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Infection control of *Staphylococcus aureus* - *spa* typing to elucidate transmission

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Till Pål, Astor och Silve – för att ni förgyller varje dag av mitt liv

En stor glasburk och två koppar kaffe

En professor stod inför sina filosofistudenter med några föremål på bordet framför sig. När lektionen började lyfte han under tystnad upp en mycket stor och tom glasburk och fyllde upp den till kanten med golfbollar.

Han frågade sedan sina studenter om burken var full. Studenterna samtyckte till att den var det.

Då lyfte professorn upp en ask med småsten och hällde dem i burken. Han skakade burken lätt. Småstenarna rullade ner i tomrummen mellan golfbollarna.

Återigen frågade han studenterna om burken var full. De höll med om att den var det. Därefter lyfte professorn upp en ask med sand och hällde sanden i burken. Naturligtvis fyllde sanden upp resten av tomrummen.

Han frågade ännu en gång om burken var full. Studenterna svarade med ett enhälligt "ja".

Då lyfte professorn upp två koppar kaffe som stått under bordet och hällde hela deras innehåll i burken, vilket effektivt fyllde upp det återstående tomrum som kunde finnas kvar mellan sandkornen. Studenterna skrattade.

"Nu", sa professorn medan skratten klingade ut, "vill jag att ni påminns om att den här burken representerar ert liv. Golfbollarna representerar de viktiga sakerna. Familj, barn, hälsa och annat som ligger er varmt om hjärtat. Sådant som, om allt annat gick förlorat och bara dessa återstod, ändå skulle uppfylla och berika ert liv."

"Småstenarna representerar de andra sakerna som betyder något, som ett hem, jobb och bil. Sanden representerar allt annat, småsakerna."

"Om ni lägger sanden i burken först", fortsatte professorn, "går det inte att få plats med golfbollarna eller småstenarna. Samma sak är det med livet. Om du lägger all tid och energi på småsakerna finns det inte plats för det som är viktigt för dig."

”Så, var uppmärksam på det som är oumbärligt för din lycka. Umgås med dina barn. Ta med din partner ut på middag. Ägna lite mer tid åt det som gör dig lycklig. Tids nog kan du städa huset och vika tvätten. Ta hand om golfbollarna först, sakerna som verkligen betyder något. Återställ det som är viktigast i ditt liv. Resten är bara sand."

En av studenterna räckte upp handen och frågade vad kaffet representerar.

Professorn log. "Jag är glad att du frågar. Kaffet finns med för att visa er att hur fullt och pressat ert liv än känns, så finns det alltid plats för en fika."

Okänd

Abstract

Staphylococcus aureus is a commensal of the human flora, primarily colonizing the anterior nares and throat, but it may also cause infections ranging from mild skin and soft tissue infections to severe diseases such as endocarditis and septicemia. *S. aureus* is also a major nosocomial problem increasing with the worldwide dissemination of methicillin-resistant *S. aureus* (MRSA). The main vector for bacterial cross-transmission in healthcare settings is the hands of healthcare workers (HCWs). No *S. aureus* was detected in the air in this thesis demonstrating that transmission through air is not important. Despite the fact that good compliance with hand hygiene is essential to prevent cross-transmission the compliance is generally less than 50 %. Gold standard to track bacterial transmission in healthcare settings has for long been pulsed-field gel electrophoresis (PFGE), a method that is labor-intensive, lacks consensus protocol and relies on semi-subjective analysis. Molecular typing by sequencing of the hypervariable part of the *S. aureus* protein A gene (*spa* typing) has overcome these problems and has shown promising results in epidemiological investigations.

The aims of this thesis were to study bacterial transmission with *S. aureus* colonization of newborn infants as a model and to evaluate *spa* typing as a molecular tool. Additionally, the influence of compliance with hygiene guidelines on *S. aureus* transmission was assessed.

Analysis of 280 MRSA isolates by *spa* typing revealed excellent typeability and epidemiological concordance and satisfactory discriminatory power. Additionally, *spa* typing was considered superior to PFGE thanks to its accessibility, ease of use and rapidity. Also, *spa* typing results are registered in a global database, facilitating inter-laboratory comparison.

The prevalence of *S. aureus* ranged from 41 % to 66 % in the populations studied and males had the highest colonization rate. Throat was the premier colonization site for adults and transmission from individuals colonized in the throat only was documented, suggesting that throat cultures should be included in *S. aureus* screening programs. The umbilicus was the premier colonization site for newborn infants. Incubating the swabs in enrichment broth prior to plating increased the prevalence of *S. aureus* positive samples by 46 %, resulting in prevalence

ranging from 51 % to 70 % in the populations studied. Thus enrichment prior to plating is necessary to determine more truthful *S. aureus* colonization rates. There were no indications of an institutional flora, as the colonization rates, *spa* type distribution and antibiotic resistance prevalence were similar among parents and HCWs.

Direct observations and self-reporting by HCWs were both validated as tools for monitoring compliance with hygiene guidelines. The compliance with hygiene guidelines was significantly higher following a 10-point hygiene intervention as compared to baseline. The compliance was also higher three years after the intervention in three of four participating departments. These data show that it is possible to markedly improve the compliance with hygiene guidelines, but to achieve a long-term effect, continuous and varied reminders seems necessary.

Both at baseline and following the intervention almost 60 % of the colonized infants were colonized with an *S. aureus* of the same *spa* type as isolated from their own family. At baseline approximately 25 % of the colonized infants received their *S. aureus* from non-family individuals, indicating transmission directly or indirectly from HCWs. Despite the improvement in compliance with barrier precautions from 41 % at baseline to 86 % following the hygiene intervention, the transmission from non-family did not decrease. This indicates that other factors may have a prominent impact on bacterial transmission. One factor might be the quality of hand hygiene technique which therefore needs to be studied further. However, to ensure patient safety it is still recommended that all HCWs comply with hygiene guidelines at all time.

Populärvetenskaplig sammanfattning

Staphylococcus aureus (*S. aureus*) eller den gula stafylokocken, är en del av normalfloran hos många människor och finns vanligtvis i näsa och svalg. Bakterien kan även ge olika sorters infektioner så som hud- och mjukdelsinfektioner samt blodförgiftning. *S. aureus* är ett stort problem inom hälso- och sjukvården där den orsakar många vårdrelaterade infektioner. Forskning har visat att en god handhygien bland vårdpersonal minskar risken för spridning av bakterier inom hälso- och sjukvården. Trots detta är personalens följsamhet till hygienrutiner ofta lägre än 50 %. För att kunna följa hur *S. aureus* sprids mellan patienter behöver man typa bakterien. Detta innebär att man delar in bakterier som tillhör samma art (*S. aureus* en art) i flera olika undergrupper. Idag görs detta genom att studera bakteriens genetiska skillnader, t.ex. med hjälp av pulsfält gelelektrofores (PFGE) eller *spa* typning.

Syftena med den här avhandlingen var att studera hur *S. aureus* sprids i sjukhusmiljö, genom att undersöka hur *S. aureus* etablerar sig hos nyfödda och att utvärdera *spa* typningens användbarhet för att beskriva spridningen. Samt att se hur personalens följsamhet till hygienrutiner påverkar spridningen av *S. aureus*.

Analys av 280 *S. aureus* visade att *spa* typning var överlägsen PFGE tack vare dess användarvänlighet, snabbhet och tillgänglighet. Dessutom finns resultaten i en internationell databas, vilket underlättar jämförelser mellan olika laboratorier.

Förekomsten av *S. aureus* hos de personer som lämnade prov var 50 % med högst förekomst hos män. *S. aureus* förekom oftast i navel hos barn och i svalget hos vuxna. Dessutom påvisas spridning av *S. aureus* från svalg. Detta indikerar att man bör ta odlings-prover även från svalg i de *S. aureus* screeningprogram som finns inom sjukvården. *S. aureus* återfanns i 46 % fler av proverna då man anrikade proverna före det att man odlade ut dem på agarplattor. Detta resulterade i att andelen personer som bar på *S. aureus* ökade till 59 %. Anrikning är därför nödvändig för att få veta hur många personer som verkligen är bärare.

För att bestämma personalens följsamhet till hygienrutiner används idag observationer, som innebär att några personer (observatörer) ur personalgruppen på en avdelning studerar hur deras kollegor följer hygienrutinerna. Det finns även möjlighet att låta personalen själva få uppskatta sin följsamhet efter ett vårdmoment (självskattning). Båda metoderna visade sig säkra när det gällde att bestämma personalens följsamhet.

Utan något speciellt fokus på handhygien (baslinjemätning) var följsamheten till hygienrutinerna 41 %. För att förbättra följsamheten genomfördes en hygienkampanj. Ett knappt år efter kampanjen var följsamheten 86 %. Ytterligare två år senare var följsamheten fortfarande högre än vid baslinjemätningen vid tre av fyra deltagande kliniker. Detta bevisar att man kan förbättra följsamheten till hygienrutiner avsevärt men för att uppnå en långvarig effekt måste man kontinuerligt arbeta med frågan.

Både vid den lägre följsamheten vid baslinjemätningen och vid den högre följsamheten vid första uppföljningen bar nästan 60 % av de koloniserade barnen på en *S. aureus* som kunde härledas till någon i den egna familjen. Vid båda mätningarna fick ungefär 25 % av de koloniserade barnen sin *S. aureus* från en person utanför familjen. Detta betyder att personalens ökade följsamhet till hygienrutiner inte gav någon effekt på spridningen av *S. aureus* till nyfödda. Andra faktorer kan därför ha en stor effekt på spridningen av *S. aureus*. En faktor kan vara hur väl personalen utför handhygienen inte bara att de utför den. Ytterligare studier behövs för att förstå hur spridningen av *S. aureus* kan förhindras. För att säkerställa patientsäkerheten i vården rekommenderas fortfarande all personal att följa hygienrutinerna i alla vårdmoment.

List of publications

This thesis is based on the following publications, referred to by roman numerals in the text.

I. **Melin S***, Haeggman S, Olsson-Liljequist B, Sjölund M, Nilsson PA, Isaksson B, Löfgren S, Matussek A. Epidemiological typing of methicillin-resistant *Staphylococcus aureus* (MRSA): *spa* typing versus pulsed-field gel electrophoresis. *Scand J Infect Dis.* 2009;41(6-7):433-9.

II. **Mernelius S**, Svensson PO, Rensfeldt G, Davidsson E, Isaksson B, Löfgren S, Matussek A. Compliance with hygiene guidelines: the effect of a multimodal hygiene intervention and validation of direct observations. *Am J Infect Control.* 2013 May;41(5):e45-8.

III. **Mernelius S**, Löfgren S, Lindgren PE, Blomberg M, Olhager E, Gunnervik C, Lenrick R, Thrane MT, Isaksson B, Matussek A. The effect of improved compliance with hygiene guidelines on transmission of *Staphylococcus aureus* to newborn infants: the Swedish Hygiene Intervention and Transmission of *S. aureus* study. *Am J Infect Control.* 2013 Jul;41(7):585-90.

IV. **Mernelius S**, Löfgren S, Lindgren PE, Matussek A. The role of broth enrichment in *Staphylococcus aureus* cultivation and transmission from the throat to newborn infants: results from the Swedish hygiene intervention and transmission of *S. aureus* study. *Eur J Clin Microbiol Infect Dis.* 2013 Dec;32(12):1593-8.

* The author's maiden name is Melin.

Paper II is available at:
http://www.sciencedirect.com/science/article/pii/S0196655312012497
Paper III is available at:
http://www.sciencedirect.com/science/article/pii/S0196655312012564

Abbreviations

APRES	Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance study
ASC	Active screening culture
bp	Base-pair
BURP	Based upon repeat pattern
CA	Community-associated
CC	Clonal complex
CI	Confidence interval
Clf	Clumping factor
D test	Double disk diffusion test
HA	Hospital-associated
HCW	Healthcare worker
HITS	The Swedish hygiene intervention and transmission of *Staphylococcus aureus* study
IgG	Immunoglobulin G
MHC	Major histocompatibility complex
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSCRAMMs	Microbial surface components recognizing adhesive matrix molecules
MSSA	Methicillin-susceptible *Staphylococcus aureus*
NGS	Next generation sequencing
OR	Odds ratio
PFGE	Pulsed-field gel electrophoresis
PVL	Panton Valentine leukocidin
SAg	Superantigen
SE	Staphylococcal enterotoxins
SCC*mec*	Staphylococcal cassette chromosome *mec*
SpA	Staphylococcal protein A
SSSS	Staphylococcal scaled skin syndrome

ST	Sequence type
TNFR1	Tumor necrosis factor-α receptor
TSS	Toxic shock syndrome
TSST-1	Toxic shock syndrome toxin-1
VISA	Vancomycin-intermediate MRSA
VRSA	Vancomycin-resistant MRSA

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Introduction

General characteristics of *Staphylococcus aureus*

Staphylococcus aureus is a 1 μm, gram-positive, facultative anaerobic cocci, which characteristically grows in irregular clusters like grapes. The bacterium was first described in 1880 by the English surgeon Sir Alexander Ogston. When examining pus from a surgical abscess from a knee joint under a microscope, he detected spherical bacteria growing in clusters and named them staphylé, after the Greek word for a bunch of grapes. Four years later, Friedrich Rosenbach isolated yellow bacteria and named them *S. aureus*, referring to the Latin word 'aureus' meaning golden.

Swedish guidelines recommend direct plating onto a solid medium for detection of bacterial growth from skin- and soft-tissue infections, from where *S. aureus* is often isolated (Föreningen för Medicinsk Mikrobiologi vid Svenska Läkaresällskapet & Folkhälsomyndigheten 2012). Broth enrichment prior to plating is not recommended, but several studies have shown a substantial increase in samples positive for *S. aureus* when incubating the swabs in enrichment broth prior to plating (Wanten *et al.* 1998; Andrews *et al.* 2009; Mernelius *et al.* 2013b). *S. aureus* is a halophile and therefore a medium with a high concentration of NaCl has been used to select for the bacteria when cultured. The staphylococcal genus consists of several species, of which the majority are coagulase-negative, e.g., *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus.* However, *S. aureus* is coagulase-positive, i.e., it will clot plasma, and a coagulase test can therefore be used to differentiate *S. aureus* from other staphylococcal species. *S. lugdunensis* can produce bound but not free coagulase, whereas *S. aureus* produces both. The tube coagulase test will be negative for *S. lugdunensis* and *S. lugdunensis* can thereby be differentiated from *S. aureus*. *S. aureus* is also DNAse-positive and will form a clear zone on DNAse agar. These classical diagnostic tests have mainly been replaced by automated species identification, e.g., VITEK and MALDI-ToF mass spectrometry (Föreningen för Medicinsk Mikrobiologi vid Svenska Läkaresällskapet & Folkhälsomyndigheten 2012).

Virulence factors

Bacterial virulence, i.e., bacteria's ability to cause disease, is mainly determined through five different characteristics (Batzing 2002).

1. The *microbial physiology,* which determines the location of establishment, e.g., an anaerobic bacterium will not establish itself in a superficial skin wound where aerobic conditions prevail.
2. *Adherence* of the bacteria to the host cell can be mediated through adhesins, i.e., surface molecules that bind to specific host cell receptors.
3. The bacteria also have to be able to *escape the antimicrobial control of the host*, an example of this is the capsule produced by some bacteria which enables them to evade phagocytosis.
4. Some bacteria are intracellular and must therefore be able to *invade the host cell*.
5. Perhaps the primary virulence factor is the ability of many pathogenic bacteria to produce *toxins*. The exotoxins are produced within the bacteria and are secreted during cell growth. Endotoxins are lipopolysaccharides which are part of the cell wall of gram-negative bacteria and are released when these bacteria die and lyse.

S. aureus produces and expresses a vast number of virulence factors, of which some are described in detail below.

Factors involved in adhesion to host cells

Adhesins are bacterial surface components that mediate bacterial adhesion to host cells. The main types of adhesins are the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). Important MSCRAMMs of *S. aureus* are protein A, clumping factor (clf) A and B, and fibronectin-binding protein A and B. *S. aureus* also produce secreted adhesines commonly referred to as secreted expanded repertoire adhesive molecules, e.g., coagulase (Chavakis *et al.* 2007).

The *S. aureus*-specific staphylococcal protein A (SpA) is a microbial surface protein with anti-opsonic and anti-phagocytic effects. SpA was first identified as an immunoglobulin G (IgG) binding protein (Forsgren & Sjoquist 1966), where the N-terminal mediates the interaction between the protein and the Fc region of the IgG molecule. *S. aureus* circumvent opsonization by antibodies by hiding the Fc portion of the IgG molecule from the Fc receptors on macrophages and neutrophils and thereby manages to evade phagocytosis. Recent studies have revealed that SpA can also bind von Willebrand factor (Hartleib *et al.* 2000) and a platelet cell protein (Nguyen *et al.* 2000). The interaction between *S. aureus* and platelets on the cardiac valve surface is of

major importance in the induction of infective endocarditis (Sullam *et al.* 1996). The tumor-necrosis factor-α receptor (TNFR1) is also a receptor for SpA. It has been demonstrated that the interaction between SpA and TNFR1 plays an important role in the pathogenesis of staphylococcal pneumonia (Gomez *et al.* 2004).

ClfA and ClfB are also microbial surface proteins but the genes encoding ClfA and ClfB are distinct from one another. It has been shown that ClfA is involved in the pathogenesis of experimental endocarditis (Moreillon *et al.* 1995; Entenza *et al.* 2000). ClfA is also involved in causing arthritis in mice (Josefsson *et al.* 2001), and impedes phagocytosis of *S. aureus* by macrophages (Palmqvist *et al.* 2004). Both ClfA and ClfB bind and activate platelets, through fibrinogen-dependent and fibrinogen-independent pathways, which may have an impact on the pathogenesis of invasive disease (O'Brien *et al.* 2002a). ClfB is involved in nasal colonization with *S. aureus* through the interaction with nasal epithelial cells (O'Brien *et al.* 2002b).

Coagulase is a secreted adhesive molecule that binds prothrombin and forms a proteolytic active complex. This complex cleaves fibrinogen into fibrin, thereby promoting coagulation on the *S. aureus* surface, inhibiting phagocytosis (Panizzi *et al.* 2004; Chavakis *et al.* 2007). This process can lead to abscess formation (Cheng *et al.* 2010). An association between coagulase-positive *S. aureus* and the development of blood-borne staphylococcal pneumonia in mice has been demonstrated (Sawai *et al.* 1997). However, another study could not identify coagulase as a virulence factor in an experimental endocarditis model (Moreillon *et al.* 1995).

Toxins

The α-, β-, γ- and δ- toxins of *S. aureus* are all cytolytic (Chavakis *et al.* 2007; Zecconi & Scali 2013). Included in the toxin group are also the exfoliative toxins A and B (Zecconi & Scali 2013), responsible for staphylococcal scaled skin syndrome (SSSS), which causes skin layers to separate and scale off. SSSS occurs primarily in infants and children and outbreaks have been reported from maternity units (El Helali *et al.* 2005; O'Connell *et al.* 2007).

The staphylococcal superantigen (SAg) family consists of toxic shock syndrome toxin-1 (TSST-1) and the staphylococcal enterotoxins (SE) (Zecconi & Scali 2013). In T cell activation, antigen-presenting cells internalize and process the invading microorganism or antigen and then the major histocompatibility complex (MHC) class II molecules carry the antigen and present it on the surface. The antigen-MHC class II complex is recognized by the T cell receptor, and the activated T cell releases cytokines (figure 1). This process activates less than 0.01 % of the available T cells. The SAgs are able to circumvent the process of internalization and bind directly

to the outside of the MHC class II protein and the T cell receptor of all major types of T cells (figure 1). As this binding is nonspecific it activates 5 % to 25 % of the T cells, inducing a massive release of cytokines, which in turn can cause extensive and systemic inflammation (Batzing 2002). TSST-1 is associated with the rare illness toxic shock syndrome (TSS), characterized by fever, hypotension, rash, multi-organ (≥3) involvement and peeling of skin (Bohach *et al.* 1990). In 1980, 97 % of the TSS cases occurred in women and primarily in menstruating women. It was demonstrated that the usage of certain brands of highly absorbent tampons was correlated with the development of TSS. This correlation was mainly due to three risk factors. First, the high concentration of nutrient-rich blood provides a perfect environment for *S. aureus* growth. Second, the tampons may cause minor cuts that give *S. aureus* and toxins access to the bloodstream. Third, some brands of tampons absorb magnesium and low concentrations of magnesium triggers toxin production (Batzing 2002). The SEA-E and SEG-I are the cause of staphylococcal food poisoning, causing vomiting and diarrhea shortly after ingestion (Proft & Fraser 2003). *S. aureus* also produces SAg-like proteins, which help *S. aureus* to evade the human innate immune response. *S. aureus* also produces SE-like proteins with unknown function (Zecconi & Scali 2013).

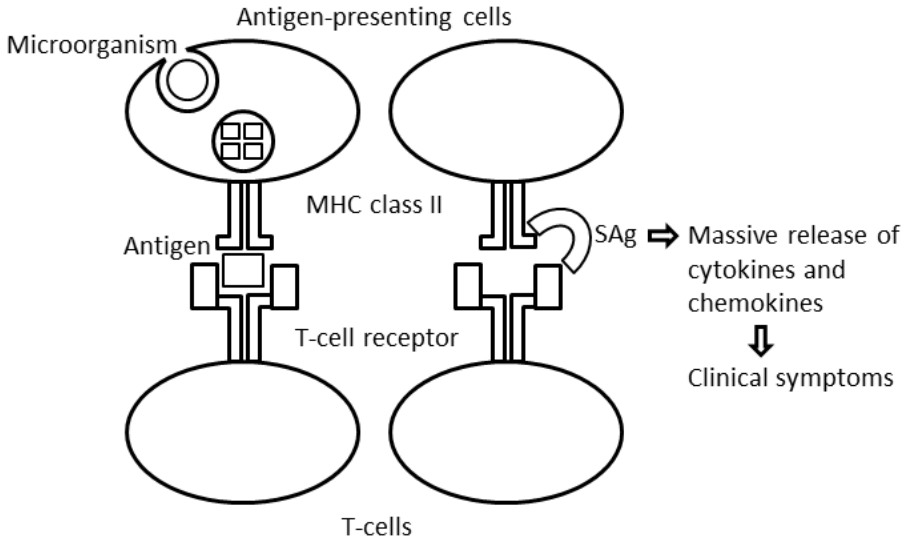

Figure 1. T-cell activation by an antigen and an SAg.

Panton-Valentine leukocidin (PVL) is a pore-forming bicomponent toxin (leukocidin D, E and M are also bicomponent toxins produced by *S. aureus*). It stimulates and lyses neutrophils and macrophages and is involved in necrotizing pneumonia (Chavakis *et al.* 2007; Zecconi & Scali 2013).

Enzymes and other proteins

S. aureus produce a large number of different enzymes and proteins, which are also regarded as virulence factors. Some of these enzymes and proteins are required for survival, persistence and nasal colonization, others have an antiphagocytic effect and some inhibits the complement (Chavakis *et al.* 2007).

Antibiotic resistance

Bacteria that develop resistance to antibiotics are an increasing problem in healthcare settings and the community. Antibiotic resistance correlates with increased mortality and morbidity, as well as increased costs due to prolonged hospital stay and the requirement for more expensive antibiotics. The development of antibiotic resistance will obviously impede treatment of several bacterial infections. It will also affect other elements of modern hospital care, e.g., cancer treatment, transplantations and advanced surgery, disciplines that are highly reliant on the use of antibiotics to defeat bacterial infections (Barriere 2015).

The development and approval of new antibiotics has declined since the 1980s (U.S. Department of Health and Human Services & Centers for Disease Control and Prevention 2013). This may be due to the high costs of development and the subsequent risk of producing a drug with no bacteriostatic or bactericide effect due to development of resistance and thereby no way of regaining the invested money.

Antibiotic resistance in *S. aureus*, primarily from skin and wound infections, has been voluntarily registered in Sweden since 2001 through the national surveillance system ResNet (Public Health Agency of Sweden 2014). Resistance to all antibiotics tested has generally been low since the introduction of the surveillance system (figure 2).

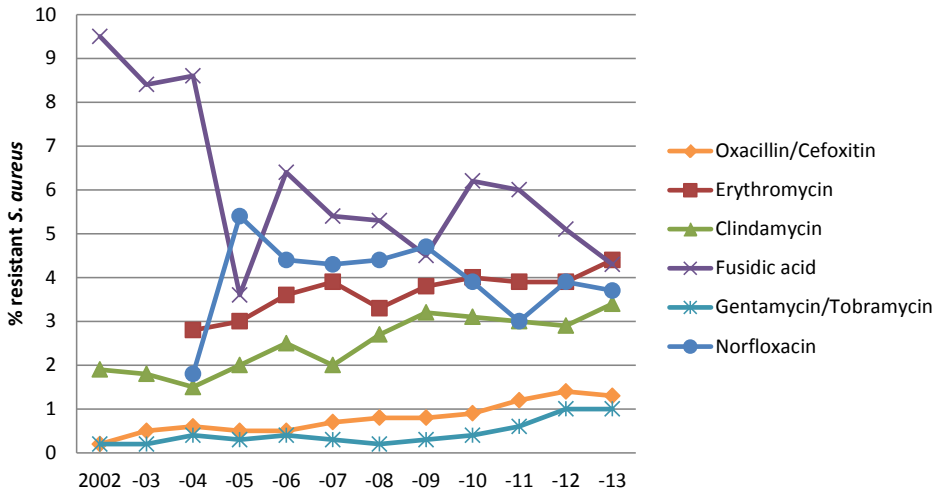

Figure 2. Prevalence of resistance in Swedish S. aureus *isolated from primarily skin and wound infections. Oxacillin/Cefoxitin is used for detection of methicillin resistance. Modified from documents provided by the Public Health Agency of Sweden and published with permission (Public Health Agency of Sweden 2014).*

Penicillin resistance

The discovery of penicillin in 1928 by Alexander Fleming marked the beginning of a new era in medicine. Or as he wrote himself:

"When I woke up just after dawn on September 28, 1928, I certainly didn't plan to revolutionize all medicine by discovering the world's first antibiotic, or bacteria killer. But I guess that was exactly what I did."

When antibiotics were first introduced they were called "wonder drugs" and they could cure previously lethal infectious diseases. In 1942, Anne Miller, who suffered from septicemia, was the first civilian successfully treated with penicillin. The first report of penicillin-resistant *S. aureus*, through the production of penicillinase, was published in 1944 (Kirby 1944). When Alexander Fleming was subsequently awarded the Nobel Prize in Physiology or Medicine in 1945, he warned that the overuse of penicillin might result in bacterial resistance. Six years after the introduction of penicillin approximately 25 % of the *S. aureus* isolates recovered from hospitalized patients were already resistant (Chambers 2001). Another ten years later the prevalence of penicillin-resistant *S. aureus* recovered from blood cultures in Denmark had reached 75 % (Jessen *et al.* 1969). This level of resistance was simultaneously seen in *S. aureus* from other countries around the world (Jeljaszewicz & Hawiger 1966; Ross *et al.* 1974). Following the dramatic increase of penicillin resistance in *S. aureus* among hospital strains the same trend was observed for community strains (Chambers 2001).

Methicillin resistance

A penicillinase-resistant penicillin namely methicillin, which showed a bactericidal effect on penicillin-resistant *S. aureus*, was introduced in 1959. Unfortunately, only two years later, the first observation of methicillin-resistant *S. aureus* (MRSA) was published (Jevons 1961). The first MRSA-epidemic was seen in the late 1960s in several European countries (Keane & Hone 1974; Kayser 1975; Frimodt-Moller *et al.* 1997), with a decline again in the early 70s (Kayser 1975; Frimodt-Moller *et al.* 1997). In the mid-70s outbreaks of MRSA infections were also being reported more frequently from the USA (Boyce & Causey 1982; Haley *et al.* 1982). In the late 80s a new pattern was seen for MRSA; infections emerged primarily among young people with no known risk-factors for MRSA and was subsequently defined as community-associated (CA)-MRSA. The first cases were reported from Australia (Udo *et al.* 1993) but subsequently CA-MRSA spread throughout the world. CA-MRSA has often been reported in groups of people in close contact, e.g., sports team participants (Begier *et al.* 2004), military recruits (Zinderman *et al.* 2004) and correctional facility inmates (Pan *et al.* 2003). Initially, certain genetic and phenotypic

traits were claimed to define and differentiate CA-MRSA from hospital-associated (HA)-MRSA. Most CA-MRSA appear to be more virulent than HA-MRSA and express PVL, which is associated with necrotic pneumonia and necrotic infections of the skin and subcutaneous tissues (Lina *et al.* 1999). Also, CA-MRSA are usually resistant only to β-lactam antibiotics (David & Daum 2010), whereas HA-MRSA are often multidrug-resistant, i.e., resistant to ≥3 classes of antibiotics. As the distinction between HA-MRSA and CA-MRSA based on genetic and phenotypic traits began to blur, and to simplify comparison of different studies the Centers for Disease Control and Prevention Active Bacterial Core Surveillance sites (Minnesota Department of Health 2004) defined CA-MRSA as:

"MRSA that has been isolated from patients who have *no*:

1. history of positive culture for MRSA from any body-site obtained more than 48 h after admission to a hospital (if hospitalized);
2. prior MRSA infection or colonization;
3. hospitalization, surgery, residency in a long-term care facility, hemodialysis, or peritoneal dialysis within the past year or
4. current indwelling percutaneous devices or catheters."

Attempts have been made to reach consensus regarding definitions of MRSA acquisition in the Nordic countries, resulting in six categories; acquisition abroad, hospital-acquired and last community-detected with four different types of associated risk-factors (Skov *et al.* 2008).

The predominant CA-MRSA clone in Europe today is PVL-positive, sequence type (ST) 80 and usually t044 (Stegger *et al.* 2014). A recent study, using whole-genome sequencing, suggests that this European CA-MRSA developed from a sub-Saharan methicillin-susceptible *S. aureus* (MSSA) in the 1980s (Stegger *et al.* 2014). The USA300 clone is widely disseminated throughout the USA and accounted for nearly 80 % of the CA-MRSA in a San Francisco-based study (Liu *et al.* 2008). The USA300 clone is PVL-positive, ST8 and often t008 (David & Daum 2010). Between the years of 2005 and 2011 the estimated number of invasive infections due to CA-MRSA in the USA was stable at almost 20 000 cases per year, whereas HA-MRSA decreased from approximately 90 000 cases in 2005 to 70 000 cases in 2011 (U.S. Department of Health and Human Services & Centers for Disease Control and Prevention 2013).

Resistance to methicillin and all other β-lactam antibiotics in *S. aureus* is primarily mediated by the *mecA*, a gene encoding the penicillin-binding protein 2a. *mecA* is located on the mobile genetic element Staphylococcal Cassette Chromosome *mec* (SCC*mec*) and there are currently 11

types registered. Historically type I-III has been associated with HA-MRSA. These types are large and carry several different antibiotic resistance genes. Types IV and V are smaller, they only carry the *mecA* and have historically been associated with CA-MRSA (Hiramatsu *et al.* 2013). In 2011, a *mecA* homologue, subsequently named *mecC*, was described in the UK and Denmark (Garcia-Alvarez *et al.* 2011). The *mecC* is associated with SCC*mec* XI, the most recently described SCC*mec* type. Also *mecB* has been described, however, this has not yet been identified in staphylococcal species (Hiramatsu *et al.* 2013). It was initially suggested that all MRSA types are descendants of a single ancestral MSSA that acquired *mecA* (Kreiswirth *et al.* 1993). However, recent studies indicate that *mecA* has probably been introduced into several successful lineages of MSSA, as *mecA* is present in several genetically distinct genotypes of MRSA (Fitzgerald *et al.* 2001; Enright *et al.* 2002).

The all inpatient costs are higher and the hospital stay is longer for patients with MRSA bacteremia as compared to MSSA bacteremia (Reed *et al.* 2005). Although difficult to assess, it seems more cost-effective to implement intensive MRSA control programs, i.e., the search and destroy policy, than to defeat an outbreak (Bjorholt & Haglind 2004). A meta-analysis also showed that the mortality rate for MRSA bacteremia is significantly higher than for MSSA bacteremia (Cosgrove *et al.* 2003). In 2011, MRSA was estimated to cause >11 000 deaths in the USA alone (U.S. Department of Health and Human Services & Centers for Disease Control and Prevention 2013). It has also been shown that MRSA infections add to the burden of MSSA infections, rather than substituting MSSA infections, as shown in figure 3 (Health Protection Agency 2005).

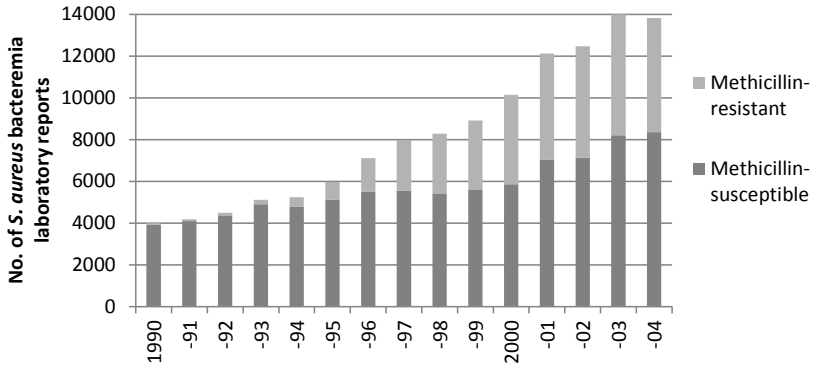

Figure 3. Number of MRSA and MSSA bacteremia laboratory reports from England and Wales 1990 through 2004 (Health Protection Agency 2005).

S. aureus resistant to methicillin or other β-lactam antibiotics is included in the Swedish Communicable Disease Act and have been mandatorily notifiable since the year 2000 (Public Health Agency of Sweden & National Veterinary Institute 2013). Since then the prevalence of MRSA has been consistently below 2 % in Sweden, both among invasive *S. aureus* isolates (European Centre for Disease Prevention and Control 2014), and among *S. aureus* isolates from skin and wound infections (figure 2, page 6). Also, no commensal MRSA was reported from Sweden in the "Appropriateness of Prescribing Antibiotics in Primary Health Care in Europe with Respect to Antibiotic Resistance Study" (APRES), a large European study performed in 2010 and 2011, involving 32 000 patients from nine countries, generating nearly 7 000 *S. aureus* isolates. The prevalence of commensal MRSA in the other eight countries in the APRES study ranged from 0.8 % in the Netherlands to 2.1 % in Belgium (den Heijer *et al.* 2013). In the same time-period, the prevalence of invasive MRSA in some European countries reached more than 50 % (European Centre for Disease Prevention and Control 2014). The low prevalence of MRSA in infections in Sweden, the other Nordic countries and the Netherlands, has been attributed to the restrictive prescribing of antibiotics and the search and destroy policy used in these countries. This policy includes screening of high-risk patients upon admission to hospital and isolation or cohort nursing until the patient is declared negative for MRSA colonization and/or infection. Patients with MRSA are treated in single-bed rooms and screening of patients and healthcare workers (HCWs) who have been in contact with the patient is recommended. Eradication of MRSA carriage and treatment of infections should be offered to patients and HCWs (Åhren & Larsson 2014). In a low-endemic setting, e.g., Sweden, it is more cost-effective to use chromogenic culture media than PCR for MRSA screening (Wassenberg *et al.* 2011). However, a recent meta-analysis showed a higher sensitivity for screening with PCR compared to chromogenic culture media (Luteijn *et al.* 2011). It has also been demonstrated that the turn-around time is substantially lower and that the price is higher for screening with PCR compared to chromogenic culture media (Danial *et al.* 2011).

Individuals colonized with MRSA rarely need systemic antibiotic treatment. Intensified skin and wound treatment and removal of all possible foreign devices, e.g., catheters, will generally be enough, but decolonization using mupirocin ointment and/or soap containing chlorhexidine may be necessary. When treating infections, the selection of antibiotic should always be based on the resistance pattern obtained by the microbiology laboratory and depends on the focus of infection. Minor skin and soft tissue infections will usually heal without antibiotic therapy, whereas more serious infections can be treated with clindamycin or sulfamethoxazole/trimethoprim. Clindamycin can also be used to treat pneumonia and will reduce the PVL production, associated with necrotizing pneumonia caused by CA-MRSA. The

first-line recommendation for treatment of invasive infections and infections of the central nervous system is vancomycin. Quinolones and rifampicin should be used with caution and always in combination with another antibiotic, as bacteria can develop resistance very quickly, even during a course of treatment, to these compounds (Hagberg 2014). β-lactam antibiotics in combination with a β-lactamase inhibitor, e.g., clavulanic acid, can be used to treat infections caused by β-lactam antibiotic-resistant bacteria.

Vancomycin resistance

The primary choice of treatment for invasive infections caused by MRSA is vancomycin. Fortunately, vancomycin intermediate/resistant MRSA (VISA/VRSA) is still uncommon, but sporadic cases have been documented (Sievert *et al.* 2008; Melo-Cristino *et al.* 2013). Between 2002 and 2011, 13 cases of VRSA were reported from the USA, all of which were multidrug-resistant (U.S. Department of Health and Human Services & Centers for Disease Control and Prevention 2013). It is of the utmost importance to monitor and prevent the dissemination of VISA/VRSA, in order to keep vancomycin as a treatment option for MRSA.

Fusidic acid resistance

Fusidic acid is the primary antibiotic used for topical treatment of impetigo, eye infections and infections correlated to a variety of dermatological disorders caused by *S. aureus*. It should be used systemically with caution. Fusidic acid has excellent bone penetration and is therefore used to treat osteomyelitis, but should be used in combination with another antibiotic, due to the high risk of resistance development in bacteria. During the late 90s an increase in fusidic acid-resistant *S. aureus* was observed in the UK (Brown & Thomas 2002). Subsequent studies showed a clonal dissemination throughout Europe of a fusidic acid-resistant *S. aureus* correlated to impetigo primarily in young children (Osterlund *et al.* 2002; Tveten *et al.* 2002; El-Zimaity *et al.* 2004). This epidemic is reflected in the Swedish national statistics on resistance (figure 2, page 6). A dramatic increase in fusidic acid resistance among *S. aureus* was also reported from New Zealand, from 17 % in 1999 to 29 % in 2013. This was also a clonal dissemination, although with a different clone than in Europe. In parallel to this clonal dissemination, a significant increase in dispensing rates for topical fusidic acid was seen in New Zealand (Williamson *et al.* 2014). The correlation between previous topical use of fusidic acid and resistance in *S. aureus* has previously been shown in dermatology patients (Heng *et al.* 2013). Although fusidic acid is not in clinical use in the USA, resistance has been reported (Jones *et al.* 2011). In the APRES study, fusidic acid resistance was detected in 2.8 % (range: 0.1 % to 7.8 %) of the European *S. aureus* isolates (den Heijer *et al.* 2014).

Clindamycin resistance

Inducible clindamycin resistance in erythromycin-resistant *S. aureus* is detected by the double disk diffusion test (D test) where the clindamycin and erythromycin discs are placed 12 mm to 20 mm apart (edge to edge) in the bacterial inoculum on the agar plate. If the circular clindamycin inhibition zone is blunted, and thereby resembles a D, on the side facing the erythromycin disc the isolate exhibits inducible clindamycin resistance (The European Committee on Antimicrobial Susceptibility Testing 2014, Version 4.0). An *S. aureus* displaying inducible clindamycin resistance, as detected by the D test is shown in figure 4.

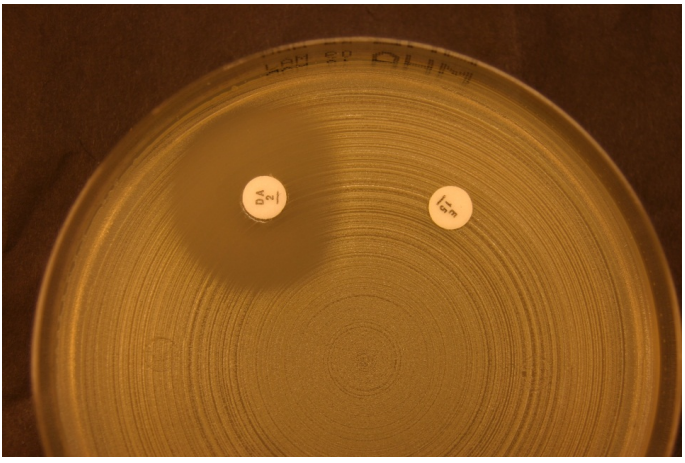

Figure 4. Inducible clindamycin resistance as revealed by the D test. DA=clindamycin, E=erythromycin.

Carriage and clinical aspects of *S. aureus*

Carriage and colonization

The bacterial flora of the skin is divided into resident and transient flora. The resident flora is regarded as the "true" skin flora as it is more or less permanent and is seldom shed to the environment. It is therefore not considered to contribute significantly to cross-infections (exogenous infections). The resident flora is not affected by repeatedly scrubbing or extensive exposure to disinfectants. It contributes to colonization resistance, meaning that it is more difficult for other potentially more pathogenic bacteria to colonize the skin when they have to compete for a colonization site with an already established flora. The transient flora is readily acquired from and shed to the environment, including other people. It is carried superficially on the skin and is the main target during hand hygiene procedures as it is considered to contribute greatly to cross-transmission and infections (Price 1938; Gould 2012).

S. aureus is a commensal of the human flora, colonizing the skin and mucosal surfaces and the anterior nares is considered the premier colonization and carriage site. A thorough review of 18 studies from 1944 until 1994 showed that the mean *S. aureus* carriage rate in the general population was 37 % (Kluytmans *et al.* 1997). More recent studies have shown nasal colonization rates of 20 % to 30 % (Andersen *et al.* 2012; Gamblin *et al.* 2013; Mernelius *et al.* 2013b; Olsen *et al.* 2013; Mehraj *et al.* 2014). Nasal colonization rates of up to nearly 60 % have been reported among HIV-positive patients (Kotpal *et al.* 2014), patients on hemodialysis (Duran *et al.* 2006), intravenous drug addicts and patients with insulin dependent diabetes (Kluytmans *et al.* 1997). Several studies have shown that carriage and colonization in the throat is more common than colonization of the anterior nares (Nilsson & Ripa 2006; Hamdan-Partida *et al.* 2010; Mernelius *et al.* 2013b). It has also been suggested that transmission of *S. aureus* may occur from the throat although transmission from the anterior nares is more common (Mernelius *et al.* 2013b). Exclusive throat carriage, i.e., colonization of the throat only, does exist (Mertz *et al.* 2007; Mertz *et al.* 2009) and age ≤30 years has been determined a risk factor and exposure to the healthcare system a protective factor for exclusive throat carriage (Mertz *et al.* 2009). By including screening cultures from the throat the sensitivity of detecting colonization significantly increases and it has therefore been suggested that *S. aureus* screening programs should include sampling of the anterior nares as well as the throat (Mertz *et al.* 2007). Other anatomical sites, e.g., the axilla, groin, skin and the intestinal tract, are also colonized with *S. aureus* (Acton *et al.* 2009; Vento *et al.* 2013)

S. aureus nasal carriers are usually divided into three distinct groups (Gould & McKillop 1954; Kluytmans *et al.* 1997).

1. Persistent carriers are almost always colonized with *S. aureus* of a single genetic type.
2. Intermittent carriers are characterized by an on-and-off colonization with *S. aureus* of different types.
3. The last group is composed of the non-carriers, who never carry *S. aureus*.

The prevalence of the three different carriage patterns varies greatly between different studies (Eriksen *et al.* 1995; VandenBergh *et al.* 1999; Muthukrishnan *et al.* 2013). This variation is probably due to variation in study populations, sample collection, culture methods and how the different carriage patterns are defined. A carrier index (number of samples positive for *S. aureus* in one individual/number of samples collected from that individual) of ≥0.8 has been suggested to define persistent carriage. A carrier index of 1.0 is rarely used, in order to avoid misclassification if there is one negative culture caused by low levels of bacteria (van Belkum *et al.* 2009). It does, however, seems that the carrier index should be 1.0 for true persistent carriage if the follow-up period is short or if the number of consecutive samples collected is low (VandenBergh *et al.* 1999). Although included in early definitions, the requirement of identical genotypes of the isolates is rarely included in more recent studies (van Belkum *et al.* 2009). In a recent study, individuals of all three carrier patterns were decolonized and subsequently recolonized with a cocktail of different *S. aureus* strains. This study showed higher serum levels of antistaphylococcal antibodies, longer *S. aureus* nasal survival and more CFU per swab sample in persistent carriers compared to both intermittent and non-carriers. There were no differences regarding these parameters between intermittent and non-carriers (van Belkum *et al.* 2009). It has been demonstrated that persistent carriers suffer a greater risk of *S. aureus* infection than intermittent carriers (Nouwen *et al.* 2005). These data combined suggest that there are actually only two carrier states; persistent carriers and other. If the carriers termed other are in fact intermittent carriers incidentally characterized as non-carriers, or non-carriers who are temporarily contaminated with *S. aureus* is still unclear (van Belkum *et al.* 2009).

Colonization with *S. aureus* is more prevalent in males than females (Mernelius *et al.* 2013b; Mehraj *et al.* 2014). There are conflicting results regarding this gender-associated difference among infants (Lebon *et al.* 2008; Mernelius *et al.* 2013b).

The primary colonization site in newborn infants is the umbilicus (Cursino *et al.* 2012; Mernelius *et al.* 2013b) and it has been shown that infant colonization begins directly after birth and reaches adult proportions after 24 h (Mernelius *et al.* 2013b). *S. aureus* nasal carriage decreases in the first year of life (Peacock *et al.* 2003; Lebon *et al.* 2008), but increases again around the age of three, peaking at approximately 50 % in the pre-teen years (Bogaert *et al.* 2004; Datta *et al.* 2008). In children the prevalence of *S. aureus* appears inversely related to the prevalence of *Streptococcus pneumonia* (Bogaert *et al.* 2004). Whereas persistent carriage appears almost non-existing in infants (Lebon *et al.* 2008) it has been shown that persistent carriage is more prevalent among older children and adolescents than adults (Armstrong-Esther & Smith 1976).

It has been demonstrated that more infants were colonized with *S. aureus* transmitted from HCWs rather than from their own parents (Matussek *et al.* 2007). A more recent study shows that this relationship has changed and most infants are colonized with the same *S. aureus* as their parents (Mernelius *et al.* 2013a). This relationship is probably dependent on the level of compliance with hygiene guidelines. An increased risk of colonization has been demonstrated for infants with colonized parents as compared to non-colonized parents (Mernelius *et al.* 2013b). This has also been shown for infants with colonized mothers (Peacock *et al.* 2003). Other factors that determine infant *S. aureus* carriage have been extensively studied. Breastfeeding (Peacock *et al.* 2003), number of older siblings (Peacock *et al.* 2003; Chatzakis *et al.* 2011) and maternal smoking (Chatzakis *et al.* 2011) have all been demonstrated to increase the odds for infant carriage. However, these factors were not associated with infant carriage in the study by Lebon *et al.* (2008).

Infection

S. aureus acts as a two-edged sword, as it is by far the most pathogenic for humans of all the staphylococcal species. *S. aureus* is widely associated with skin- and soft tissue infections and it is also the causative microorganism of several severe conditions, e.g., endocarditis, osteomyelitis, and bacteremia. It was shown as early as the 1950s that *S. aureus* nasal carriers have an increased risk of developing postoperative wound infections, as compared to non-carriers (Weinstein 1959; Williams *et al.* 1959). This has more recently been verified in patients undergoing orthopedic (Skramm *et al.* 2014) and cardiac surgery (Kluytmans *et al.* 1995). Also *S. aureus* nasal carriers have a greater risk of developing bacteremia (Wertheim *et al.* 2004a). It has also been demonstrated that the mortality rate attributed to *S. aureus* bacteremia is lower in carriers compared to non-carriers (Wertheim *et al.* 2004a). Studies have shown that >80 % of patients with *S. aureus* bacteremia were previously colonized in the anterior nares with an identical genotype, strongly indicating endogenous infection (von Eiff *et al.* 2001; Wertheim *et al.* 2004a). Studies using microarray have failed to identify genes specific to invasive or carriage isolates (Lindsay *et al.* 2006; Stark *et al.* 2009). These studies further emphasize the role of colonizing *S. aureus* in the development of invasive disease. They also suggest a genetic predisposition to whether or not the carrier strain will cause invasive disease. Given the correlation between *S. aureus* nasal carriage and increased risk of endogenous infections, eradication of nasal carriage has been suggested as a strategy to decrease staphylococcal infection rates. Decolonization, using intranasal treatment with mupirocin ointment, has proven highly efficient in reducing *S. aureus* carriage (Perl *et al.* 2002). Pre-operative eradication of nasal carriage significantly reduced the rates of surgical-site infections in cardiothoracic patients as compared to a historical control group (Kluytmans *et al.* 1996b). These results could not be replicated in a large, double-blind, randomized, placebo-controlled study on surgical patients (Perl *et al.* 2002). A more recent study showed a decreased risk for hospital-acquired *S. aureus* infection among patients who underwent nasal and skin decolonization prior to surgery (Bode *et al.* 2010). Also in non-surgical patients contradictory results regarding the efficacy of nasal decolonization on nosocomial *S. aureus* infections (Wertheim *et al.* 2004b) and *S. aureus* bacteremia (Kluytmans *et al.* 1996a) have been published.

Newborn infants with heavy *S. aureus* colonization of the umbilicus have an increased risk of subsequent *S. aureus* infection compared to non-colonized infants (Stark & Harrisson 1992). Newborn infants colonized with MRSA also have a significantly higher risk of developing an MRSA infection than non-colonized infants (Huang *et al.* 2006b). A recently published meta-analysis determined the risk of developing an MRSA infection to be 24 times higher for infant

MRSA carriers than non-carriers (Zervou *et al.* 2014). Endogenous MRSA infections have also been demonstrated for newborn infants, with indistinguishable isolates collected from the site of infection and colonization (Huang *et al.* 2006b). The incidence of umbilical infections in developing countries can reach >20 % and is associated with sepsis (Mir *et al.* 2011). Topical application of chlorhexidine to the umbilical cord of neonates has proven effective in reducing infection and mortality in developing countries (Soofi *et al.* 2012). Umbilical infections are, however, rare in developed countries, and there is no evidence that application of antiseptics is needed in these countries (Imdad *et al.* 2013).

S. aureus is also of major concern in the healthcare environment, as one of the main causes of nosocomial infections. *S. aureus* frequently causes nosocomial surgical site infections, pneumonia and sepsis (Kampf *et al.* 2009), increasing the mortality and morbidity of the patients as well as the costs for the hospital.

Typing

Pathogenic bacteria thrive in different reservoirs, e.g., humans, animals, water and food. Dissemination of bacteria from any of these reservoirs can produce clusters of colonization or infection, also termed outbreaks. In healthcare settings, typing is primarily used for two purposes; in surveillance of infectious diseases and in outbreak investigations. Surveillance cultures and typing of organisms of extra importance in infection control can detect clusters of these organisms and thereby function as an early warning system for detection and prevention of potential outbreaks. In case of an increased incidence of infections or colonization with a certain bacterial species, typing is used to determine if the isolates are of one type, i.e., an outbreak, or of several different types, indicating an accumulation of sporadic cases. In case of an outbreak, typing is used to determine the extent of the outbreak, detect the source and clarify transmission routes (van Belkum *et al.* 2007).

To evaluate and validate typing methods six performance criteria and six convenience criteria have previously been set up (van Belkum *et al.* 2007). The following are the performance criteria.

1. The marker assessed should be *stable* over time, it must not vary to such a degree that it confuses the epidemiological picture.
2. All isolates should be *typeable* by the method.
3. The ability to *discriminate* between different isolates is assessed by the discriminatory power of the method and it is considered ideal if it is >0.95. The discriminatory power of a method refers to the probability that two unrelated isolates picked at random from a certain population will be assigned to different types.
4. The discrimination must also be *concordant* with the epidemiological data.
5. Independent of the laboratory technician, time and place, the results should be *reproducible*.
6. Finally, to adequately assess the potential of a typing method a well-defined and appropriate *test population* must be used.

The following convenience criteria should also be considered.

1. The *flexibility*, i.e., the range of bacterial species that can be studied with minimal modifications of the typing method, is recommended to be high.
2. *Rapid* typing results are desirable; it is preferential if results are obtained within a working day.
3. Reagents, equipment, knowledge and skill should be easily *accessible.*

4. The method should also be *easy to use.*
5. The method should be *cheap* to initiate, maintain and perform.
6. The last convenience criterion, the possibility for *computerized analysis and use of electronic databases*, is of the utmost importance in longitudinal comparisons or studies involving a large number of isolates.

Outbreaks are often located in a single hospital or long-term care facility or to people living in close proximity; therefore, typing related to outbreak investigations can be performed at the local microbiology laboratory. For regional or national surveillance, typing can be undertaken at the reference laboratory. To monitor and define globally disseminated bacterial clones international collaborations are needed. Different typing methods, with different levels of discriminatory power, are required for each level of investigation (van Belkum *et al.* 2007). Many researchers have made great efforts to find the best typing method, but ultimately there is no such method. There are only methods that are better or worse at answering certain questions.

The importance of being able to communicate typing results between laboratories has been discussed previously. In the spirit of this there is also an ongoing debate on the vocabulary used in the world of bacterial typing. The main discussion involves the terms *isolate*, *strain*, *type* and *clone* and van Belkum *et al.* (2007) defined the terms as follows. An isolate is "a population of bacterial cells in pure culture derived from a single colony". A strain is "an isolate or group of isolates that can be distinguished from other isolates of the same genus and species by phenotypic or genotypic characteristics." However, no definite set or number of characteristics has been established to define a strain. By these definitions two isolates can represent the same strain, but two strains cannot be the same isolate. The word 'type' should only be used when the isolate has been characterized by an existing typing scheme, e.g. *spa* type. Finally the term 'clone' is defined as "bacterial isolates that, although they may have been cultured independently from different sources in different locations and perhaps at different times, still have so many identical phenotypic and genotypic traits that the most likely explanation for this identity is a common origin". The term 'clone' is commonly used to name widespread multidrug-resistant and/or highly virulent bacterial strains (David & Daum 2010).

One of the main tasks for clinical microbiology laboratories is to perform antibiotic susceptibility testing on isolated pathogenic bacteria. The antibiotic susceptibility pattern can often give a first indication of a possible outbreak, and can thereby be considered the first line phenotypic typing method. It has been proven that routine molecular typing is important in detecting outbreaks, transmission routes and the source of the outbreak (Mellmann *et al.* 2006; Boers *et al.* 2011).

Phage typing

Historically, phage typing has been used for epidemiological typing of *S. aureus*. This is a phenotypic typing method which characterizes *S. aureus* into more than 20 different phage types. *S. aureus* is inoculated on the agar of a gridded petri dish, with different phages inoculated in each of the squares in the grid. If one of the phages is specific for the tested *S. aureus* the phage will reproduce within the bacteria, resulting in no bacterial growth and a clear zone in that square, i.e., a plaque is formed. Each phage has a unique name (consisting of a number, sometimes in combination with a letter), and the phage in the grid where a plaque forms determines the specific phage type of that *S. aureus* (Batzing 2002). As a vast number of different phages had to be kept in stock at each laboratory performing phage typing this typing method was restricted to reference laboratories. It was thereby impossible to obtain rapid typing results to aid in epidemiological investigations. Another problem with phage typing was the large number of untypeable isolates. Due to these inconveniences in combination with the recent development in genetic analysis phage typing is no longer in clinical use.

Pulsed-field gel electrophoresis

In conventional gel electrophoresis the electrophoretic conditions will separate DNA fragments based on size. This is accomplished by a sieving effect, where smaller fragments more easily travel through the gel matrix than larger ones, which results in a pattern based on the size of the fragments. However, fragments larger than 20 000 base-pairs (bp) travel equally fast through the gel matrix, resulting in no resolution of the fragments. Pulsed-field gel electrophoresis (PFGE) of macrorestricted DNA was developed to enable studies of entire yeast and bacterial genomes. To exclude non-specific restriction of DNA, bacterial cells are first welded into an agarose plug. The embedded cells are subsequently lysed and cell debris, proteinases and nucleases are enzymatically removed and washed away. By the use of a restriction enzyme that cleaves DNA infrequently (for *S. aureus* usually *SmaI* (Mulvey et al. 2001; Murchan et al. 2003)) DNA fragments ranging in size from tens to hundreds of kbps are produced. By applying an alternating electric field to the agarose gel containing the DNA, the fragments are forced to change both their conformation and orientation, resulting in a size-dependent separation of the fragments (Peters 2009). The procedure of the PFGE method is outlined in figure 5.

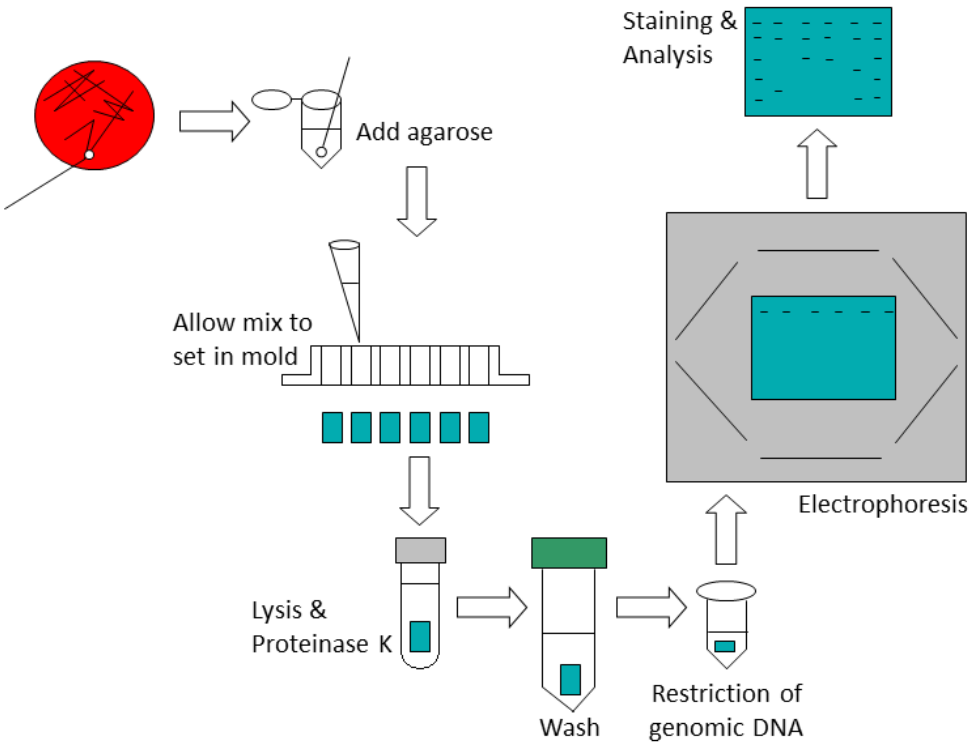

Figure 5. Schematic illustration of the PFGE method.

The Tenover criteria have been, and still are, widely used for interpretation of restriction fragment patterns (Tenover *et al.* 1995). Briefly, isolates with the exact same patterns are defined as indistinguishable, those that differ by 2-3 fragments are defined as closely related, by 4-6 fragments as possibly related and by ≥7 fragments as different. Interpretation of restriction fragment patterns by the naked eye is subjective and impractical when working with large datasets; therefore software for analysis of restriction fragment patterns is now frequently used. This has improved the objectivity of the analysis but PFGE is still afflicted by several other problems. Results obtained by different protocols are difficult to compare therefore attempts to harmonize protocols have been made (Mulvey *et al.* 2001; Murchan *et al.* 2003). The lack of a consensus protocol, as well as the nature of the data, makes transferring of results between laboratories difficult. Also, PFGE is technically demanding. The cost per sample is low (Vainio *et al.* 2011), but the number of samples that can be run simultaneously is restricted. The method is highly discriminatory, with an index of diversity often above 0.95 (Cookson *et al.* 2007). Moreover, it has excellent typeability (Hallin *et al.* 2007) as well as epidemiological concordance (Strommenger *et al.* 2008).

PFGE has long been considered gold standard for typing of most bacterial species, including *S. aureus* (Murchan et al. 2003). Due to the problems with gel-based methods discussed above and development in the field of sequencing in recent years, this has now changed and in studies on epidemiological surveillance and outbreak investigations (Melin *et al.* 2009) as well as in national reference laboratories (Vainio *et al.* 2011) sequence-based methods are used to determine the clonal relationship between *S. aureus* isolates.

Single locus sequence typing

Gel-based typing methods are afflicted by several problems that are overcome with sequence-based methods. These methods could be based either on sequencing a single locus or multiple loci. Several different typing schemes based on sequencing part of a single *S. aureus* gene has been established, e.g., *spa* and *clfB* typing. In *clfB* typing the R-domain of the gene is sequenced. The R-domain consists of a variable number of 18 bp long repeats (Kuhn *et al.* 2007). However, *spa* typing is the most well-established and used method today.

spa *typing*

S. aureus protein A is a surface protein encoded by the spa gene (figure 6). The gene consists of a cell wall attachment sequence (X_c), the variable number tandem repeat region (X_r), the IgG-binding regions (A-D), a region homologous to A-D (E) and a signal sequence segment (S) (Uhlén *et al.* 1984). *spa* typing is based on amplification and sequencing of the X_r region, which consists of a variable number of 24 bp repeats (rare cases of 21 bp and 27 bp repeats have also been documented) where each repeat has a unique sequence. Different *spa* types arise from point mutations in the repeats, as well as from deletion and duplication of the repeats. Therefore, the sequence of each *spa* type is unique and there are *spa* types of variable length. *spa* typing was initially based on amplification of the X_r region and subsequent gel-based analysis of the length of the amplicon (Frenay *et al.* 1994). This method obviously gave no information regarding the sequence, and two isolates could be classified as indistinguishable based on being equally long, whereas they in fact had repeats differing in sequence. When sequencing became more readily available, the method of *spa* typing was modified (Frenay *et al.* 1996) and nowadays it is based on sequencing of the polymorphic X_r region. The highly conserved regions flanking the X_r region enables annealing of the primers necessary for amplification and sequencing.

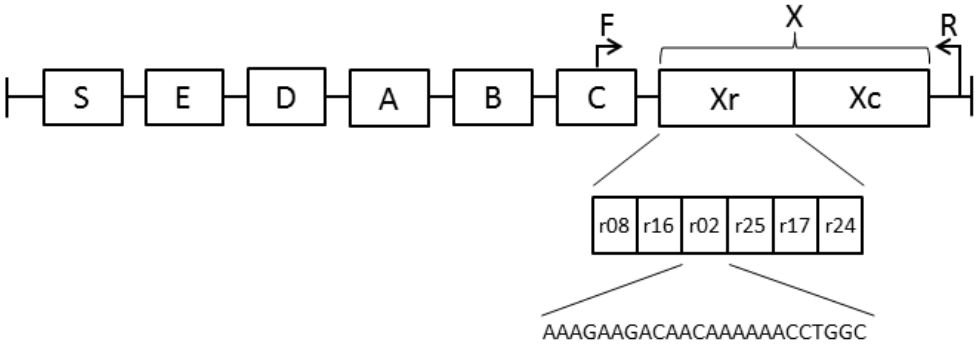

Figure 6. Schematic illustration of the spa gene. The repeat succession outlined represents spa *type t138. F and R represent the sites where the forward and reverse primers bind, respectively.*

Analysis of sequence data and attribution of *spa* types to isolates is easily performed using the Ridom StaphType software. The software is associated with a freely available web-based database (Ridom GmbH 2014) which allows researchers from all over the world to submit and obtain repeat sequences and the repeat succession of *spa* types. Therefore, both researchers who do and those who do not have access to the software have access to the standardized terminology of *spa* typing (Harmsen *et al.* 2003). On March 23, 2015 there were 14 777 *spa* types and 665 repeats registered in the database. At that time, the most prevalent *spa* type in the database was t032 (10.44 %), in many cases classified as the epidemic MRSA-15.

The Ridom StaphType software assigns names to the repeats and types according to the Ridom nomenclature, where each repeat is assigned a numeric code, e.g., r01 and r02 and the order of the repeats is combined into a *spa* type, e.g., t001 and t084 (Ridom GmbH 2014). There is also an alternative nomenclature, the Kreiswirth nomenclature, where the repeats are assigned a letter- and numeric code, e.g., A1 and D2 and the order of the repeats is combined into a *spa* type, e.g., 1 and 4 (Koreen *et al.* 2004). This nomenclature is not as widespread and has not had the same impact around the world as the Ridom nomenclature. The standardized nomenclature used in *spa* typing is one of its major advantages especially over band-based methods, e.g., PFGE, which makes comparison and exchange of data between laboratories easy. Additionally, *spa* typing results are acquired relatively quickly. One study showed that it took just under 10 h to perform *spa* typing on 24 isolates, whereas typing 12 isolates by PFGE took 40 h (Vainio *et al.* 2011).

spa typing shows great typeability (Koreen *et al.* 2004; Strommenger *et al.* 2008), epidemiological concordance (Melin *et al.* 2009) and reproducibility (Shopsin *et al.* 1999; Strommenger *et al.* 2008) as well as high discriminatory power (Cookson *et al.* 2007; Hallin *et al.* 2007; Babouee *et al.* 2011). The X_r region of *spa* is also stable over time, both *in vivo* and *in vitro* (Frenay *et al.* 1996).

The Based Upon Repeat Pattern (BURP) algorithm was developed to enable long-term epidemiological studies using *spa* typing. BURP combines *spa* types with similar repeat patterns into clonal complexes (*spa* CCs). The similarity of the *spa* types is based on the parsimony assumption, i.e., the hypothesis with the fewest assumptions is most likely to be the correct one. In one study the algorithm gave a similar evolutionary signal as multilocus sequence typing (MLST) and microarray data, indicating its potential as a method for longitudinal studies (Mellmann *et al.* 2007).

spa typing has been extensively studied for its appropriateness as a typing tool in epidemiological investigations. Some recommend the method for local outbreak investigations (Strommenger *et al.* 2008) and for detection of *S. aureus* transmission (Matussek *et al.* 2007). Others have shown that *spa* typing alone is not discriminatory enough to demonstrate the endemic establishment of MRSA (Fossum Moen *et al.* 2014). There are also indications that *spa* typing must occasionally be combined with the detection of other genetic markers, e.g., SCC*mec* or resistance or virulence genes (Hallin *et al.* 2007; Fossum Moen *et al.* 2014). There is also evidence that the discriminatory power increases when combining typing of two loci, e.g., *spa* and *clfB* (Kuhn *et al.* 2007).

Multilocus sequence typing

In national, global and long-term epidemiological studies typing should be based on a genetic marker which slowly accumulates genetic variation. For many bacterial species the most widely used typing method to do this is MLST, based on sequencing of several highly conserved genes, i.e., housekeeping genes. The *S. aureus* MLST scheme is based on sequencing the internal fragments, approximately 500 bps in length, of each of the following seven housekeeping genes: carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glp*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqiL*). Through a web-based server the sequences of each of the seven fragments are assigned a distinct allele number. The combination of these seven allele numbers constitutes the allelic profile, or the ST, of the isolate (Enright *et al.* 2000; Larsen *et al.* 2012). For a schematic illustration of the MLST analysis see figure 7. Due to the vast number of different alleles at each locus, it is highly unlikely that two unrelated isolates would have the same ST by chance; therefore, isolates with the same ST can safely be defined as of identical or highly similar genotypes. The discriminatory power of MLST is generally slightly lower than for PFGE and *spa* typing and therefore the method is highly useable for long-term studies and for the determination of global dissemination of certain genetic linages of *S. aureus* (Cookson *et al.* 2007). As MLST is a sequence-based method it has the advantage of producing unambiguous results, which are easy to incorporate into electronic databases and transfer between laboratories. As the method requires sequencing of seven genes it is expensive and time-consuming, especially compared to single locus sequence typing where only one locus per isolate needs to be sequenced.

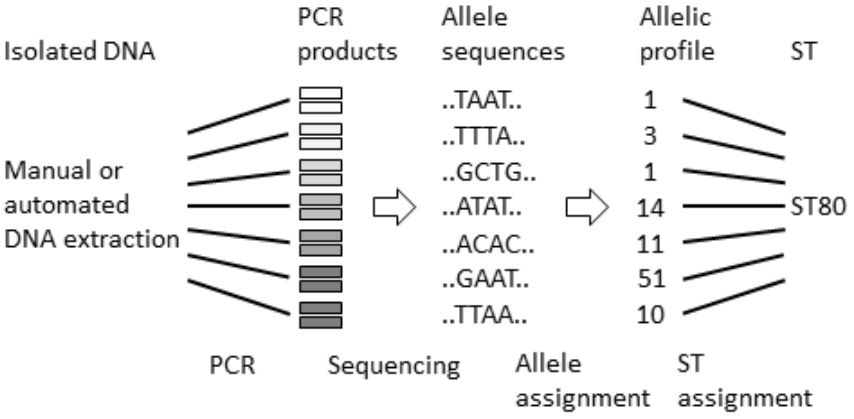

Figure 7. Schematic illustration of the MLST analysis. Modified from: http://beta.mlst.net/Instructions/default.html.

The application Based Upon Related Sequence Types (eBURST) was developed to describe the relationship of closely related isolates based on MLST data. The method initially forms groups of STs that share identical alleles in at least six of seven loci with at least one other ST in the group. The primary founder of the group is the ST differing from the largest number of other STs in only a single locus. Using these settings all isolates of a group are part of the same CC and the isolates are considered to have recently diverged genetically from each other. STs that have different alleles in more than two loci compared to any other ST cannot be assigned to a group and are called singletons (Feil *et al.* 2004).

The ST obtained by MLST in combination with the SCC*mec* type is currently used to name MRSA, e.g., the epidemic clone EMRSA-16 is known as ST36-MRSA-II, an MRSA of ST36 carrying SCC*mec* type II (Deurenberg *et al.* 2007).

Next generation sequencing

Next generation sequencing (NGS) or second generation sequencing differs from the original sequencing approach using Sanger sequencing in its ability to produce millions of reads (35-700 bps in length) in a single run to a reasonably low cost. To generate the complete sequence of a genome the short sequence reads are assembled by overlapping sequences (*de novo* assembly) or compared to previously sequenced reference genomes (re-sequencing). With its full coverage of the genome it has been deemed the end-point in typing, with the possibility of absolute resolution between isolates. The method produces large amounts of data and requires heavy computer resources and the requests for well-trained bioinformaticians will increase (Sabat *et al.* 2013).

Although the cost associated with NGS is still high it continues to decline (Harris *et al.* 2013). For NGS to be implemented in routine outbreak investigations and surveillance typing continuous efforts have to be made to shorten the time required for sequencing and analysis (Eyre *et al.* 2012; Harris *et al.* 2013). In addition to this, analysis tools and standard procedures to determine the variation between genomes have to be established. There are different approaches used today. The extended MLST produces an allelic profile of the entire core genome, based on hundreds to thousands of alleles, compared to seven for traditional MLST. The pan-genome approach compares the presence or absence of genes of the entire genome between different isolates. The number of single-nucleotide polymorphisms that differs between isolates can also be used. Despite the drawbacks of high costs, prolonged sequencing and analysis time and lack of standard analysis procedures, NGS has recently become widely available to reference and clinical laboratories. Several studies have demonstrated that NGS is highly useful in investigations of MRSA outbreaks (Eyre *et al.* 2012; Harris *et al.* 2013). NGS has also been used to demonstrate that an HCW, initially unknown to be colonized with MRSA, was the potential source of an MRSA outbreak (Harris *et al.* 2013). The importance of interpreting whole-genome data in combination with epidemiological information has been emphasized (Eyre *et al.* 2012; Diep 2013). Colonizing and infecting *S. aureus* isolates from a single individual, indistinguishable by *spa* typing and PFGE, have been demonstrated to differ by whole-genome sequencing (Schijffelen *et al.* 2013). It has also been suggested that the genetic information revealed by NGS will provide insights into the genetic basis of transmissibility and virulence (Diep 2013).

A summary of the advantages, disadvantages and applications of the typing methods for *S. aureus* described above is given in table 1.

Table 1. Advantages, disadvantages and applications of described typing methods for S. aureus.

Method	Advantages	Disadvantages	Applications	References
Phage typing		Many untypeable isolates Only available at reference laboratories Few phenotypes	Not in clinical use today	
PFGE	Excellent typeability Excellent discriminatory power High epidemiological concordance Inexpensive Examine large portion of genome	Technically demanding Labor-intensive Time-consuming Difficult to standardize Partly subjective analysis Limited portability of data	Outbreak investigations National and international surveillance	(Tenover *et al.* 1995; Murchan *et al.* 2003; Cookson *et al.* 2007; Hallin *et al.* 2007; Strommenger *et al.* 2008; Vainio *et al.* 2011)
spa typing	Produces unambiguous data Excellent typeability High discriminatory power High epidemiological concordance Excellent reproducibility Standardized nomenclature High throughput Full portability of data	Less discriminatory than PFGE Characterizes part of a single gene	Outbreak investigations National and international surveillance	(Harmsen *et al.* 2003; Cookson *et al.* 2007; Strommenger *et al.* 2008; Melin *et al.* 2009; Vainio *et al.* 2011)
MLST	Produces unambiguous data Full portability of data	Expensive Labor-intensive	Long-term surveillance MRSA clone designation	(Cookson *et al.* 2007; Deurenberg *et al.* 2007; Sabat *et al.* 2013)
NGS	Ultimate discriminatory power Genome-wide single-nucleotide resolution	Expensive Time-consuming Requires heavy computer resources and well-trained bioinformaticians Lack standard procedures for analysis	Probably the future method of choice for local, national and international surveillance, outbreak investigations and characterization	(Sabat *et al.* 2013)

Infection control

In the 1840s Ignaz Semmelweis laid the foundation for modern infection control when he was working at the General Hospital of Vienna. He observed that the maternal mortality was noticeably higher in one of the two obstetrics clinics. The clinic with the higher mortality rate was staffed by medical doctors, who carried out both deliveries and autopsies during the same shift, without any routines for hand-washing between the two tasks. The other clinic was staffed by midwives, who did not perform autopsies. Semmelweis therefore required the doctors to clean their hands with a chlorinated lime solution when entering the labor room. Following the intervention, the maternal mortality rate in the clinic staffed by doctors decreased to the same level as in the clinic staffed by midwives.

The impact of hand hygiene on transmission of *S. aureus* to newborn infants was studied in 1962. An index infant, colonized with a phage-typeable *S. aureus*, was placed in a bassinet at one end of the study room. On either side (A and B) of the room three infants were placed in individual bassinettes. All contact with the A and B babies was performed after contact with the index baby. Whereas the nurses caring for the A babies always washed their hands after touching the index baby and between touching the A babies, the nurses caring for the B babies did not perform any hand-washing procedure after touching the index baby or between touching the B babies. This study showed a significantly higher rate of acquisition of the index strain among the B babies, who had been attended to by nurses with unwashed hands, as compared to the A babies, attended to by nurses with washed hands. Also, it required an exposure time to the index strain that was nearly four times as long before the infants in the group handled with washed hands acquired the index strain as compared to those handled with unwashed hands. The study also demonstrated that transmission of *S. aureus* via the hands of HCWs is of greater importance than transmission via the air (Mortimer *et al.* 1962).

There are several different approaches to identify bacterial colonization and minimizing transmission and nosocomial infections. Detection of bacterial colonization of patients admitted to hospitals can be done in two ways. The first is by the use of active screening cultures (ASC), with cultures taken upon admission to the hospital and thereafter on a regular basis throughout hospitalization. ASCs can be applied to all patients, i.e., universal screening, or selectively, i.e., to patients with certain risk factors, e.g., skin lesions, catheters or diarrhea. The other way to detect colonization is through clinical microbiological cultures, performed on clinical indications. Comparing ASCs with clinical microbiological cultures revealed that solely relying on the latter resulted in a great underestimation of the number of patients colonized with MRSA (Salgado &

Farr 2006). To avoid transmission and nosocomial infections decolonization can be used, either universally targeting all patients in a hospital or ward, or selectively targeting only the patients colonized or infected with the microorganism of interest.

The strategy of choice must be based on the prevalence of the microorganism of interest in that specific geographic area. For instance in areas with a low prevalence of MRSA (5 %) use of ASCs in combination with selective decolonization appears to be the most cost-effective strategy to prevent MRSA transmission and infection whereas for areas with higher prevalence (12 % or 20 %) universal decolonization seems to be more cost-effective (Gidengil *et al.* 2015). However, it is also important to take into account the potential of resistance development when implementing strategies involving decolonization.

The search and destroy policy, based on ASCs, isolation and decolonization, has been adopted by the Nordic countries and the Netherlands and has helped to keep the prevalence of MRSA low. The decolonization strategy for MRSA usually involves application of mupirocin ointment intranasally twice a day for five days, and/or daily scrubbing with a chlorhexidine gluconate-containing soap (Åhren & Larsson 2014).

Nosocomial infections

Nosocomial infections are defined as infections acquired during hospital care or as a result of diagnostic procedures, treatment or nursing in any part of the healthcare system. Infections in HCWs caused by their work are also considered nosocomial (Swedish Association of Local Authorities and Regions 2015). An infection acquired more than 72 h after admission to a hospital is usually classed as nosocomial. Although nosocomial infections have become almost synonymous with hospital-acquired (or associated) infections, they can also arise in non-hospital environments, such as long-term care facilities.

Nosocomial infections obviously cause a tremendous amount of suffering among patients, but are in Sweden also estimated to cause 750 000 extra patient days annually and cost approximately 6.5 million SEK. Point prevalence measurements of nosocomial infections have been performed biannually in Sweden since 2008, showing a stable prevalence of approximately 9 % (Swedish Association of Local Authorities and Regions 2015), which is in line with reports from other European countries (Chalmers & Straub 2006). It has been shown that improved compliance with hygiene guidelines can reduce the frequency of nosocomial infections by up to 40 % (Pittet *et al.* 2000).

Nosocomial infections originate either from the commensal flora of the patient itself, through endogen transmission, or through cross-transmission, where the infectious agent originates from someone other than the patient him/herself. There are no clinically significant differences between endogenous infections and cross-infections, but there are differences in the epidemiology and how to prevent the two types of infections.

Bacterial transmission

Transmission of microorganisms from person to person is mediated through contact transmission, droplet transmission or airborne transmission. In direct contact transmission microorganisms are transmitted from a colonized or infected person to a susceptible host through body-surface to body-surface contact. In indirect contact transmission microorganisms are transmitted to the host via a contaminated object, e.g., a needle, dressing or the hands of an HCW. Within healthcare settings contact transmission via the hands of HCWs is considered the main transmission route (Weinstein 1991). Far from all transmissions lead to an infection, but a large German study revealed that 10 % of the nosocomial infections among ICU patients could be attributed to cross-transmission (Grundmann *et al.* 2005). As it is impossible to know the outcome of transmission (ranging from colonization to life-threating infections) the aim must be to hinder all bacterial transmission.

The prevention strategies for cross-transmission are subdivided into two categories. The first category is isolation of the contagious patient from other patients in the same ward. Although this is effective in hindering transmission if done properly, it is expensive and labor-intensive. It is also inconvenient and uncomfortable both for the patient and HCWs. The second way to prevent cross-transmission is to break all possible transmission routes. Cleaning will remove visible dirt mechanically. Soaps and detergents have no antimicrobial activity themselves, but aid in dissolving the dirt. Generally, non-sporulating bacteria and viruses need organic matter to survive, on clean surfaces they will dry out and die. Sterilization *per se* means to make something sterile, i.e., no microorganisms should be present on the object after the process of sterilization. Reducing the number of microorganisms by a factor of more than 10^6 is, however, considered sufficient for sterilization. The best effect is reached on clean objects, i.e., those free of visible dirt. To remove or kill microorganisms on surfaces disinfectants are used. Depending on the circumstances of disinfection the antimicrobial activity and the toxicity of the compound have to be appropriate balanced. As the main vector for microorganisms in healthcare settings is the hands of HCWs (Weinstein 1991), hand disinfection is the most important factor in preventing bacterial transmission.

Outbreaks

An outbreak is defined as a "temporal increase in the incidence of infection (or colonization) by a certain bacterial species, caused by enhanced transmission of a specific strain" (van Belkum *et al.* 2007). Multiple strains can be included in an outbreak, if the outbreak has been going on for some time or if there is extensive person-to-person transmission (Barrett *et al.* 2006). ASCs can identify colonization prior to any clinical infections are seen and thereby identify an outbreak early on. Outbreak investigations include several different strategies. The source of the outbreak and the route of transmission are determined by epidemiological typing of the causative microorganism. Antibiotic therapy can be changed to eliminate the source of the outbreak and hygiene efforts are reviewed and improved to prevent any further transmission.

Hygiene guidelines

The Swedish National Board of Health and Welfare has published a regulation on hygiene guidelines in healthcare, mandatory for all HCWs performing examinations, patient care and treatment, i.e., bedside tasks (The National Board of Health and Welfare 2007). The regulation comprises the following eleven points:

1. Work clothes must be short-sleeved.
2. Work clothes must be changed daily or more often if needed.
3. Hands and forearms must be free from wristwatches and jewelry.
4. Hands must be disinfected with an alcohol-based disinfection agent, or other agent with equal effect, immediately prior to and after every direct contact with patients.
5. Hands must be disinfected prior to and after use of gloves.
6. Hands must, if visually soiled, be washed with soap and water prior to disinfection.
7. When nursing a patient with gastroenteritis, the hands must always be washed with soap and water prior to disinfection.
8. Hands must be dry when applying disinfection agent.
9. A disposable plastic apron or protective gown must be used if there is a risk the work clothes will come into contact with body fluids or other biological materials.
10. Disposable gloves must be used in contact, or if there is a risk of contact, with body fluids or other biological materials.
11. Gloves must be removed directly after, and changed between different, bedside tasks.

Hand hygiene

Originally, hand-washing was the recommended method for cleaning hands in healthcare settings. Unfortunately, the compliance with hand-washing has usually been unacceptably low, seldom exceeding 40 % (Widmer 2000). There is evidence that promotion campaigns to improve hand hygiene including the introduction of alcohol-based hand rub have been more successful than trying to improve compliance with already existing hand-washing guidelines (Bischoff *et al.* 2000; Pittet *et al.* 2000). Also the antimicrobial effect of alcohol-based hand rub is significantly better than that of hand-washing (Ayliffe *et al.* 1978), which only reduces the transient flora of the hands. Although early studies showed conflicting results regarding the bactericidal effect of alcohol (Price 1939), this effect is now well established (Price 1939; Salvage *et al.* 2014). The improved bactericidal effect when scrubbing the hands with alcohol as compared to just dipping them in alcohol was established early (Price 1939). Hand-washing requires 1-2 min to perform correctly, whereas hand disinfection requires less than 30 seconds

of working time. During disinfection HCWs can perform other healthcare-related tasks, such as reading a radiograph or introducing themselves to the patient, activities more difficult to perform while washing the hands. Additionally, whereas hand-washing dries out the skin, hand disinfection with an alcohol-based hand rub has no such effect. However, the application of a moisturizer is recommended both after hand-washing and hand disinfection. These drawbacks of hand-washing have led to the recommendation and introduction of waterless disinfection with alcohol-based hand rub in healthcare settings (Widmer 2000). Hand-washing is still recommended when the hands are visibly soiled and when handling patients with gastroenteritis or infected with spore-forming bacteria, since non-enveloped viruses and spores are highly tolerant to alcoholic disinfectant solutions.

Compliance with hygiene guidelines in recent studies, mainly reflecting the use of alcohol-based hand rub has generally been found to be below 50 % (Costers *et al.* 2012; Tromp *et al.* 2012; Biswal *et al.* 2014). Compliance with hygiene guidelines is generally thought to be low when the activity index (number of opportunities for hand hygiene per hour of care) is high, which was confirmed by Pittet *et al.* (2000). The apprehension that compliance is lower during night shifts than during day shifts has, however, not been confirmed (Pittet *et al.* 1999). Some studies have shown a significantly lower compliance with hygiene guidelines among physicians compared to other professions (Randle *et al.* 2010; Scheithauer *et al.* 2011; Costers *et al.* 2012). A more recent study did not show any general differences in compliance rates between physicians, midwives and registered nurse assistants (Mernelius *et al.* 2013c). Compliance with disinfection of the hands prior to patient contact generally renders lower compliance than disinfection after patient contact (Mernelius *et al.* 2013c; Biswal *et al.* 2014).

Monitoring compliance with hygiene guidelines

Gold standard for monitoring compliance with hygiene guidelines is direct observations (Boyce 2008), a method where HCWs (observers), from the own department or unit, monitor their colleagues compliance with hygiene guidelines. The observers are educated by infection control nurses and infection control practitioners on infection control and hygiene guidelines. The components monitored to determine compliance with hygiene guidelines in Sweden are described in table 2.

Table 2. The components monitored to determine compliance with hygiene guidelines in Sweden.

Barrier precautions*	Dress code†
B1. Hand disinfection before patient contact	D1. Short-sleeved work clothes
B2. Hand disinfection after patient contact	D2. Hands and forearms free from watches and jewelry
B3. Correct use of gloves	D3. Short or restrained hair
B4. Correct use of plastic apron or protective gown	

** Variables B1 and B2 "yes" or "no" only, variables B3 and B4 also "not applicable" for situations where gloves and/or apron or gown are not necessary.*
†Variables D1 to D3 "yes" or "no" only.

Even though direct observations are gold standard for monitoring compliance with hygiene guidelines, the method is considered expensive, and self-reporting has therefore been suggested as an alternative method. However, both methods are time-consuming and many have therefore searched for a substitute marker to indirectly measure compliance with hygiene guidelines, e.g., the usage of hand hygiene products (Haas & Larson 2007). Eckmanns *et al.* (2006) showed a positive correlation between hand hygiene compliance and hand rub consumption. The efficacy of hand hygiene monitoring technology, i.e., systems that count alcohol-based hand rub or soap dispensing events or in other ways estimate compliance and/or provide hand hygiene reminders, has recently been reviewed. To generally adopt hand hygiene monitoring technology could not be recommended based on the seven studies included and the need for further and scientifically better studies was emphasized (Srigley *et al.* 2015).

Validation of direct observations has been performed on a small scale, showing no difference in results between different observers (Tromp *et al.* 2012). This was confirmed in another study, which also concluded that self-reporting by HCWs is also a valid way to monitor compliance with hygiene guidelines (Mernelius *et al.* 2013c).

Interventions to improve compliance with hygiene guidelines

The hands of HCWs is the main vector for transmission of microorganisms in healthcare settings (Weinstein 1991) and to reduce the number of nosocomial infections several studies have attempted to improve the compliance with hygiene guidelines, especially hand hygiene. The most successful way to accomplish this has proven to be by applying a multimodal hygiene intervention (Pittet *et al.* 2000; Kirkland *et al.* 2012; Tromp *et al.* 2012; Mernelius *et al.* 2013c; Biswal *et al.* 2014). Hygiene interventions that are multidisciplinary, including all disciplines of HCWs, also physicians, also appear to be more successful (Mernelius *et al.* 2013c). Attempts have been made to clarify which of the initiatives of an intervention result in the greatest improvement of compliance with hygiene guidelines (Kirkland *et al.* 2012). This has resulted in the conclusion that a combination of initiatives is probably the key to success (Pittet *et al.* 2000). Improved compliance with hygiene guidelines has resulted in reductions in healthcare-associated infections, healthcare-associated *S. aureus* infections (Kirkland *et al.* 2012), MRSA acquisition rate and colonization pressure (Chun *et al.* 2014) as well as the prevalence of nosocomial infections and the incidence of MRSA infections and bacteremia (Pittet *et al.* 2000).

Many studies have shown a long-term effect of hygiene interventions on compliance with hygiene guidelines (Kirkland *et al.* 2012; Tromp *et al.* 2012; Mernelius *et al.* 2013c; Biswal *et al.* 2014) whereas others have demonstrated the opposite (Gould & Chamberlain 1997). All studies reporting on a long-term effect were multimodal, whereas the study reporting no long-term effect only featured a single intervention. It appears as if continuous reminders or minor sequential interventions need to be implemented for a sustained effect (Pittet *et al.* 2000; Kirkland *et al.* 2012).

The involvement of patients in improving the compliance with hand hygiene among HCWs has also been studied, with a comprehensive review recently published on the subject (Davis *et al.* 2015). Some studies show that patients were more willing to remind HCWs to conduct hand hygiene when encouraged to do so by an HCW (Davis *et al.* 2011; Davis *et al.* 2013) or if prompted in multiple ways (Pittet *et al.* 2011). Patients in general appear to be more willing to confront nurses than physicians regarding their hand hygiene (Davis *et al.* 2011; Pittet *et al.* 2011). Very few patients did, however, actually confront HCWs about their hand hygiene (Pittet *et al.* 2011; Schwappach *et al.* 2011).

Aims of the thesis

The main aims of this thesis are to study bacterial transmission, with *S. aureus* colonization of newborn infants as a model, and to evaluate the impact of compliance with hygiene guidelines on transmission.

The specific aims of this thesis were to:

- Evaluate the usefulness of *spa* typing in epidemiological investigations (paper I).
- Establish *S. aureus* colonization rates, antibiotic susceptibility patterns and *spa* type epidemiology (papers III & IV).
- Determine the effect of the inclusion of an enrichment broth prior to plating, on *S. aureus* recovery (paper IV).
- Elucidate from which sites transmission of *S. aureus* occurs (paper IV).
- Validate direct observations and self-reporting by HCWs for monitoring compliance with hygiene guidelines (paper II).
- Improve the compliance with hygiene guidelines by a multimodal and multidisciplinary hygiene intervention (paper II).
- Determine the effect of different rates of compliance with hygiene guidelines, on *S. aureus* transmission (paper III).

Outline of the Swedish HITS study (papers II, III & IV)

The Swedish Hygiene Intervention and Transmission of *S. aureus* (HITS) study is a study in three parts aiming at clarifying the correlation between different compliance rates with hygiene guidelines and transmission of *S. aureus* to newborn infants. The study setting was four departments of obstetrics and gynecology, at four different hospitals. Characteristics of the four departments and patients are given in table 1, paper III.

Part one – Baseline (spring 2008)

Compliance with hygiene guidelines (barrier precautions and dress code, according to table 2, with the exception of D3, page 37) was monitored through direct observations. Samples to identify *S. aureus* colonization were collected from the anterior nares and throat of HCWs in the participating departments at the start of their shift, from parents upon arrival at the delivery ward and from visiting adults. Mothers were also sampled from the vagina. Samples from the anterior nares and umbilicus were collected from newborn infants at the age of 2 h and subsequently every 24 h until discharge, when the final samples were collected. Visitors were sampled from the anterior nares and throat if older than 18 years. All adults and visiting siblings were also sampled from any skin lesions (table 3). All samples were collected using cotton swabs. From the departments environmental and air samples were also collected, by contact plates and a surface air sampler, respectively.

Table 3. Number of samples collected from each site in each population at baseline.

	Infants				Fathers	Mothers	HCWs	Visitors
	2 h.	24 h.	48 h.	Discharge				
Umbilicus	213	147	60	205				
Nares	213	147	62	203	215	215	390	36
Throat					215	215	390	
Vagina						205		
Skin lesion					22	12	24	5

Transmission routes were established using *spa* typing. We evaluated four possible transmission routes for *S. aureus* colonizing newborn infants. First, if the infant and one of its family members were colonized with *S. aureus* of the same *spa* type, regardless of whether the *spa* type was also present elsewhere, the infant's own family was considered the origin of transmission. Second, HCWs and/or other families were considered the source of transmission if the infant's *spa* type was found among HCWs, any of the other infants simultaneously cared for in the department, or any family member of those infants. Third, the environment was considered the source of transmission if the infant's *spa* type could only be detected in the environment. And finally, if the *spa* type of the *S. aureus* colonizing an infant could not be detected among its own family, any other family, HCW or in the environment, the source of transmission was considered unknown.

Part two – The Intervention (autumn 2008)

A multimodal and multidisciplinary hygiene intervention was launched in each department and continuous monitoring of the compliance with hygiene guidelines was performed. The intervention comprised the following parts: a lecture, a workshop, safety briefings, posters, an infection control audit, supporting visits and phone calls from infection control nurses, feedback on the progress and compliance rates, education of more observers and training in, and assessment of, the hand hygiene technique.

Part three – The post-interventional phase (2009-2010)

When the departments had reached and stabilized at a level of compliance with hygiene guidelines of ≥80 % (point of stability), sampling (table 4) and identification of transmission routes were performed in the same way as during baseline. The compliance with hygiene guidelines was also assessed three years after the intervention was initiated (follow-up).

Table 4. Number of samples collected from each site in each population at the post-interventional phase.

	Infants				Fathers	Mothers	HCWs	Visitors
	2 h.	24 h.	48 h.	Discharge				
Umbilicus	233	167	60	215				
Nares	232	166	60	216	230	233	318	45
Throat					229	229	319	3
Vagina						233		
Skin lesion					30	12	26	1

Performance of bacteriological methods

Enrichment broth (paper IV)

In paper IV, 2 371 samples were examined for *S. aureus* growth, out of which 488 samples were positive by direct plating and an additional 225 samples by incubating the swabs in enrichment broth prior to plating. This corresponds to a total increase of *S. aureus*-positive samples by 46 % using enrichment broth prior to plating (table 1, paper IV). The largest increase in *S. aureus*-positive samples was seen for vaginal samples with a 267 % increase in positive samples when plating after incubation in enrichment broth. This corresponds to an increase from three to eleven positive samples, out of 157, so due to the small sample size these results need to be interpreted with caution. It has been demonstrated that *S. aureus* rectovaginal colonization in mothers does not pose a risk for early onset neonatal sepsis (Tomlinson *et al.* 2011). The premier colonization site for adults was the throat (47 %, $p<0.001$ compared to other sites sampled) and the number of *S. aureus*-positive samples from throat was doubled after enrichment compared to direct plating. Also for samples from the nares of infants the number of positive samples more than doubled after enrichment. Incubating the swabs from the other sites cultured (skin lesions, nares of adults and umbilicus of newborn infants) in enrichment broth prior to plating all generated more positive samples than direct plating. These results indicate that incubation of swabs in enrichment broth prior to plating is necessary to determine more truthful *S. aureus* colonization rates. A study on pregnant women and their newborn infants identified an increased *S. aureus* yield by the addition of an enrichment broth prior to plating (Andrews *et al.* 2009). An increased sensitivity of *S. aureus* detection when using an enrichment broth was also demonstrated by Wanten *et al.* (1998). The recommendation to use an enrichment broth prior to plating is not included in the Swedish guidelines for culturing samples from skin and soft tissue infections, from which *S. aureus* is often isolated (Föreningen för Medicinsk Mikrobiologi vid Svenska Läkaresällskapet & Folkhälsomyndigheten 2012).

Typing methods (papers I, III & IV)

In paper I, 280 MRSA isolates from colonized or infected individuals were characterized by antibiotic susceptibility testing, *spa* typing, PFGE and PVL gene presence/absence. Also, MLST was performed on representative isolates from the collection. A thorough epidemiological investigation of each MRSA case was possible thanks to the low prevalence of MRSA in Sweden. Therefore the epidemiological data was considered gold standard when evaluating the performance of *spa* typing and PFGE.

All isolates were typeable both by *spa* typing and PFGE, resulting in 100 % typeability. The index of diversity was 0.94 (95 % confidence interval [CI] 0.92-0.95) for *spa* typing and 0.95 (95 % CI 0.93-0.96) for PFGE, both in the order of the desired diversity index of 0.95 (van Belkum *et al.* 2007). Of the 35 outbreaks detected, only one consisted of isolates of different *spa* types (table I, paper I), rendering great epidemiological concordance (99.5 %). One isolate in this outbreak was of a *spa* type that differed by one point mutation in the eighth repeat from the predominant *spa* type (table 5), indicating a close genetic relationship between the two types. In six of the outbreaks, isolates of two or more distinguishable, but >80 % similar and therefore considered closely or possibly related, PFGE patterns were observed. Due to the interpretation criteria used the epidemiological concordance of PFGE was 100 % (p=0.33 compared to epidemiological concordance for *spa* typing).

Table 5. Repeat succession of the two spa *types observed in one outbreak in paper I and the repeat sequences of the repeats differing between the* spa *types.*

spa type	Repeat succession	Repeat no.	Repeat sequence
t032	26-23-23-13-23-31-29-**17**-31-29-17-25-17-25-16-28	r17	AAAGAAGACG**G**CAACAAGCCTGGT
t1377	26-23-23-13-23-31-29-**110**-31-29-17-25-17-25-16-28	r110	AAAGAAGACG**A**CAACAAGCCTGGT

spa typing results can be obtained within 12 h starting with a pure bacterial culture (Vainio *et al.* 2011). Although this is slightly slower than optimal for ultimate clinical use of epidemiological typing (van Belkum *et al.* 2007) it is faster than PFGE, where the run-time on the instrument alone (the process in which fragments are separated on the gel) usually takes about 24 h (Murchan *et al.* 2003). DNA has successfully been prepared directly from frozen bacteria (S. Mernelius, unpublished data) and by omitting culturing; the process of *spa* typing has been speeded up further. For analysis by PFGE, bacteria have to be cultured, as the DNA concentration in the plug has to be standardized to enable inter-laboratory comparison of results (Murchan *et*

al. 2003). *spa* typing can easily process samples in a 96-well format, whereas for PFGE, the maximum number of samples that can be run on one gel, and thereby the maximum number of samples that can be processed simultaneously, is 39. For in-house sequencing the set-up cost for *spa* typing is quite heavy, but this is also true for the instrumentation needed to perform PFGE. Sequencing performed by commercial sequencing companies can save a laboratory a lot in investment costs. As *spa* typing is based on amplification by PCR, the reagents for this method are more expensive than for PFGE, which is based on macrorestriction of unamplified material. PFGE, on the other hand, is more labor-intensive, and therefore the staff cost for PFGE exceeds that for *spa* typing. Data analysis of PFGE restriction patterns requires an experienced technician, and includes moderately subjective interpretation of weak bands and double bands. In contrast, data analysis of *spa* data is very easy and requires a minimum of interpretation as only isolates with exactly identical sequences are considered clonal and are assigned the same *spa* type. Therefore *spa* typing is superior to PFGE when analyzing a large number of isolates.

Another advantage of *spa* typing over PFGE is the standardized nomenclature and thereby the transportability of data between laboratories. Paper I includes MRSA from four different counties. The microbiology laboratories in each county could have performed *spa* typing in-house and easily transferred the results electronically to one coordinating laboratory, whereas PFGE had to be performed at one central laboratory for optimal comparability.

Both *spa* typing and PFGE have shown high discriminatory power (Cookson *et al.* 2007) and 100 % typeability (Hallin *et al.* 2007). In paper I, both methods performed comparably well regarding discriminatory power, typeability and epidemiological concordance. *spa* typing was considered superior to PFGE thanks to its accessibility, ease of use and rapidity. This is in line with previous studies that have found *spa* typing well suited for outbreak investigations (Shopsin *et al.* 1999; Strommenger *et al.* 2008). A recent study from Norway, a low-prevalence country for MRSA like Sweden, detected clonal dissemination of a single *spa* type (t304), and recommended supplementary typing methods in addition to *spa* typing for this situation (Fossum Moen *et al.* 2014). Other studies have also implied that *spa* typing should be used in combination with the analysis of additional markers, e.g. SCC*mec* or virulence or resistance genes, to fully clarify epidemiological connections (Hallin *et al.* 2007; Strommenger *et al.* 2008). Isolates of identical *spa* types were observed in different outbreaks in paper I. The presence or absence of PVL among these isolates could in some cases differentiate these outbreaks from one another.

In papers III and IV *spa* typing was used to determine transmission routes of *S. aureus* to newborn infants. The origin of the *S. aureus* colonizing the infants could be identified as another person or the environment in 83 % to 84 % in these studies, although there were approximately 10 000 different *spa* types registered in the Ridom Spa Server database at that time (Ridom GmbH 2014). The large proportion of certain transmission routes despite the vast number of *spa* types present reflects the potential of *spa* typing as a valid epidemiological typing tool. A previous study has also used *spa* typing to reveal transmission of *S. aureus* among newborn infants (Matussek *et al.* 2007).

In line with the results in paper I, several studies have shown that the discriminatory power of MLST is lower than that of *spa* typing and PFGE (Cookson *et al.* 2007; Hallin *et al.* 2007). MLST is therefore not suitable for local outbreak investigations as isolates from different outbreaks would be assigned the same ST and thereby confuse the epidemiological conclusions.

S. aureus epidemiology

Antibiotic resistance (papers I & III)

The prevalence of resistance in *S. aureus* in the Swedish HITS study (paper III), in skin and wound infections in Sweden and from the Swedish data in the APRES study are shown in table 6. Two isolates resistant to cefoxitin, the antibiotic used to screen for MRSA, were detected in paper III and one was confirmed to be an MRSA by the detection of *mecA*. The colonizing *S. aureus* isolated from healthy individuals in paper III were significantly less resistant to cefoxitin and fusidic acid than the *S. aureus* causing infections collected nationally. (Statistics based on the assumption that 15 clinical microbiology laboratories delivered data on 100 isolates each.) This was also true for clindamycin and erythromycin in the years of 2009 and 2010, whereas no difference was seen for these antibiotics in 2008. Resistance to fusidic acid was also significantly lower among *S. aureus* in paper III, compared to the Swedish data in the APRES study. There were no differences in resistance to cefoxitin, clindamycin or erythromycin in paper III and the APRES study, both including colonizing *S. aureus*. The level of antibiotic resistance in Sweden is generally low which is reflected in paper III. This is probably due to the restrictive antibiotic prescription policy in Sweden.

Table 6. Prevalence of resistance (%) among S. aureus *from the Swedish HITS study (paper III), the Swedish national database and the APRES study.*

Year		Cefoxitin	Clindamycin	Erythromycin	Fusidic acid
2008	Baseline*	0.1	2.0	2.1	0.9
2008	National data†	0.8	2.7	3.3	5.3
2009-10	Follow-up*	0.0	1.4	1.5	0.7
2009	National data†	0.8	3.2	3.8	4.5
2010	National data†	0.9	3.1	4.0	6.2
2010-11	APRES study‡	0.0	1.5	1.6	1.9

** Data from paper III*
† Data from (Public Health Agency of Sweden 2014)
‡ Data from (den Heijer et al. 2013; den Heijer et al. 2014)

In paper III, 32 erythromycin-resistant isolates were detected, of which 30 (94 %) exhibited inducible clindamycin resistance, the other 2 (6 %) were clindamycin-susceptible. The prevalence of inducible clindamycin resistance varies greatly in different studies (Siberry *et al.* 2003; Vandana *et al.* 2009). Clindamycin can be used to treat pneumonia and more serious skin and soft tissue infections caused by MRSA (Hagberg 2014). Treatment failure of an MRSA infection with clindamycin due to inducible clindamycin resistance has been documented (Siberry *et al.* 2003). Treatment with clindamycin of infections caused by inducible clindamycin-resistant *S. aureus* should be avoided, as the exposure of clindamycin to these bacteria might provoke further resistance development. Inducible clindamycin resistance can be detected by the D test (The European Committee on Antimicrobial Susceptibility Testing 2014, Version 4.0) and to discriminate between isolates exhibiting inducible resistance and susceptibility this method has to be applied to all clinical isolates of *S. aureus* (Vandana *et al.* 2009).

The distribution of resistant isolates was similar between parents, infants and HCWs (paper III). This indicates that more resistant strains of *S. aureus* are not accumulated in healthcare settings and that HCWs do not harbor a specific institutional flora, more resistant to antibiotics than the flora present in the community.

The MRSA incidence in Jönköping County between 2009 and 2013 was among the highest in Sweden (Public Health Agency of Sweden & National Veterinary Institute 2013). From this, one may draw the conclusion that infection control was not as efficient in this county, compared to other counties. The national point prevalence measurements on compliance with hygiene guidelines show that Jönköping County has been among the best since the measurements began in 2010. In 2014 the compliance with hygiene guidelines in Jönköping County was the highest in Sweden (86.6 %) (Swedish Association of Local Authorities and Regions 2014). Instead the high MRSA incidence may imply a well-functioning infection control program, with extensive screening of risk patients as well as contact tracing.

The prevalence of multidrug resistance (resistance to ≥3 classes of antibiotics) among Swedish *S. aureus* isolates was 0.2 %, compared to 2 % to 3 % in the *S. aureus* from the other eight participating European countries in the study by den Heijer *et al.* (2013). In paper I, where all isolates were methicillin-resistant, 109 (39 %) were classified as multidrug-resistant whereas no multidrug-resistant isolates were identified in paper III. It has been shown that MRSA isolates are more often multidrug-resistant compared to MSSA isolates (Erami *et al.* 2014). This is not surprising as some of the SCC*mec* types are known to carry several antibiotic-resistance genes.

Prevalence of *S. aureus* (papers III & IV)

In paper III a total of 6 530 samples were collected from mothers (n=448), fathers (n=446), newborn infants (n=449, 215 girls), HCWs (93 % females, n=711) and visiting siblings (n=79) as well as relatives (n=3). Environmental samples (n=496) were collected with contact plates and a total of 24 000 L of air was sampled using a surface air sampler. For detailed sampling schemes see table 3 (page 41) and table 4 (page 42).

The colonization rates ranged from 44 % to 66 % among adults (table 2, paper III), showing that fathers were colonized to a larger extent than mothers, HCWs and infants ($p<0.01$). Also in paper IV adult males were significantly more often colonized than women, an expected result as the 2 434 samples in paper IV were part of the collection in paper III, only differing in the culture technique used. The procedure of using an enrichment broth prior to plating (paper IV) revealed colonization rates ranging from 51 % to 70 % among adults. A gender-associated difference in colonization, with males being more frequently colonized compared to females, has been demonstrated in previous studies (Bischoff *et al.* 2004; Herwaldt *et al.* 2004; Sangvik *et al.* 2011; Olsen *et al.* 2012). Initially, the reason for this association was unknown and scarcely studied (Bischoff *et al.* 2004). More recent research has shown an inverse correlation of *S. aureus* colonization and carriage to serum concentrations of vitamin D among non-smoking men. It has been suggested that vitamin D up-regulates the antibacterial immune defense and high concentrations of vitamin D would thereby kill off *S. aureus* and reduce colonization (Olsen *et al.* 2012). There are indications that the immune defense is somehow boosted by female sex hormones (Marriott & Huet-Hudson 2006). This could explain the lower *S. aureus* colonization rates among females compared to males described in paper III and previous studies (Bischoff *et al.* 2004; Herwaldt *et al.* 2004; Sangvik *et al.* 2011) as well as why the association between colonization and carriage status and serum concentration of vitamin D was more prominent among males (Olsen *et al.* 2012). Due to the few hours of sun-light during the winter in Scandinavia, low serum concentrations of vitamin D, and thereby a higher prevalence of *S. aureus*, can be expected during this period. This was also demonstrated in a Swedish study, where *S. aureus* colonization rates were approximately 20 % higher in March compared to August (Nilsson & Ripa 2006). The gender-associated difference so prominent among adults was not detected among infants (table 2, paper III). It has been shown previously that male infants are colonized to a larger extent than female infants (Lebon *et al.* 2008). There was no statistically significant difference in colonization rates between mothers and HCWs, who were mainly females (paper III). These differences indicate that *S. aureus* colonization patterns are not influenced by pregnancy.

The *S. aureus* colonization of infants increased from approximately 40 % (table 2, paper III) to 61 % when incubating the swabs in enrichment broth prior to plating (table 2, paper IV). Infant colonization began immediately at birth, with 17 % of the infants being colonized already at the age of 2 h, climbing to 47 % at the age of 24 h (figure 1, paper IV). Paper IV show that newborn infants were more commonly colonized in the umbilicus (53 %) than in the anterior nares (42 %, $p<0.05$). In paper III, 25 % of the newborn infant carriers were detected by analyzing the anterior nares only and 95 % by analyzing umbilicus only. Similarly to this, a previous study showed that 73 % of the newborn infant *S. aureus* carriers were detected by culturing the umbilical only, whereas only 36 % were detected by nares culture only (Andrews *et al.* 2009).

Prevalence of *S. aureus* in different sites in male and female adults and infants was determined in paper IV and is shown in figure 8. For adults the most prevalent colonization site was the throat (47 %, $p<0.001$ compared to other sites sampled). This has been shown in previous studies (Nilsson & Ripa 2006; Hamdan-Partida *et al.* 2010) and it has been suggested that the throat should be included in *S. aureus* screening programs (Wanten *et al.* 1998). Previously, the anterior nares have been considered the premier colonization site and therefore much of the *S. aureus* research has been performed on nasal carriers (Kluytmans *et al.* 1997).

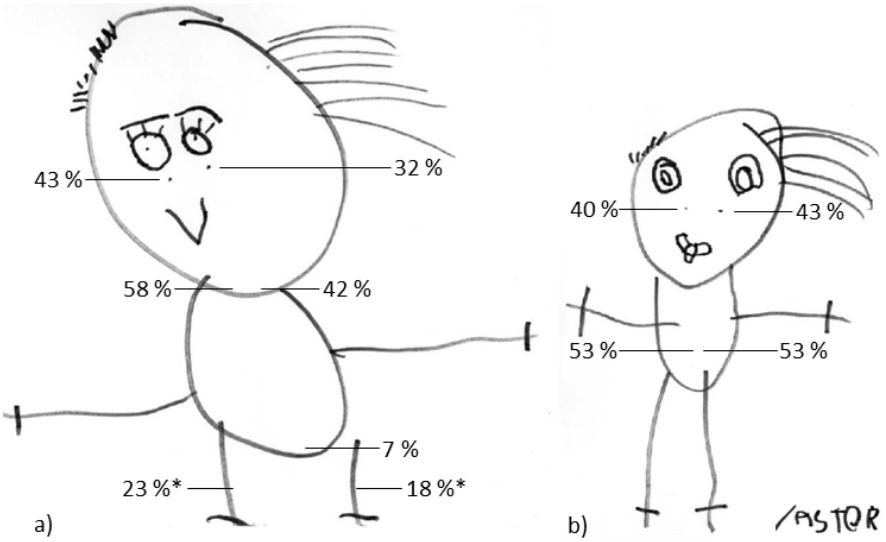

Figure 8. Prevalence of S. aureus *in the sites sampled in a) male (left) and female (right) adults and b) male (left) and female (right) infants.*
** Skin lesions*
Drawing by Astor Mernelius, after idea by Elisabeth Norén.

A recent European study revealed that Sweden had the highest prevalence of *S. aureus* nasal carriage (30 %) among the nine participating countries (den Heijer *et al.* 2013). The prevalence of nasal colonization among adults was 27 % (paper IV) when using direct plating, the same method as used in the paper by den Heijer *et al.* (2013). When incubating the swabs in enrichment broth prior to plating the prevalence of nasal *S. aureus* colonization was 37 % (paper IV), thereby being the second most common colonization site among adults.

S. aureus was detected in 17 (3.4 %, 10 at baseline and 7 at follow-up) of the 496 environmental samples collected in paper III. *S. aureus* was found in lavatories (n=4), areas exclusively used by HCWs (n=5), ward rooms (n=2), a milk kitchen (n=1), on medical equipment (n=4) and on a Pilates ball used by the mothers (n=1). No *S. aureus* was detected in the 24 000 L of air sampled. The focus of environmental and air contamination in hospitals has primarily been on MRSA, rather than MSSA. The prevalence of MRSA on surfaces and in the air in hospitals is low, even in countries where MRSA is endemic (Creamer *et al.* 2014; Mirzaii *et al.* 2014). Despite this, it has been demonstrated that patients admitted to rooms previously occupied by patients with MRSA has an increased risk of acquiring MRSA compared to patients admitted to rooms previously occupied by non-MRSA patients (Huang *et al.* 2006a). It has also been shown that a prolonged MRSA outbreak was stopped by intensified cleaning (Rampling *et al.* 2001).

spa type distribution and cluster analysis (papers III & IV)

The probability of isolating two indistinguishable strains from two individuals with connections in time and space but with no known transmission events is a function of the prevalence of those strains in the corresponding community. Knowing the prevalence of different strains in the community will therefore aid in the understanding of the increased prevalence of specific strains in the healthcare setting. If a strain assumes endemic proportions in the community, a high prevalence in the healthcare setting might not indicate an outbreak or increased transmission. This situation requires additional typing and characterization of the isolates to resolve potential outbreaks. Increased prevalence in healthcare settings of fairly uncommon community strains does, however, imply intra-healthcare transmission and a possible ongoing outbreak.

A total of 314 different *spa* types were identified in paper III. Of these, 49 % were unique to single individuals. The most common *spa* types among newborn infants, parents, HCWs, relatives and siblings at baseline and follow-up are shown in figure 1, paper III. The *spa* type most commonly isolated was t084 (n=104), followed by t012 (n=65), t015 (n=50), t021 (n=42) and t002 (n=41). The four most common *spa* types in paper III were also the four most common in paper IV (figure 2, paper IV). A previous study from partly the same geographical area showed that t012, t015 and t021 were the most common *spa* types (Matussek *et al.* 2007), indicating that major clones do not fluctuate greatly over time. In the same study, t084 was not detected at all (Matussek *et al.* 2007) which could indicate that the molecular epidemiology of *S. aureus* is somewhat dynamic. t084 has recently been described as one of the most common *spa* types in studies both from northern Norway and Nigeria (Sangvik *et al.* 2011; Kolawole *et al.* 2013; Olsen *et al.* 2013).The high prevalence of t084 both among adults and infants indicates that it is highly transmittable and a successful colonizer. *S. aureus* of *spa* type t084 was also the predominant colonizing type, but also the cause of four cases of bacteremia, in the neonatology department in one of the hospitals participating in the Swedish HITS study (paper III). The t084 in the neonatology department had unfortunately also acquired tobramycin resistance. The introduction into healthcare settings of such a transmittable type, carrying virulence factors or resistance genes, poses a great threat to healthcare.

No specific *spa* types were correlated to male or female gender, or to the nares or throat among adults (paper IV). Gender-specific *spa* types have been identified previously (Sangvik *et al.* 2011). The study demonstrating this correlation was, however, larger than ours. A significant association between HCWs and a specific *spa* type (t160) was identified in paper IV, which is in line with a previous study, which, however, identified an association between two other

spa types, namely t012 and t015, and HCWs (Olsen *et al.* 2013). t160 was also the most common *spa* type in nursing homes in the same geographical area as the studies in papers III and IV (Stark *et al.* 2014). This adds to the indication already suggested, based on the high prevalence of the previously undetected t084 in the region, that some strains are more prone to transmission and are more successful colonizers.

Simultaneous *S. aureus* colonization in more than one site was observed in 140 individuals of which 42 (30 %) were colonized with isolates of different *spa* types in at least two sites (paper IV). These individuals were termed multiclonal individuals. The phenomenon of multiclonal individuals has also been described in elderly persons living in nursing homes (Stark *et al.* 2014). Multiclonal colonization could mislead epidemiological conclusions. In paper III, 17 % of the *S. aureus* transmissions to newborn infants were of unknown origin; these transmissions might to some extent be explained by multiclonal colonization. Antibiotic treatment may also be hampered by the presence of an undetected resistant strain in clinical samples. A mathematical model predicted that approximately 7 % of the individuals would be colonized by more than one *S. aureus* strain, based on the typing of three colonies from the anterior nares (Cespedes *et al.* 2005). A recent study on multiclonal colonization in children in which four to 15 colonies were typed from each sample revealed that 30 % contained two or three different strains of *S. aureus* (Mongkolrattanothai *et al.* 2011). However, typing of several colonies from each sample is time-consuming and labor-intensive. Therefore a culture independent method to clarify multiclonal colonization in one site has been described (Matussek *et al.* 2011).

BURP cluster analysis of the 170 different *spa* types detected in paper IV revealed 19 *spa* CCs and nine singletons. The four largest *spa* CCs contained 52 % of the *spa* types and 62 % of the isolates. For details of these *spa* CCs see table 7. Of the detected *spa* types, twelve were shorter than five repeats, and were by default excluded from the analysis. Shorter s*pa* types contain little genetic information and the reliability of the evolutionary analysis based on these *spa* types is limited (Mellmann *et al.* 2007). Of the 29 most commonly identified *spa* types (figure 2, paper IV), 12 were found in the largest *spa* CC (*spa* CC 015). This *spa* CC has previously been identified as the largest *spa* CC among elderly people living in nursing homes in southern Sweden (Stark *et al.* 2014). Only two of the 29 most common *spa* types (t084, the most commonly identified *spa* type, and t085) were found in the second largest *spa* CC.

Table 7. The number and percentage of spa *types and isolates in the four largest* spa *CCs in paper IV.*

	Number of	
	spa types (%)	isolates (%)
spa CC 015	53 (31 %)	297 (38 %)
spa CC 084	13 (8 %)	113 (14 %)
spa CC 349	13 (8 %)	33 (4 %)
spa CC 002	10 (6 %)	46 (6 %)

Only nine of the 42 individuals (21 %) that were colonized with two different *spa* types in different sites, were colonized with *spa* types from the same *spa* CC, indicating close genetic relatedness. Even lower prevalence of congruence between *spa* types in samples collected from individuals over time has been reported (Stark *et al.* 2014). The colonization with genetically different *spa* types in different sites indicates that there is a close match between the bacteria and not only the host, but also the specific site of colonization in that host.

Infection control in healthcare settings

Validation of direct observations and self-reporting by HCWs (paper II)

Direct observations are considered gold standard for monitoring compliance with hygiene guidelines (Boyce 2008). As the method is considered time-consuming and expensive other methods, e.g., self-reporting by HCWs or alcoholic hand rub consumption, have been suggested. The validity of direct observations and self-reporting by HCWs seems scarcely studied. Therefore direct observations and self-reporting by HCWs using the Swedish protocol for monitoring compliance (table 2, page 37) were validated in three ways in paper II.

1. Double appraisal. This was used to determine the concordance between two observers, who simultaneously and blinded to the reporting of the other observer monitored the compliance of the same HCW performing a bedside task.
2. Multi appraisal. This was used to determine the concordance between several observers as well as between observers and the infection control nurses. The compliance with hygiene guidelines for a prerecorded bedside task was monitored by all observers (n=226) present at an educational meeting for observers.
3. Self-reporting by HCWs. An experienced observer monitored the compliance with hygiene guidelines of an HCW. This HCW was subsequently asked to fill out a compliance form for the same, recently performed, bedside task.

In the double appraisal 69 observed bedside tasks were evaluated. The concordance between observers was 99 % for barrier precautions and 97 % for the dress code, as the compliance was judged identical by 68 and 67 observer-pairs, respectively. Within observer-pairs there were 4 discrepancies registered regarding hand disinfection after patient contact, 6 regarding glove use and 1 each regarding short sleeves and short or restrained hair. The high concordance between observers demonstrated is in line with a previous small-scale validation of direct observations (Tromp *et al.* 2012).

In the multi appraisal, 95 % to 100 % of the observers correctly judged the seven different components of the hygiene guidelines, showing great concordance between observers as well as between observers and the experts, i.e., the infection control nurses. There were no statistical significant differences in compliance rates when determined by experienced observers or new observers.

For self-reporting by HCWs 49 bedside tasks were evaluated. When the compliance with barrier precautions and the dress code was registered by self-reporting HCWs it was 76 % and 96 %, respectively, and when registered by the observers it was 71 % and 94 %, respectively. The differences in compliance rates were not statistically significant. The concordance between the self-reporting HCWs and the observers was 94 % for barrier precautions (3 discrepancies registered) and 98 % for the dress code (2 discrepancies registered). These results indicate that self-reporting by HCWs is a valid method to monitor compliance with hygiene guidelines. However, self-reporting by HCWs demands a very good knowledge on hygiene guidelines by all HCWs. Anecdotal evidence on self-reported compliance with hygiene guidelines by HCWs show that HCWs perceive themselves to be 90 % compliant with hygiene guidelines, whereas they believe their coworkers to be only 50 % compliant (Walker *et al.* 2014).

Compliance with hygiene guidelines (papers II & III)

Baseline compliance in papers II and III reflects the compliance in the participating departments when no extra focus was spent on hygiene guidelines. It was established based on 217 direct observations on barrier precautions and 227 regarding the dress code, reaching compliance rates of 41 % and 93 %, respectively. The compliance rates for barrier precautions of each participating department are shown in figure 1, paper II.

Following the initiation of the 10-point hygiene intervention (for details see page 42), observers in each department performed ten direct observations each week. The compliance rates were continuously evaluated by infection control nurses and graphical feedback was sent to and posted in the departments weekly. It took seven months to one year to reach and stabilize at a compliance rate of ≥80 %. In paper II, this evaluation point was termed 'point of stability' and the compliance rates, based on 556 direct observations on barrier precautions and 580 regarding the dress code, were 85 % and 99 %, respectively. In paper III this evaluation point was termed 'follow-up' and the compliance rate was 86 % with barrier precautions and 99 % with the dress code (316 and 342 direct observations were performed, respectively). The compliance rates were significantly higher at point of stability (paper II) and follow-up (paper III) compared to baseline, both regarding barrier precautions and the dress code. This shows that it is possible to markedly improve the compliance with hygiene guidelines by a multimodal and multidisciplinary hygiene intervention, which confirms previous data (Boyce & Pittet 2002). A hospital-wide study by Kirkland *et al.* (2012) also demonstrated a statistically significant improvement in compliance with hygiene guidelines approximately one year after initiation of monitoring and giving feedback on compliance rates. This might indicate that the mere attention to the issue of unsatisfactory compliance could be encouragement enough for many to improve their behavior. Studies have shown that compliance with hygiene guidelines improves when introducing an alcohol-based hand rub, but not when promoting hand-washing (Bischoff *et al.* 2000; Pittet *et al.* 2000). This may be due to the shorter time required to perform hand disinfection compared to hand-washing or the lack of unfavorable side effects of alcohol-based hand rub compared to hand-washing.

To establish the long-term effect of the intervention an evaluation of compliance rates (follow-up, paper II) was undertaken three years after the hygiene intervention was launched. Over the entire study period (from baseline to follow-up) a significant improvement in compliance was seen in three of the departments (A, B and D), whereas it was close to significant in department C ($p=0.07$). By performing an extensive educational hygiene intervention and

promoting alcohol-based hand rub, Pittet *et al.* (2000) managed to significantly improve the compliance with hygiene guidelines over a period of three years. Also, they used continuous reminders in the form of posters emphasizing the need for good compliance with hygiene guidelines and replaced them weekly. The use of continuous and varied reminders might be necessary to achieve a sustained long-term effect on the compliance. The compliance with barrier precautions was also evaluated three years after follow-up (six years after the hygiene intervention was launched) and ranged from 82 % to 99 % in departments B, C and D. This was significantly higher than at baseline ($p<0.001$). Compliance rates for this evaluation point were not available for department A. Recent studies have shown continued improved compliance even after the interventions themselves have ended (Kirkland *et al.* 2012; Tromp *et al.* 2012).

The effect on the compliance of the individual parts in the hygiene intervention was not established (paper II). The intention was, however, to improve the compliance with hygiene guidelines, not to evaluate what specific part of the hygiene intervention that did this. In line with the results in paper II, previous studies have shown that multimodal hygiene interventions are successful in improving compliance with hand hygiene (Pittet *et al.* 2000; Pittet 2001; Tromp *et al.* 2012). The HCWs participating in the workshops expressed that it gave them an improved understanding for the importance of compliance with hygiene guidelines, which was also the aim of the workshops. The concept of workshops has attracted attention at national and international conferences on infection control and is today used routinely by the infection control nurses locally as well as in other parts of Sweden.

From the patients' point of view correct hand hygiene prior to patient contact is more important than hand hygiene after patient contact, which is primarily performed to protect the HCW. Despite this, the compliance with disinfection of hands prior to patient contact was significantly lower than with the other components of barrier precautions at baseline ($p<0.001$) in paper II. Similar results were obtained by Biswal *et al.* (2014), thus demonstrating the importance of focusing on improving the compliance with hygiene guidelines prior to patient contact in hand hygiene interventions. Disinfection of hands prior to patient contact was also one of the components where the compliance significantly declined from point of stability to follow-up (the other one being proper use of plastic aprons or protective gowns), indicating that without continuous and varied reminders on the importance of hand hygiene, HCWs fall back into old behavioral patterns. Although the posters from the hygiene intervention remained on display in the departments from the time the intervention started until follow up they were not changed at any point, potentially becoming "invisible" to the HCWs. In a large previous study, posters with messages on hygiene guidelines and hand hygiene promotion were replaced weekly (Pittet *et al.* 2000).

The correct use of gloves is a common issue of debate at educational meetings for observers (PeO Svensson, personal communication). It has been demonstrated that many nurses go from dirty to clean work and touch commonly used objects and the environment, with contaminated gloves still on (O'Boyle *et al.* 2001). Therefore the issue of glove use needs to be thoroughly discussed with HCWs.

Compliance with the dress code is necessary to be able to perform hand hygiene correctly. Long sleeves and jewelry on the hands and forearms will impair the hand hygiene, leaving bacteria on the sleeves with the risk of recontamination of the hands and unsatisfactory disinfection of the skin under rings and watches. In paper II, the compliance with the dress code was already high at baseline (average 93 %) and continued to be so throughout the study (point of stability and follow up 99 %), indicating a lesser need to focus on the improvement of the compliance with the dress code in future hygiene interventions. The reason for the difference in compliance with barrier precautions and the dress code is most likely due to the fact that HCWs only have to change their cloths and remove their jewelry once per shift. Barrier precautions, on the other hand, usually have to be performed several times per hour of work. A study by Tromp *et al.* (2012) also demonstrated a continuously high compliance with the dress code in a nursing ward but a significant increase, from relatively low levels, in the outpatient clinic of the same department.

Papers II and III also show that the high compliance with hygiene guidelines can be reached and maintained without external funding, which is in line with previous work (Pittet *et al.* 2000), emphasizing a high compliance with hygiene guidelines at all time.

Transmission routes and sources (papers III & IV)

A study from 1962 reported that only 10 % of the newborn infants were colonized with the same *S. aureus* as the infant's mother and 70 % with an *S. aureus* colonizing an HCW. During post-birth hospitalization during the 60s infants were still nursed by the nurses, primarily seeing their mothers when being fed (Mortimer *et al.* 1962). This situation has changed dramatically over the years and now infants are roomed in with their mothers and the nursing is mainly performed by the infant's parents. In the study by Matussek *et al.* (2007) more infants were still colonized with the same *S. aureus* as the HCWs than with *S. aureus* from their own family. The HCWs in the study by Matussek *et al.* (2007) registered the number of times they touched the newborn infants daily, revealing more physical contact between HCWs and newborn infants than necessary during a day (Gunhild Rensfeldt, unpublished data). Efforts have subsequently been made in the departments to minimize unnecessary physical contact between HCWs and newborn infants, in favor of physical contact between parents and infant. For instance, the father now carries the newborn infant from the mother to the scale and examination table for the first medical examination. This probably have had an effect on transmission as in the Swedish HITS study most newborn infants were colonized with the same *S. aureus* as their own family (table 3, paper III). Newborn infants of parents colonized with *S. aureus* had a significantly increased odds ratio (OR) for colonization than infants of non-colonized parents (OR_{MH}=2.8, 95 % CI 1.8-4.5, adjusted for gender of adult, $p<0.001$). The increased risk of infant colonization has previously been related to maternal colonization (Peacock *et al.* 2003).

Paper III revealed a marked improvement in compliance between baseline (41 % for barrier precautions and 93 % for the dress code) and follow-up (86 % for barrier precautions and 99 % for the dress code). Despite the improvement in compliance the transmission of *S. aureus* from HCWs did not decrease. Both at baseline and follow-up approximately 25 % of the colonized newborn infants were colonized with an *S. aureus* of the same *spa* type as an HCW and/or someone from another family (table 3, paper III), indicating transmission directly or indirectly from HCWs. Studies have shown that the greatest benefits from hand hygiene occur during the increase from very low levels to approximately 20 % (Cooper *et al.* 1999; Beggs *et al.* 2008). Also, a mathematical model showed that most staphylococcal outbreaks could be prevented if the compliance rate was approximately 55 % (Beggs *et al.* 2008). With the relatively high compliance rates already at baseline in paper III an effect on transmission might be difficult to detect.

Although the compliance with hygiene guidelines is unknown from the time of the study by Matussek *et al.* (2007), it is thought to be lower than at baseline in paper III. To improve the compliance with hygiene guidelines a large national campaign was launched between the study by Matussek *et al.* (2007) and the study described in paper III, which probably increased the compliance already prior to the baseline measurement. This supposedly improvement might also have had the largest effect on transmission.

The quality of the hand hygiene was not monitored during direct observations and it is therefore possible that although compliance improves over time the technique is continuously poor. The hygiene intervention did include hand hygiene technique practice and assessment (by an infection control nurse) with visualization of the coverage of hand rub on the hands using a fluorescent additive to the hand rub and an ultraviolet device (paper II). At baseline the HCWs were unaware of when the direct observations were performed. The hygiene intervention revealed that monitoring of the compliance would henceforth be performed weekly by direct observations. It has been shown that a key factor in improving compliance with hygiene guidelines is that the HCWs are aware that they are being monitored (Walker *et al.* 2014). The Hawthorn effect, i.e., the possibility that HCWs change their behavior when they know they are being observed, has also been demonstrated to have an effect on compliance rates (Srigley *et al.* 2014). It is therefore possible that this effect have led to an overestimation of compliance rates at point of stability and follow-up, in papers II and III, respectively. However, it is assumed that the Hawthorn effect only has a transient effect on the human mind (Srigley *et al.* 2014). The compliance rates slowly improved after the hygiene intervention and stabilized at a satisfactory level more than six months later. During this time the Hawthorn effect most likely would have subsided and therefore the compliance rates at follow-up (paper III), when the transmission routes were re-examined, most likely do reflect the true compliance with hygiene guidelines.

Paper III only reports the transmission of *S. aureus* from healthy individuals to newborn infants. Therefore, the transmission patterns from infected individuals could not be evaluated here. Infected individuals are more likely to have higher loads of bacteria, thereby posing an increased risk for transmission. Also, the effect on nosocomial infections by the improved compliance with hygiene guidelines could not be evaluated. However, the incidence of nosocomial infections had probably been low, as the obstetric departments primarily handles healthy patients, and a detectable decrease might have been difficult to identify. Decreased incidence of nosocomial infections by improved compliance rates has previously been described (Pittet *et al.* 2000; Kirkland *et al.* 2012). The main advantage of the HITS study (paper III) is the lack or very few bacteria present on the newborn infant as this makes the tracking of colonization easy. It is also the disadvantage, as infants most likely very easy get colonized with the first bacteria they

encounter. It is therefore unknown to what extent newborn infants substitute their initial flora once they have been living at their home for a period of time. The commensal flora of an adult act as a barrier to colonization with other bacteria or strains. It appears as if the match between the bacteria and the host is very specific (van Belkum *et al.* 2009) which might indicate that the definite flora is not established directly at birth. This theory is further emphasized by the fact that persistent carriage is almost non-existing in newborn infants (Lebon *et al.* 2008).

No *S. aureus* were detected in the 24 000 L of air sampled in paper III and although there were indications of transmission from the environment (table 3, paper III) this seems to have been a minor problem. Therefore the truth that has been accepted and preached in infection control over the last few decades, that the main bacterial transmission route within healthcare settings is through the hands of the HCWs, also appears to be true here.

It has been shown that persistent nasal carriers have an increased risk of infection as compared to non-carriers (Kluytmans *et al.* 1995; Wertheim *et al.* 2004a; Skramm *et al.* 2014) and that >80 % of the infections are caused by the carrier strain, as a result of endogenous transmission (von Eiff *et al.* 2001; Wertheim *et al.* 2004a). Eradication of nasal carriage has proven effective in the reduction of endogenous infections (Talon *et al.* 1995; Kluytmans *et al.* 1996a; Kluytmans *et al.* 1996b). Infections caused by strains other than the one detected in the nares of the patient self, have been suggested to originate from HCWs and/or fellow patients (Perl *et al.* 2002). However, paper IV demonstrates that 7 % of the *S. aureus* transmissions originated from individuals colonized in the throat only. As 34 % of the transmissions originated from nares-only carriers (table 3, paper III) an increased risk of transmission from the nares as compared to the throat was demonstrated (OR=4.8, 95 % CI 1.8-12.7). The majority of transmissions (61 %) did, however, occur from individuals colonized in multiple sites. The fact that transmission from throat is demonstrated may indicate a risk for droplet transmission, from HCWs and parents by coughing or sneezing. The high prevalence of throat colonization in combination with the indication that transmission from the throat occurs, further emphasizes the importance of including throat samples in screening programs. Eradication of throat colonization may also be necessary to prevent endogenous *S. aureus* infections and nosocomial transmission, as suggested in a recent review article (van Rijen *et al.* 2008).

Concluding remarks and future perspectives

Colonization of newborn infants with *S. aureus* was used as a model for bacterial transmission in a healthcare setting and to evaluate the impact of compliance with hygiene guidelines on transmission.

Performance of bacteriological methods

To determine more truthful *S. aureus* colonization rates, incubation of swabs in enrichment broth prior to plating is necessary, as this procedure increased the number of *S. aureus* positive samples, regardless of which site was cultured. This may have an impact on routine diagnostic procedures at microbiology laboratories.

spa typing is well suited for epidemiological investigations in routine microbiological laboratories thanks to its great discriminatory power and epidemiological concordance as well as its accessibility, ease of use and rapidity.

S. aureus *epidemiology*

The prevalence of *S. aureus* colonization was higher in men compared to women and the throat was the premier colonization site among adults, followed by anterior nares. Also, transmission from the throat was demonstrated. This highlights the importance of including throat cultures in *S. aureus* screening programs for adults.

Infant colonization began immediately at birth and reached adult prevalence within 24 h to 48 h. To determine *S. aureus* colonization among newborn infants, cultures from the umbilicus is most important, as 95 % of the colonized infants were detected by exclusive analysis of the umbilicus compared to 25 % by exclusive analysis of anterior nares.

No institutional *S. aureus spa* type prone to transmission was detected, as the *spa* type distribution and antibiograms were similar among newborn infants, parents and HCWs. Also pregnancy did not appear to influence *S. aureus* colonization, as colonization rates, *spa* type distribution and antibiograms were similar among mothers and HCWs (mainly women).

To evaluate true transmission in healthcare settings knowledge of the distribution of strains, e.g., through *spa* typing, in the community is required.

The commensal flora is considered to act as a barrier to colonization with other bacteria or strains. It would be interesting to investigate what *S. aureus spa* types colonizes the infants a period of time after birth to determine whether the initial colonizing flora act as a barrier to colonization also for infants.

Infection control in healthcare settings

Direct observations and self-reporting by HCWs are both valid methods to monitor compliance with hygiene guidelines.

The compliance with hygiene guidelines was markedly improved by a multimodal and multidisciplinary hygiene intervention and a long-term effect was achieved, but might require continuous and varied reminders to be completely sustained. For instance, workshops, one part of the hygiene intervention, are now routinely used by infection control nurses locally as well as in other parts of Sweden.

The compliance with barrier precautions is not the only factor to be considered in prevention of bacterial transmission, as the increase of compliance from 40 % to more than 80 % did not reduce the transmission of *S. aureus* from HCWs to newborn infants. Factors that might affect transmission are the timing and technique of hand hygiene as well as correct glove use. The fact that transmission from throat was demonstrated may indicate a risk also for droplet transmission, not studied in this thesis.

As this thesis focuses on transmission from healthy colonized individuals to newborn infants it may not reflect transmission patterns from infected individuals. As the load of bacteria most likely affects the risk of transmission, infection would increase the risk of transmission. Therefore, it is of utmost importance that all HCWs comply with hygiene guidelines at all time to ensure patient safety, especially as the high compliance can be reached and maintained at a regular budget.

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