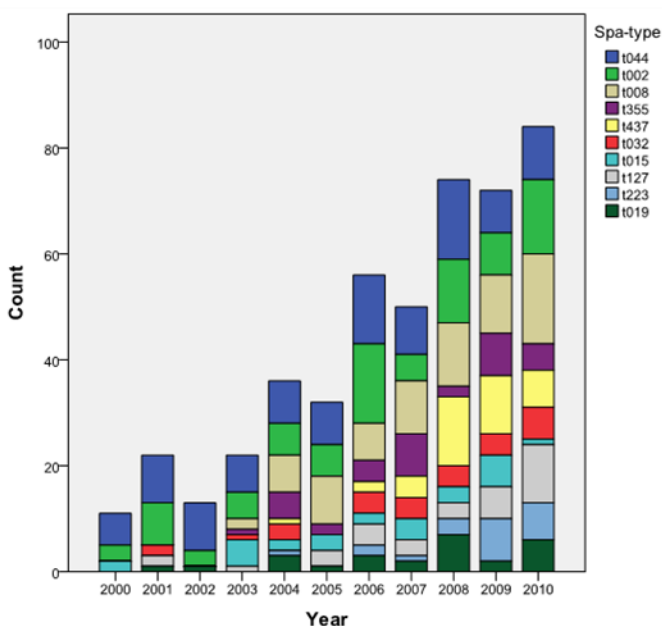


Figure 8. Number of index MRSA cases with the 10 most common MRSA *spa*-types in the years 2000 – 2010 in the Skåne Region, Sweden

Acquisition of MRSA - related to foreign country of origin, healthcare abroad and foreign travel (Fig. 8 and 9)

In our study, as well in studies from Denmark (91), Norway (92) and Germany (93), there was an association between acquisition of MRSA and a foreign origin or traveling/staying abroad. Only 24 % of the index cases in our study were both of Swedish origin and had contracted their MRSA in Sweden. The patients of foreign origin mostly contracted MRSA when travelling to their former home countries, but acquisition in Sweden was also common, probably reflecting contact with their fellow citizens that had recently been in their country of origin or visitors to Sweden. A Swedish study showed characteristic differences in MRSA acquisition depending on the region from which they originated (94). Similarly, our study showed a relation between different *spa*-types and regions of MRSA acquisition. For example *spa*-type t044 was most often acquired in Sweden by immigrants from the Middle East, whereas type t008 was most often acquired by persons of Swedish origin travelling abroad, to North America and other parts of Europe. Sometimes changes in travel and immigration patterns might explain why certain *spa*-types have become more common than others. Travel to and immigration from Asia has increased during the study period (TDB, Travel Data Base and SCB, Statistical Central Bureau) and may explain why *spa*-types t437, t127 and t019, which in our study had a connection with Asia, increased. Immigration from the Middle East to Skåne County is common and increased from 1000 immigrants in year 2000 to more than 3,000 in 2008 and then declined to 2,000 immigrants in 2010 (SCB). *Spa*-type t044 was strongly related to family origin from and acquisition in the Middle East and coincided with the immigration pattern. Similarly, the increase in *spa*-types t355 and t223 could be explained by increased immigration from the Balkan states.

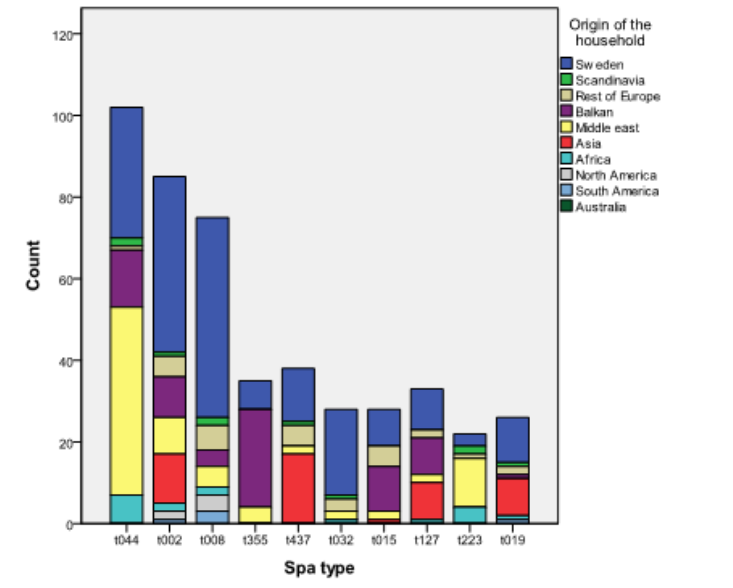


Figure 9. The ten most frequent spa types. The origin of the household of the index cases

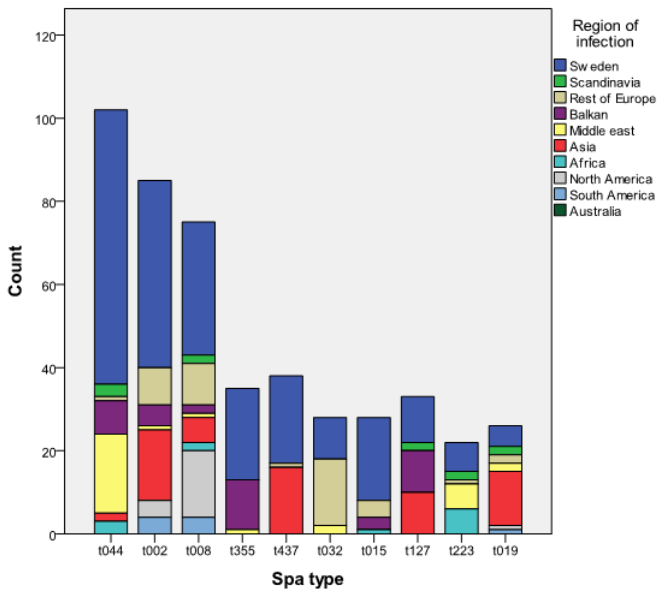


Figure 10. The ten most frequent spa types. The region of acquisition of the index patients

Differences between the spa types' ability to cause infection (Table 2)

The PVL toxin is a well known virulence factor in *S. aureus* (80). Similar to what has been reported in several other studies, the PVL-positive strains in our study caused more clinical infections than PVL-negative strains (80). In particular, PVL-positive MRSA of *spa*-types t008, t019 or t044 caused significantly more skin infections than other types. PVL negative strains of some *spa*-types (t032, t127, t002 and t437), caused significantly fewer clinical infections. However, another European study concluded that *spa*-typing of MRSA isolates is unsuitable for predicting the likelihood of an infection with MRSA (95).

Table 2. All MRSA cases with *spa*-types. Numbers, age, and the presence of PVL gene and MRSA cultured from a skin lesion (representing a skin infection)

Spa-type	Frequency n (%)	Median age (min-max)	PVL n (%) +=positive -=negative	MRSA cultured from skinlesions representing a clinical infection n (%)	OR* (odds ratio)	P-lue *
t044	209 (11.7)	22 (0-93)	+ 209 (100) - 0 (0)	113 (54)	1.75	0.005
t002	214 (12.0)	29 (0-101)	+ 25 (12) - 189 (88)	16 (64) 59 (31)	1.13 0.18	1 <0.001
t008	132 (7.4)	28 (0-88)	+ 95 (72) - 35 (26)	62 (65) 17 (49)	3.09 1.08	<0.001 1
t355	69 (3.9)	23 (0-86)	+ 68 (99) - 1 (1)	34 (50) 0 (0)	1.22	1
t437	69 (3.9)	33 (0-93)	- 34 (49) - 35 (51)	21 (62) 7 (20)	2.40 0.083	0.18 0.008
t032	61 (3.4)	52 (1-93)	+ 1 (2) - 60 (98)	0 (0) 23 (38)	0.38	0.03
t015	59 (3.3)	35 (0-93)	+ 0 (0) 58 (98)	22 (38)	0.85	1
t127	50 (2.8)	30 (0-98)	+ 5 (10) - 45 (90)	3 (60) 7 (16)	0.21	0.005
t223	52 (2.9)	16 (0-69)	+ 0 (0) - 51 (100)	18 (35)	0.69	0.96
t019	46 (2.6)	32 (0-76)	36 (86) 0 (0)	36 (86)	3.02	0.012
Other	827 (46.3)	32 (0-97)	+ 151 (18) - 670 (81)	68 (45) 165 (25)		
All	1788	30 (0-101)	630 1158	353 (56) 318 (27)		

Note * Logistic regression model and a post-hoc analysis comparing the proportion of clinical infections. In the analysis types with less than 10 cases and the heterogenous group "other" were excluded.

Spa types and the ability to spread in the household (Table 3)

It is recognized that MRSA can be shared among household contacts (85). In a study from the United States (96), 23 % of the household contacts to children with a community-acquired infection were colonized in the nose. In an American study of

315 patients with *S. aureus* infection where 812 household contacts were cultured for *S. aureus*, 405 were found to be colonized with MRSA and *spa*-type t008 showed the greatest potential for spread in the household (97). This might be one of the reasons why *spa*-type t008 has spread so successfully in the community around the world. In our study, 35% of the screened household contacts were positive for MRSA with none of the *spa*-types being significantly more prone to spread. A weakness in the statistical analysis of the ability to spread to household contacts is the absence of adjustments for important confounders such as size of living area, duration of contacts and age distribution.

Table 3. MRSA index cases with *spa*-types and number community acquired. Household contacts traced and number MRSA positive.

<i>spa</i>	Frequency n (%)	Community acquired n (%)	Number of household- contacts mean	N of household contacts traced for MRSA	N of contacts positive for MRSA (%)*
t044	102 (10,5)	74 (73)	3.3	339	105 (31)
t002	85 (8,7)	44 (52)	2.7	227	100 (44)
t008	75 (7,7)	47 (63)	1.6	124	54 (44)
t355	35 (3,6)	21 (60)	2.9	102	34 (33)
t437	38 (3,9)	27 (79)	2.1	81	29 (36)
t032	28 (2,9)	10 (34)	1.4	38	13 (34)
t015	28 (2,9)	19 (68)	2.3	65	28 (43)
t127	33 (3,4)	6 (18)	1.8	61	24 (39)
t223	22 (2,3)	11 (50)	3.1	69	28 (41)
t019	26 (2,7)	16 (62)	1.8	47	19 (40)
Other	504 (51,4)	218 (44)	1.8	974	303 (31)
All	976	493 (51)	2.3	2127	737 (35)

Note * A logistic regression model found significant differences between the proportions of family infections among different *spa*-types (excluding "other", since this is a heterogenous group by definition). Due to observed overdispersion, a quasibinomial model was used with estimated dispersion 1.68 (Fobs=4.48, denom.df=10, nom.df=353, p<0.001). Unfortunately, the post-hoc analysis with all pairwise comparisons failed to identify which *spa*-types that differed.

Our results supports screening for MRSA in patients treated in health-care settings abroad and taking cultures from patients with skin infections contracted outside Sweden. It also supports continued contact tracing of household contacts. Knowledge of the *spa*-type may give valuable clues in the process of contact tracing.

Eradication of MRSA colonization in the throat (paper III)

Because of difficulties in including patients the study was ended before recruiting as many patients as planned. Fifty-two patients in 42 households were evaluable for the study. In group I who received systemic antibiotic together with topical treatment 28 patients in 22 households were evaluable. Of these, the majority, 21 patients in 15 households, received clindamycin and 7 patients in 7 households received trimethoprim-sulfamethoxazole together with rifampicin due to resistance or allergy to clindamycin. In group II, who received topical treatment alone, 24 patients in 20 households were evaluable.

The patients in our study was quite young, healthy and a majority had been colonized with MRSA for more than one year. Almost one third of the patients had gone through earlier attempts to eradicate MRSA but failed. (Table 4)

Table 4. Patient characteristics

Treatment	Group I (Systemic + topical n=28)	Group II (Topical n=24)
Age (median years)	40	36
Female gender n (%)	15 (54)	18 (75)
Foreign-born n (%)	10 (36)	13 (54)
Chronic diseases n (%)	5 (18)	6 (25)
Household contacts with MRSA n (%) (at the end of the study)	7 (25)	9 (37)
Carriage time before inclusion median years(min-max)	1,2 (0.3-11)	1,2 (0.2-5)
Location for carriage -the throat +/- the nares (%)	23 (82%)	20 (83%)
Location for carriage -the throat and the perineum +/- the nares (%)	5 (18%)	4 (17%)
Eradication attempts prior to the study (%)	8 (29%)	8 (33%)

The majority of the patients that only received topical treatment MRSA eradication failed with only 15 % of the households being negative 2 weeks and 10 % 6 months after the end of the treatment. With addition of systemic antibiotics the result was significantly better with 68 % of the households being negative 2 weeks and 50 % 6 months after the end of the treatment. (Fig.10a and b). Thus the difference in outcome between the 2 groups was 40 % (95 % CI 15 % -65 %, P=0.007). A possible explanation is that insufficient levels of the topical treatment is reached in the crypts of the tonsils, and that *S. aureus* can be internalized by human cells and can survive intracellularly (101). Systemic antibiotics, especially rifampicin, may achieve better tissue and intracellular levels leading to higher MRSA eradication rates (102). Nasal application with mupirocin is well established and shown to be effective for nasal decolonization (66). Treatment with systemic antibiotics is less studied. Among existing studies there is strongest evidence for the use of a combination therapy with rifampicin being part of it (66). In a study with rifampicin and doxycycline combined with nasal mupirocin and chlorhexidine washings for 7 days MRSA was successfully eradicated from 74 % of the patients at a follow up of 3 months (100). Cultures from the throat were not taken. In accordance with our result failure in treating throat carriage with topical treatment has been described (68, 98, 99).

This study suggests that MRSA colonization in the throat is possible to eradicate but that it is difficult. It shows that topical treatment is not sufficient to eradicate MRSA carriage in the throat and that addition of systemic treatment substantially increases the success rate.

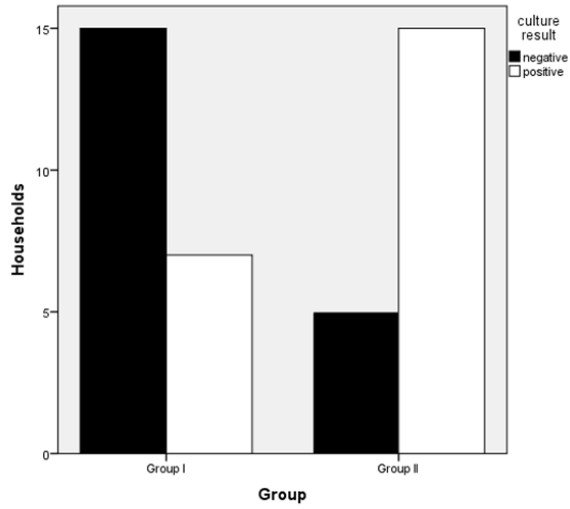


Figure 10a. Culture results 2 weeks after end of treatment

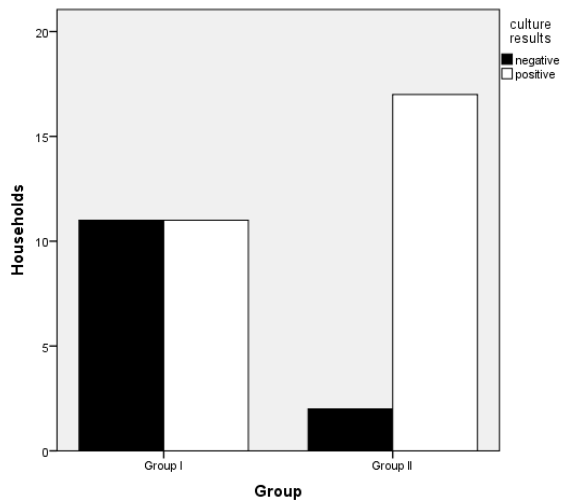


Figure 10b. Culture results 6 months after end of treatment

The epidemiology of MRSA with *mecC* in Skåne County (paper IV)

The discovery of MRSA carrying the *mecC* gene has raised questions about the epidemiology of these strains. In a retrospective study we described 45 patients with *mecC* MRSA in Skåne County between 2005 and 2014.

The median age of the patients with *mecC* MRSA in our study was high (60 years) (table 5) as compared with the median age of patients with clinical symptoms of *mecA* MRSA (32 years) in a previous study from the same geographical region in 2005 to 2010 (103). The majority of patients with *mecC* MRSA in our study were of Swedish origin (table 5). In the previous study of patients with *mecA* MRSA (103) only 50 % of the patients with clinical symptoms of *mecA* MRSA were of Swedish origin. The same pattern is described in Denmark (70), and Belgium (104) indicating a different epidemiology compared to *mecA* MRSA. This might indicate that import does not contribute to the emergence of *mecC* MRSA in our county. The majority of the *mecC* patients had an underlying chronic disease (table 5) which has not been the case with the *mecA* patients in the same region (103). A common denominator was having a chronic wound and most often the patients without underlying diseases had an existing wound and no primary infection was seen in contrast to what we have seen in patients with *mecA* MRSA. This might reflect that *mecC* MRSA do not possess the PVL genes and other virulence genes as described in previous studies (69).

The *mecC* MRSA isolates in our study can be grouped in two MLST clonal complexes, CC130 and CC2361 (table 5), which is in concordance to the Danish study. The most common *spa*-type, t373, is previously described only from Denmark and Ireland (70, 105). The second most common *spa*-type in our material, t843, is so far the most commonly described. Of the *spa*-types detected in dairy cattle in Sweden, t524, t911 and t843 (74, 75), only t843 is described in our material. The origin of *mecC* MRSA is not yet clear but there is evidence that contact with animals poses a risk and that it can be transmitted between species (69). *MecC* MRSA have been detected from a diverse range of species, dairy cattle, seal, chaffinch, dog, sheep, wild hare, cat, wild brown rat, hedgehog and otter (69). Most of the patients with *mecC* MRSA lived in rural areas, at least one person worked with cattle and five persons lived in farms. These factors might indicate exposure to animals and hence exposure to *mecC* MRSA. Unfortunately, since the study is retrospective we lack full information about animal contact.

The *mecC* MRSA in our study had lower oxacillin MICs than their *mecA* counterpart, with a median MIC value of 16 mg/L. This is in agreement with other studies (69). According to EUCAST most *mecA* MRSA-strains have MIC-values above 64 mg/L and the majority 256 (106). In accordance with other reports (70), all but one *mecC* MRSA isolate were susceptible to all classes of other tested antibiotics. After the positive culture was taken, 39 patients were treated with antibiotics. The majority were treated with cloxacillin and few of these patients had their treatment switched to non beta-lactam antibiotics when the culture result was known. This might indicate that treatment with beta-lactam antibiotics can be effective, reflecting the lower MIC-values. This is also shown in a study by Mancini et al (107), who reported that treatment with flucloxacillin was effective in experimental endocarditis caused by *mecC*-positive *S. aureus*.

Our results suggest that *mecC* MRSA isolates might be less prone to be contagious compared to *mecA* MRSA since only 2 of 27 tested family members were found to be positive. In the previous study from the same region 39% of the tested family members were positive for *mecA* MRSA (103). Among patients with infection caused by *mecA* MRSA from the same geographical region the median time for carriage in clinical cases was 66 days while the patients with *mecC* were carriers for a shorter time, 21 days (7-210). Since the number of patients with *mecC* MRSA still is small, our data should be interpreted with caution.

Table 5. The 45 *MecC*MRSA cases. Median age, gender, origin, culture sites, underlying diseases, *spa* types and the median MIC value.

Median age in years (range)	60 (2-86)
Female gender (n)	22 (49 %)
Non-Swedish origin (n)	3 (7 %)
Initial culture site (n) for detection of <i>mecC</i> MRSA	Wound (42), sputum (2), nasopharynx (1)
Underlying diseases (n)	35 (78%) 11 diabetes mellitus, 9 cancer, 4 autoimmune diseases, 11 atherosclerotic disease
<i>Spa</i> types (n)	CC 130 (38): t373 (23), t843 (11), t10530 (1), t6594 (1), t7538 (1), t11205 (1) CC 2362 (7): t978 (4), t3391 (3)
MIC oxacillin median (mg/l)	16 (1-64)

Conclusions

- The median duration of MRSA colonization was 5.9 months. Having household contacts with MRSA, young age, *spa*-type t002 and colonization in 2 or more locations, was significantly associated with a longer duration of colonization. Having a clinical infection treated with antibiotics was significantly associated with a shorter carriage time. Eradication treatment was associated with a shorter carriage time.
- We saw a change in the epidemiology of MRSA in Skåne County during the period (2000–2010) with a ten-fold increase of cases. There was a strong association between MRSA acquisition and either a non-Swedish origin or travelling and staying abroad.. Different *spa* types were seen in patients who had travelled to or originated from different regions of the world. Thirty-five per cent of the household contacts were positive for MRSA The PVL-positive strains with *spa* types t008, t019 or t044 caused significantly more skin infections compared to the other *spa* types.
- MRSA colonization in the throat is possible to eradicate. Our study shows that topical treatment is not sufficient to eradicate MRSA carriage in the throat and that addition of systemic treatment substantially increases the success rate.
- *MecC* MRSA mainly affects older patients with underlying diseases or with an existing skin lesion. The *mecC* MRSA in our region appears to have a domestic origin. Data indicates that it could be a poor colonizer with few secondary cases and few people being colonized for a long time

Future perspectives

So far most intervention strategies for limiting the spread of MRSA in Sweden has focused on the health care environment. Measures taken are screening and pre-emptive isolation of patients having received health care abroad and screening of medical staff having worked or received health care abroad. For every new unexpected MRSA case found in health care, contact tracing among patients, and sometimes medical staff, is performed in order to prevent spread. All MRSA cases are followed up and contact tracing among household contacts are performed.

The increasing burden of MRSA disease from the community raises questions on what the optimal strategies are. For how long will it be possible for us to keep up with our ambitious guidelines regarding screening, contact tracing and monitoring of our MRSA patients? Regardless, general measures with basic hygiene precautions and environmental cleaning becomes more important every year due to the increasing MRSA frequencies in the community. Since staying in and travelling to foreign countries are factors connected to an increased risk of acquiring MRSA, screening of patients that have acquired a skin and soft tissue infection or a wound abroad has become a recommendation in our county as of this year. To be able to recommend adequate empirical treatment for staphylococcal infections, the frequency and resistance pattern of MRSA in wound and bloodcultures must continuously be followed. Knowledge of *spa* type is of importance and give guidance in the process of contact tracing and typing of the MRSA isolates should continue.

MecC MRSA seems to be a poor colonizer and to cause infections that most of the time is less severe and easier to treat compared to *mecA* MRSA. This might indicate that there is no advantage in handling the *MecC* MRSA patients in the same way as *mecA* MRSA patients. However, our study population of *MecC* MRSA is small and more cases has to be followed to form a basis for a decision to change the recommendations.

Controlling the spread of MRSA outside the healthcare environment is a challenge and it is not obvious which measures are the most successful. Infected skin lesions and wounds that don't heal should be cultured in order to find MRSA. Skin

infections due to MRSA should be treated to prevent colonization. On the other hand, eradication treatment of MRSA colonization shall be performed with caution, due to the risk of selection of resistant bacteria. In some situations, however, eradication treatment of MRSA colonization could be considered and if that is the case all MRSA positive household members should be treated simultaneously to avoid re-colonization.

Good hygiene is basic to reduce the risk of spreading MRSA. In order to prevent MRSA increased knowledge in the population about the importance of hygiene and other measures that the individual can take to reduce the risk of infection or further spread of MRSA in different situations is needed.

For example, crowded environments, like child day care, sport facilities, or activities with direct or indirect skin contact increases the risk of spreading MRSA. Knowledge on the risk of spreading MRSA and other bacteria in these facilities when having an open wound or boil should be disseminated.

Populärvetenskaplig sammanfattning på svenska

Bärarskap av MRSA

Staphylococcus aureus, gula stafylokocker, är en bakterietyp som finns i den mänskliga normalfloran hos 25–30 % av befolkningen. I de flesta fall orsakar bakterien ingen skada utan lever i symbios med människokroppen, oftast på huden eller i näsan. De är den vanligaste orsaken till sårinfektioner och kan också ge upphov till bölder, lunginflammation, hjärnhinneinflammation och infektion i skelett och leder. Infektioner med bakterieväxt i blodet och på hjärtklaffar är mycket allvarliga och förknippade med hög dödlighet. *S. aureus* är också en vanlig bakteriell orsak till vårdrelaterade infektioner, t.ex. sårinfektioner efter operation eller infektion i anslutning till främmande material (proteser m.m.). Meticillinresistenta *S. aureus* (MRSA) har utvecklat motståndskraft (resistens) mot betalaktamantibiotika, en grupp antibiotika som vanligen används mot stafylokockinfektioner. De innebär att behandlingsalternativen vid infektion orsakad av MRSA är begränsade och förknippade med fler biverkningar. MRSA har under 1990-talet blivit ett världsomfattande problem. De utgör nu mer än 25 % av de stafylokockstammar som orsakar blodförgiftning i många delar av världen, t ex USA och Syd- och Mellaneuropa. Däremot är frekvensen i Sverige fortfarande låg och ligger sedan länge under 1 %. Kolonisation med eller infektion orsakad av MRSA har tidigare huvudsakligen drabbat äldre individer med sjukvårdsassocierade riskfaktorer såsom sår, hudskador, främmande material som penetrerar huden som katetrar och drän samt tung antibiotikabehandling. På 1990- och 2000-talet har emellertid samhällsförvärvade stammar blivit allt vanligare och de drabbar numer även yngre, tidigare fullt friska individer. Svår infektion orsakad av MRSA har en ökad dödlighet jämfört med motsvarande infektion orsakad av meticillin-känsliga stafylokocker och medför ökade kostnader på grund av dyrare antibiotikabehandling, ökat behov av sjukvårdsresurser och förlängd vårdtid.

I detta doktorandprojekt har olika aspekter av bärarskap med MRSA studerats. Syftet med projektet var att skaffa ökad kunskap om hur individer med bärarskap av MRSA bör handläggas och behandlas inom sjukvården med det övergripande målet att minska risken för spridning av MRSA.

- Vi har undersökt hur länge bärarskap av MRSA varar och vilka faktorer som påverkar bärartiden.
- Vi har studerat epidemiologin för MRSA i Skåne under åren 2000 till 2010 och undersökt om visa av MRSA stammarna är mer benägna att spridas och ge upphov till symptomgivande infektion.
- Vi har undersökt huruvida det räcker med lokalbehandling för att behandla bort bärarskap av MRSA i svalget eller om antibiotikabehandling i tablettform krävs som tillägg.
- Vi har studerat epidemiologin för MRSA med *MecC* (en ny resistensmekanism) i Skåne.

Bärartid och faktorer som påverkar bärartiden

Medianbärartiden med MRSA var 5,9 månader. Att ha hushållskontakter med bärarskap av MRSA, låg ålder, bärarskap av *spa* typ t002 samt att vara koloniserad på två eller fler lokaler var kopplat till en längre bärartid. Att ha en symptomgivande infektion som behandlades med antibiotika (jämfört med enbart kolonisation eller obehandlad infektion) eller att ha fått eradikeringsbehandling mot MRSA-bärarskap var associerat med kortare bärartid. Våra resultat talar för att patienter kan definieras som ”MRSA-negativa” i uppföljningsprogram och därmed avskrivs från åtgärder och förhållningsregler på grund av bärarskapet. Resultaten visar också på vikten av att hushållskontakter till MRSA-bärare odlas för MRSA.

Epidemiologin för MRSA i Skåne under 2000-2010.

Antalet MRSA-fall ökade från 31 år 2000 till 315 2010. De flesta fallen var samhällsförvärvade och medianåldern var 30 år. Trettiofyra procent av fallen identifierades pga en symptomgivande infektion och de övriga genom smittspårning eller screening. Drygt en tredjedel av hushållskontakterna var MRSA-positiva. Enbart 24 % av MRSA-fallen var av svenskt ursprung och hade förvärvat sin MRSA i Sverige. Ett tydligt samband sågs mellan typ av MRSA-stam och var i världen MRSA förvärvades. De stammar som producerade giftet PVL-toxin orsakade oftare hudinfektioner än andra MRSA-stammar. Vi såg ingen skillnad i spridningsbenägenhet mellan de olika MRSA-stammarna. Våra resultat stödjer strategin att screeningodla patienter med sjukvårdskontakter utomlands, att odla patienter med hudinfektioner förvärvade utomlands samt att odla hushållskontakter till MRSA-bärare för MRSA. Kännedom om typ av MRSA-stam kan ge vägledning vid smittspårning.

Eradikeringsbehandling av MRSA-bärarskap i svalget

I vissa fall görs försök att eradikera bärarskap av MRSA med antibiotikabehandling. Det rör sig framför allt om sjukvårdspersonal med bärarskap samt patienter med återkommande infektioner orsakade av MRSA, patienter med behov av sjukvårdskontakter framöver där MRSA-bärarskapet utgör en risk för smittspridning

inom sjukvården och hos dem som får sociala konsekvenser av bärarskapet. Bärarskap av MRSA i näsan kan behandlas bort med lokalbehandling med antibiotika. Eradikeringsbehandling av svalg bärarskap av MRSA är sämre studerat, men upplevs som mer svårbehandlat. I en jämförande studie fann vi att eradikering av MRSA bakterier från svalget är möjligt men att det inte räcker med lokalbehandling. Det krävs tillägg av antibiotika i tablettform till lokalbehandlingen för att få effekt på bärarskapet.

Epidemiologi av MRSA med mecC i Skåne

För påvisning av MRSA används idag undersökning av *mec A*, den gen som kodar för meticillinresistens. Fram tills nyligen har detta varit den enda kända genen som kodar för meticillinresistens. 2011 beskrevs upptäckten av MRSA med en ny gen kodande för meticillinresistens kallad *mecC*. Vi undersökte de 45 första detekterade fallen av MRSA med *mecC* i Skåne och jämförde dem med patienter med bärarskap av *mecAMRSA*. Patienterna med *mecC* var äldre, oftare av svenskt ursprung, och de hade mer bakomliggande sjukdomar. De flesta av patienterna hade en hud- och mjukdelsinfektion vid diagnostillfället. Bärartiden var kortare och smittspridning till hushållskontakter hittades endast i ett fall. Ingen smittspridning i sjukvård sågs. De studerade *mecCMRSA* hade lägre grad av resistens mot betalaktamantibiotika jämfört med *mecAMRSA*.

Tack

Det är många jag vill tacka som på olika sätt bidragit till denna avhandling.

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