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Staphylococcus aureus colonisation and host-microbe interactions

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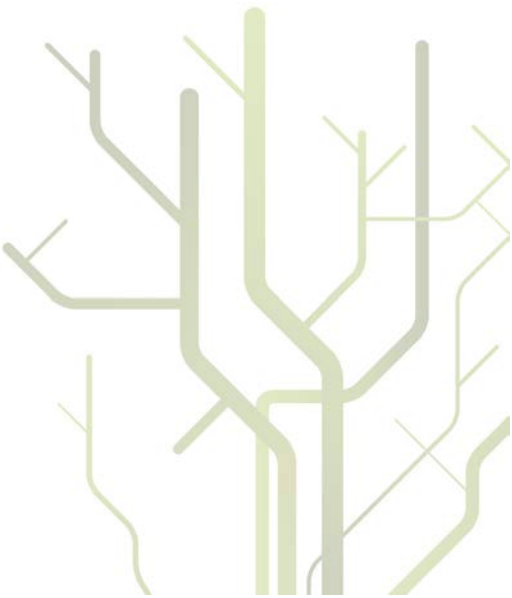

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CONTENTS

PREFACE

Why are some people carriers of *Staphylococcus aureus* while others are not? Is the pathogenesis of *S. aureus* just a mishap, or is it a well-regulated performance showing us the versatility of this commensal bacterium? Even after all these years of research, we don't know for sure, and new insights are needed to completely answer these and other questions regarding *S. aureus* colonisation and infection. As treatment of *S. aureus* infections can be challenging and may even result in treatment failure, it is of the utmost importance to prevent infections from arising. By gaining insight on *S. aureus* nasal colonisation and the close interactions between the microbe and the host, we hope that eventually, a better understanding may provide us with novel means for targeted intervention and treatment.

LIST OF PAPERS

Paper I

Maria Sangvik, Renate Slind Olsen, Karina Olsen, Gunnar Skov Simonsen, Anne-Sofie Furberg, and Johanna U. Ericson Sollid. 2011. Age- and gender-associated *Staphylococcus aureus spa* types found among nasal carriers in a general population: The Tromsø Staph and Skin Study. J. Clin. Microbiol. **49**(12):4213-4218

Paper II

Karina Olsen, Maria Sangvik, Gunnar Skov Simonsen, Johanna U. Ericson Sollid, Arnfinn Sundsfjord, Inger Thune and Anne-Sofie Furberg. 2012. Prevalence and population structure of *Staphylococcus aureus* nasal carriage in healthcare workers in a general population. The Tromsø Staph and Skin Study. 2012. Epidemiol. Infect. doi:10.1017/S0950268812000465

Paper III

Marit Sørum, Maria Sangvik, Marc Stegger, Renate S. Olsen, Mona Johannessen, Robert Skov, and Johanna U. Ericson Sollid. *Staphylococcus aureus* mutants lacking cell-wall bound protein A found in isolates from bacteraemia, MRSA-infection and a healthy nasal carrier. Submitted.

Paper IV

Fatemeh Askarian, Maria Sangvik, Anne-Sofie Furberg, Anne-Merethe Hanssen, Johanna U. Ericson Sollid and Mona Johannessen. *Staphylococcus aureus* TIR-domain protein, TirS, negatively interferes with TLR2-, MyD88- and TIRAP-mediated NF-κB signaling and increases intracellular bacterial accumulation. Submitted.

INTRODUCTION

SYMBIOSIS BETWEEN HOST AND MICROBE

In humans, mammalian cells are outnumbered by microbes by a factor of ten; on average an individual consists of 10^{13} mammalian cells and 10^{14} culturable microbial cells. Most body surfaces that are exposed to the external environment are colonized by microbes. However, the number of microbes and the variety of species present depends on the particular body site. Most of the microbes living with humans inhabit the gastro-intestinal tract, with one million times more microbes than what is typically found on the skin. The microbes found at a body site are known as the indigenous microbiota, or the normal flora of that site, and consists of bacteria, archaea, viruses, fungi and protists. When we are born, a life-long symbiosis between us and the microbes starts. There are three different types of symbiosis; mutualism – where both parts benefit, commensalism - where one part benefits and the other is left unaffected, and parasitism – where one part benefits and the other part suffers. Through a lifetime, we will encounter all the three types of symbiotic relationships with different members of our normal flora. Several factors may affect the number and types of microbes at a specific body site, including age, gender, host genotype, hormones, diet, hygiene, clothing, climate, occupation and living conditions. Elderly people often experience a decrease in the efficiency of the immune system and various organ dysfunctions, as well as being more prone to malnutrition, reduced hygiene and increasing use of medical devices such as catheters. These factors may affect the indigenous microbiota of their body. Differences in the composition of the normal flora between males and females are found, and may involve hormones, anatomy, behaviour or other physiological factors (242).

TROMSØ STAPH AND SKIN STUDY

The Tromsø Staph and Skin Study (TSSS) was initiated to investigate microbe, host and environmental factors that are involved in *Staphylococcus aureus* colonisation of healthy adults as well as subsequent infection (Figure 1). TSSS is a cross-sectional study, and was performed as a part of the sixth Tromsø Study which was undertaken from October

2007 to December 2008. The Tromsø Study is a multipurpose, longitudinal study, based on the population in the municipality of Tromsø, Norway, at 69°N, and was initiated in 1974 to determine causes of the high cardiovascular mortality. Later surveys have had increased emphasis on several chronic diseases and conditions, including cardiovascular diseases, diabetes mellitus and osteoporosis. The sixth Tromsø Study invited randomly chosen participants aged 30 to 87 years in the municipality of Tromsø to participate in a health survey, with a total of 12,984 attendees (65.7 % of the invited). Clinical examinations, blood samples, questionnaires and interviews were included, and all procedures were performed by trained technicians (89). TSSS was conducted from October 2007 through July 2008 and included all attendees aged 30-49 years and random samples of older attendees, with a relative distribution of birth cohorts as in the municipality (156). To assess *S. aureus* colonisation, baseline nasal swab cultures were collected from 4,026 participants (2,285 women and 1,741 men). A second sample was collected from 2,997 of the participants (1,712 women and 1,285 men), to determine the *S. aureus* carrier status. The median time between baseline and the second screening was 28 days. In this study, the term "colonisation" included both intermittent and persistent colonisation whereas the term "carrier" was used for participants with two positive nasal samples.

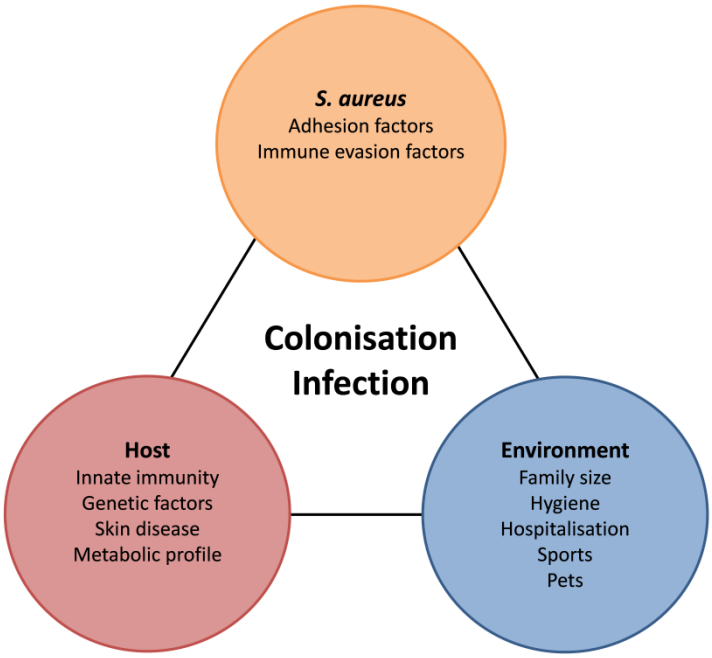


Figure 1. Host-microbe-environment interplay. Suggested interactions between microbial, host and environmental risk factors involved in *S. aureus* colonisation and infection. Based on (158).

STAPHYLOCOCCUS AUREUS

General characteristics

Staphylococci belong taxonomically to the family of *Staphylococcaceae*, and are Gram-positive, catalase-positive cocci with a GC-content of 30-35%. Currently, 47 species and 24 subspecies of the genus *Staphylococcus* have been described (http://www.bacterio.cict.fr/s/staphylococcus.html, accessed 21. Sept. 2012). Among the staphylococci, *S. aureus* is easily identified by its ability to produce coagulase and hence clot human plasma (243).

Host specificity and host range

The nares are thought to be the main ecological niche and the largest reservoir of *S. aureus* in humans, but multiple body sites can harbour this bacterium (222). *S. aureus* is a common inhabitant of the skin (241) and perineum (172), and can also be found in the axillae (172, 241), vagina (71) and the gastrointestinal tract (241). Several studies have indicated that colonisation of the throat is more prevalent than colonisation of the anterior nares (75, 110, 124, 147). *S. aureus* is also known to colonise and infect both pets and livestock, including dogs, cats, rabbits, horses, cattle and pigs (140). A major concern is the presence of methicillin resistant *S. aureus* (MRSA) in pets and livestock, as these may serve as reservoirs for human colonisation, exemplified by ST398 from pigs (229).

As human lineages of *S. aureus* are not so commonly found in animals, and vice versa, there are most likely some host range barriers. A microarray study revealed that although host specificity seems to be lineage specific, animal lineages are closely related to human lineages, and that host specificity may be attributable to only a few genes or gene combinations (209). Surprising similarity has been found among adhesion and immune evasion genes from different animal hosts, exhibiting very different target proteins, suggesting that these proteins are not essential for virulence (127). A study on isolates from farmers and cows found that the emergence of a new bovine-adapted genotype was the result of a host shift from humans to cows, indicating that host specificity is a trait that may undergo changes (184).

Clinical significance

S. aureus is a major human pathogen and is potentially able to infect any tissue of the human body, causing everything from skin infections to life-threatening diseases. The infections caused by *S. aureus* can be divided into three general types: 1) superficial lesions, e.g. surgical site- and wound infections; 2) systemic and life threatening conditions, e.g. endocarditis, osteomyelitis, pneumonia, brain abscesses, meningitis and bacteraemia; and 3) toxinoses, e.g. toxic shock syndrome, food poisoning and scalded skin syndrome (2). The hallmark of staphylococcal infection is the abscess, containing pus which consists of dead neutrophils, living and dead bacteria, necrotic tissue, and the contents of lysed host and bacterial cells (153). Immunocompetent hosts will in most cases successfully clear the infection and drain the abscess, whereas for the immunocompromised and occasionally for a healthy individual, the infection may progress into deeper tissues and become a potentially fatal invasive infection (153).

S. aureus infections usually involve a carrier, either by autoinfection - developing an infection with their own carrier strain, or by causing cross-infections – when their strain is transmitted to and infects another individual. Globally, *S. aureus* is the cause of a large proportion of bloodstream infections (22%), and skin and soft tissue infections (39%) (39). In Norway, *S. aureus* is the second most common blood culture isolate, accounting for 14.5% of the isolates when skin contaminants are excluded (148).

Methicillin resistant *S. aureus* has been a topic of concern for several years, being a large burden for most healthcare institutions around the world, with higher mortality, morbidity and financial costs compared to methicillin-susceptible *S. aureus* (MSSA) (68). The MRSA rates have been increasing rapidly worldwide during the last decades (202). However, data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) from 2002 to 2009, indicate that there is a significant reduction in the proportion of MRSA overall in the participating countries (56). MRSA infections used to be a hospital-related problem (healthcare-acquired/associated (HA) MRSA) but lately there has been an increase in MRSA infections in the community (community-acquired/associated (CA) MRSA) and from livestock (livestock-associated (LA) MRSA) (202). In general, antimicrobial resistance rates are significantly higher among HA isolates of *S. aureus* than for CA isolates, implying that the hospital isolates are

epidemiologically distinct from community isolates and that there is a resident microflora in the hospitals (165).

Transmission

A typical transmission route of *S. aureus* is from the nose to the hand of a person, then to a surface (e.g. a door knob), before being transferred via the hand to the nose of a second person. For a successful transmission, *S. aureus* must adapt to different environments, and survive stress factors like nutrient limitation, desiccation, changes in temperature, osmolarity and pH, interference from other bacteria, and the antimicrobial actions of the human body (84). Transmission of *S. aureus* from host to host is less efficient than transmission of the related human coloniser *Staphylococcus epidermidis*, illustrated by the following three points; 1) *S. epidermidis* resides on the skin and only requires direct contact between two hosts for transfer; 2) *S. epidermidis* colonises all humans, and there are no known host barriers preventing colonisation by this organism, whereas *S. aureus* has a limited number of potential hosts; 3) Interference between Agr groups in genetically diverse *S. aureus* strains may inhibit colonisation with new strains, whereas this has not been shown in *S. epidermidis* (125). The complex transmission of *S. aureus* has been hypothesized to explain its evolution and maintenance of virulence (125).

Genomic content

The genome size of *S. aureus* typically varies from 2.5 to 3.1 Mb, and contains ~2,500 open reading frames. Since the first two *S. aureus* genome sequences; N315 and Mu50, were published in 2001 (107), other genome sequences followed rapidly: MW2 (7), MRSA252 and MSSA476 (82), COL (59), USA300-FPR3737 (40), USA300-HOU-MR (81), NCTC8325 (60), ET3-1 (80), JH-1 and JH-9 (143), Newman (6) and TW20 (83). Today, full genome sequencing has become routine, and the number of sequenced genome drafts is exploding, however only a subset of these are fully annotated and completed (20). The *S. aureus* genome consists of 1) core genes, conserved between the different lineages; 2) core variable (CV) genes, genes that vary between genomes or may even be missing; and 3) mobile genetic elements (MGEs), fragments of DNA encoding toxins,

Table 1. Mobile genetic elements (MGEs) in *S. aureus*. Based on (121, 202) and references therein.

MGE	Attributes	Examples
Bacteriophages encoding toxins	Lytic: complete bacterial lysis Temperate: long-term relationship with cells Chronic: release progeny without killing the host 1-4 per strain, large impact on *S. aureus* diversity and evolution	Staphylococcal complement inhibitor (SCIN), chemotaxis inhibitory protein (CHIPS), staphylococcal enterotoxin A (SEA), Panton-Valentine leukocidin (PVL)
Pathogenicity islands (SaPIs)	Phage-like, but lack genes for capsid heads and tails necessary for horizontal transfer 0-2 per strain	Enterotoxins, toxic shock syndrome toxin (TSST)
Plasmids	Carry antimicrobial resistance determinants, toxins and/or determinants involved in metabolism	3 plasmid groups: 1) small multicopy plasmids; 2) large low-copy plasmids; 3) conjugative multiresistance plasmids
Staphylococcal cassette chromosome (SCC) elements	Large fragments of DNA, often encoding antimicrobial resistance and/or virulence determinants The CcrAB or CcrC recombinases ensure site-specific integration at the *attBscc* site within *orfX* of the *S. aureus* chromosome	SCC*mec* types I-XI, SCC mercury
Genomic islands	Flanked by a broken transposase gene upstream and partial restriction-modification (RM) system type I downstream	Three families: vSAα, vSAβ, vSAγ. Encodes eg. staphylococcal superantigen-like genes, bacteriosins and enterotoxins, and phenol-soluble modulins, respectively
Arginine catabolic mobile element (ACME)	Encodes an arginine deaminase pathway	
Insertion sequences (IS)	Can exist independently in the genome of *S. aureus*, but are often present as pairs constituting a composite transposon May cause changes in the expression of genes in the core genome	IS*256*, IS*257*
Transposons	Mainly encode antimicrobial resistance genes in *S. aureus* Inserted into the chromosome or into mobile genetic elements	Tn*554* (*erm*), Tn*1546* (*vanA*)

virulence factors and genes involved in host adaption as well as mobilisation functions (114, 115, 121).

The core genome contains approximately 80% of the *S. aureus* genes, including genes for surface proteins involved in adhesion, as well as genes encoding essential metabolic and regulatory properties (58). As a part of the core genome, the core variable (CV) genes make up 10-12% of the *S. aureus* genome, and often encode regulators of virulence genes or surface proteins involved in host interactions during nasal colonisation, such as the surface protein staphylococcal protein A (*spa*) (115).

The accessory genome accounts for the remaining 20% of the *S. aureus* genome, consisting of MGEs containing 50% of known virulence factors in *S. aureus*. The MGEs include e.g. bacteriophages, pathogenicity islands, plasmids and transposons (Table 1), and are capable of horizontal transfer between strains (58). Exchange of virulence factors between strains, resulting in different virulence factor combinations, contributes to adaption of clones specialised for infection of selected hosts or environments (80, 83).

POPULATION STRUCTURE OF *S. AUREUS*

Bacterial population structures range from clonal populations to those that are a result of free recombination, and include all variations in between. A strictly clonal population has no exchange of genetic material, and reproduces by binary fission of the mother cell into two daughter cells. The only source of variation is mutations, which are vertically inherited and accumulated and hence give rise to clonally divergent lineages. However, most bacterial species have mechanisms for exchange of genetic material, or horizontal gene transfer (HGT) and can potentially recombine with any other member of the population (72).

Bacterial population structure can be interpreted by the use of different typing methods to obtain an understanding of other characteristics of the bacterial population, such as host specificity, pathogenicity, epidemic potential and the presence of virulence genes (126). Hence, to understand *S. aureus* nasal carriage and its relation to infection of the host, the population structure needs to be defined. Several large typing studies with different methods have been performed on *S. aureus*, revealing an essentially clonal

population (46, 52, 70, 130, 141). The first study to describe the population structure of naturally occurring MSSA isolated from the nose of healthy adults revealed 3 major and 2 minor genetic clusters of *S. aureus* by using amplified fragment length polymorphism (AFLP) clustering (130). The results corresponded well to the multilocus sequence typing (MLST) based clonal complexes (CCs) defined by studies of carriage and invasive MSSA and MRSA isolates, mainly from the UK, with 5 of the main CCs being CC8, CC30, CC5, CC22 and CC45 (50, 51, 175). A microarray based on all genes from the seven current *S. aureus* sequencing projects was used to investigate isolates from 100 healthy carriers as well as 61 community-acquired isolates had a higher resolution, and identified 10 dominant lineages corresponding to MLST CC1, CC5, CC8, CC12, CC15, CC22, CC25, CC30, CC45 and CC51 (115). *S. aureus* population structures from colonised persons in different parts of the world, indicate that there is a large geographical divergence in the most commonly found CCs (49, 50, 130, 181).

A study of 37 unlinked loci including the MLST loci in 30 well-characterised diverse strains resulted in a unrooted Bayesian reconstruction of *S. aureus* phylogeny, subdividing the species into two distinct groups, with group 1 subdivided into groups 1a and 1b, supporting the population structures previously obtained using MLST genes, AFLP clustering and microarray analysis (33). To summarise, *S. aureus* has a clonal population structure with a limited number of major lineages colonising the human population.

Molecular typing

The *S. aureus* population structure has been investigated by several different methods, including multilocus enzyme electrophoresis (MLEE), pulsed field gel electrophoresis (PFGE), MLST, DNA microarrays and *spa* typing. When typing, the underlying assumption is that there is only one evolutionary history, which is true for a clonal population. Genes acquired by HGT will have another evolutionary history than those inherited from mother cell to daughter cell, and to find clonal relationships of strains, genes subject to vertical transfer, such as housekeeping genes, have been preferred (72). However, a study by Cooper and Feil compared 37 loci and found no strong association between gene function and phylogenetic reliability, indicating that not only

housekeeping genes may be used to infer intra-species lineage assignments (33). When interpreting typing results, it is important to have knowledge of the natural bacterial population structure (70).

By defining single base pair differences not found elsewhere in the MLST database as mutations, and multiple base pair differences and alleles found in unrelated CCs as recombinations, it was estimated that the MLST alleles of *S. aureus* change 15 times more often by mutation than by recombination (50). However, the 7 MLST loci do not represent the entire genome, and a recent genome-wide SNP analysis estimated a relative rate of 0.6 for homologous recombination compared to mutation rate (212).

By the use of molecular typing techniques, the spread of clones in hospitals and in the community can be identified and kept under surveillance. In outbreak situations, epidemiological typing can be used to find the transmission modes of the epidemic clones, and to monitor the reservoir of the infectious agent. For epidemiological surveillance, typing systems reveal the prevalence of pandemic, endemic or epidemic clones in the population and in different geographical areas (205). Different applications may have different requirements, but in general, a typing regime requires proper typeability, reproducibility, discriminatory power and stability, and it should be easy to interpret and use (204). Today, a range of techniques are in use for typing of staphylococci, with different strengths and weaknesses (Table 2).

From the early bacteriophage typing studies in the 1950s, based on the ability of bacteriophages to lyse different staphylococci, 'epidemic types' of staphylococci were first identified, giving us hints on the staphylococcal population structure (233). Thereafter, studies using MLEE brought insight on *S. aureus* population structure many steps further. MLEE is based on separating extracts of bacterial proteins by electrophoresis. The gel is then sliced in several layers, and the sections are stained to detect housekeeping enzymes essential for cell viability and growth. One detects allelic variants by observing changes in the electrophoretic migration compared to known alleles (191). Typically, 15-25 enzymes are selected to obtain a high level of differentiation between strains (45).

PFGE, AFLP, multiple locus variable number tandem repeat analysis (MLVA), repetitive element PCR, and random amplified polymorphic DNA (RAPD) are all methods where

variation in the nucleotide sequence is detected indirectly by primer-binding and/or restriction sites, and are often referred to as band-based methods or molecular fingerprinting (200). In PFGE, chromosomal DNA is digested with a restriction enzyme that cleaves infrequently, to obtain large fragments which after being exposed to a switching electric field on an agarose gel produce a banding pattern or fingerprint (8). As a highly discriminatory method, it has been widely used for typing of staphylococci, and has been considered to be the gold standard in typing of *S. aureus* outbreak investigations (215). However, the comparison of data from fingerprints run in different laboratories can be challenging, and in addition the interpretation of the results is still quite subjective. AFLP is a PCR-based strategy, where genomic restriction fragments are detected by PCR amplification using generic primers (227). In MLVA, gene targets with short tandem repeats are used to make DNA profiles. Several MLVA schemes for typing of *S. aureus* have been used (54, 182, 189). Repetitive element PCR, or rep-PCR, is a PCR-based method, amplifying specific regions between noncoding repetitive sequences to obtain a DNA fingerprint pattern of PCR products from 150 to about 5,000 bp (179).

DNA sequence-based typing methods are of great value for bacterial population genetics. MLST makes use of the same genetic principles as MLEE, but differentiates alleles at the DNA level, by sequencing internal fragments of housekeeping genes (120). MLST was first applied in *S. aureus* in a study by Enright *et al*. (46). The sequences of the fragments of seven housekeeping genes are compared to known alleles at the MLST website (www.mlst.net), and an allelic profile, referred to as a sequence type (ST) is obtained. As an example, *S. aureus* ST30 has the allelic profile 2-2-2-2-6-3-2. eBURST is an algorithm used to cluster related STs into clonal complexes (CCs) (51). As housekeeping genes are essential for the bacterial cells, accumulation of genetic variation is limited to keep the functionality of the proteins, and this stability makes the MLST allelic profile suitable for studies of global epidemiology. However, MLST does not have sufficient discriminatory power to be used in *S. aureus* outbreak situations (131).

spa typing is a sequence-based method, where the variable number tandem repeat (VNTR) region of Staphylococcal Protein A is analysed, and a *spa* type is assigned based on the order and number of the short, typically 24 bp repeats (Figure 2). *spa* typing has discriminatory power comparable to that of PFGE, and can be used both for outbreak

Table 2. Some examples of current typing methods for *S. aureus*. Based on (72, 202, 206).

Method	Target	Strengths	Weaknesses
spa typing	Sequence polymorphism in the variable X region of the gene encoding *S. aureus* Protein A	Rapid, high throughput, standard nomenclature, interlaboratory reproducibility	Misclassification of particular lineages due to recombination/homoplasy
Multilocus sequence typing (MLST)	Sequence determination of allelic variants of seven housekeeping genes	Interlaboratory reproducibility, standard nomenclature	Low throughput, high cost
Pulsed-field gel electrophoresis (PFGE)	Polymorphisms in restriction sites on the chromosome	High discriminatory power	Technically demanding, time-consuming, limited interlaboratory reproducibility, multiple nomenclatures
Multilocus VNTR analysis (MLVA)	Polymorphism in chromosomal VNTR elements	Rapid, high throughput	No international standard protocol or nomenclature, misclassification of some lineages
Microarray	Whole genome or selected targets in the genome, depending on the array design	Flexible, high discriminatory power	Design of arrays require knowledge of genome content and variation
Whole genome sequencing (WGS)	Whole genome	Extremely high discriminatory power	Demanding data interpretation

VNTR, variable number of tandem repeats

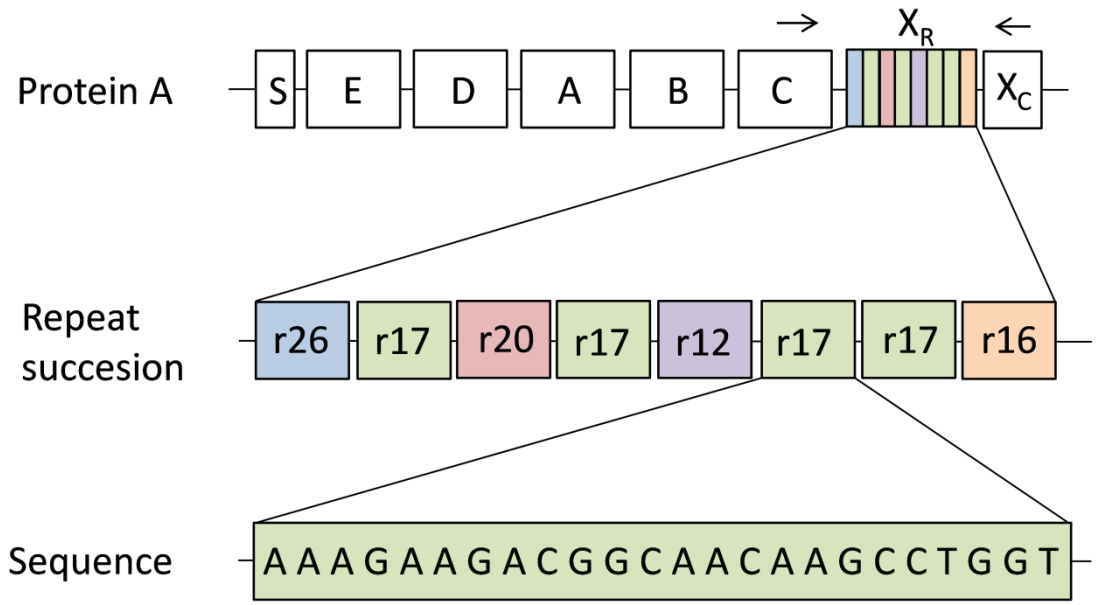


Figure 2. The principle of *spa* typing. The VNTR repeat region X_R of Protein A is the basis for *spa* typing. This region consists of a number of short repeats, and the number of repeats as well as their order determines the *spa* type. The particular repeat succession in the figure represents *spa* type t003. Arrows indicate the primers used in *spa* typing.

investigations as well as population studies due to the slow accumulation of point mutations and relatively fast changes in repeat numbers (103). However, recombination may disturb the congruence between *spa* types and sequence types/clonal complexes on some occasions (174).

Microarrays can also be used for population analysis. As the DNA microarray systems based on the whole genome of *S. aureus* provide a large amount of information for which data analysis may be complicated, several smaller DNA microarrays have been developed; focusing on e.g. detection of genes associated with virulence, antimicrobial resistance or adhesion, *agr* alleles, MSCRAMMS, capsule types or assigning isolates to an MLST CC or ST and SCC*mec* type (41, 139, 186).

Whole genome sequencing (WGS) has an extremely large discriminatory power, and has been proven to be a valuable research tool (97). WGS is rapidly evolving, and has

already been evaluated for typing *S. aureus* strains by SNP analyses (48, 77, 104). The main challenge is the need for data interpretation.

A quite recent approach is matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF-MS), which analyses surface-associated proteins by mass spectral analysis and can be used on intact bacterial cells (168). A MALDI-TOF-based typing scheme has been established, covering the most abundant HA-MRSA lineages (244).

The various typing methods differ when it comes to discriminatory power, accuracy and reproducibility, costs and technical challenges. Which method is the most appropriate to study *S. aureus*, depends on the study question asked. For local studies of population structure and short-term outbreaks, it is advantageous to use a method based on hyper-variable loci, such as *spa* typing, whereas for global population studies and long-term studies, methods based on stable housekeeping genes (such as MLST) are preferred.

S. AUREUS NASAL COLONISATION

Already in 1931, the association between *Staphylococcus aureus* nasal carriage and staphylococcal infection was reported by Danbolt (37). More recently, Feil *et al.* analysed a sample of 334 *S. aureus* isolates from carriage and community-acquired disease in the same population, and found that the isolates from carriage had a population structure similar to the isolates from disease (50). A subset of these isolates was also examined by comparative genomic hybridisation (CGH) to find the presence of putative virulence genes. However, no marker or specific lineage associated with disease was found (115). It seems as the frequency of any *S. aureus* strain in the human population influences its potential to cause invasive disease, and the importance of its virulence factors remains unclear, whereas host susceptibility is thought to play an essential role (45). Von Eiff *et al.* found, in a study of *S. aureus* bacteraemia, that in more than 80% of the cases, blood isolates were identical to isolates from the patient's anterior nares, indicating that most cases of *S. aureus* bacteraemia are of endogenous origin (226).

Epidemiology of *S. aureus* colonisation

Within a healthy population, ~20% (ranging from 12-30%) are reported to be persistently colonised in the anterior nares, whereas ~30% (16-70%) are intermittent carriers and ~50% (16-69%) are non-carriers (47, 86, 101, 157, 234). The proportions of intermittent and non-carriers have a very wide range, resulting from differences in culture methods, populations studied and interpretation guidelines (223). In 2004, a culture rule stating that 2 nasal swab cultures accurately predicted the persistent *S. aureus* carriage status was proposed (152).

In persistent carriers, the mean number of colony forming units (CFU) has been reported to be higher than in intermittent carriers (238), resulting in an increased risk of spreading staphylococci to the surroundings (239). It has also been shown that the genotypes of *S. aureus* isolated from repeated cultures from intermittent carriers differ more often than from persistent carriers (47), indicating that there may be differences in the determinants of persistent and intermittent carriage. Some individuals may even carry their resident strain for several years (223). In 2009, van Belkum *et al.* suggested a paradigm shift, where *S. aureus* nasal carriage types were reclassified to only two groups; persistent carriers and others (219). This was based on results where intermittent carriers and non-carriers shared both antistaphylococcal antibody profiles and responses to inoculation with an *S. aureus* mixture, as well as the previously described higher risk of infection among persistent carriers than in intermittent and non-carriers (151, 226, 236).

In volunteers first undergoing *S. aureus* eradication, then artificial inoculation with a mixture of *S. aureus* strains, the original persistent carriers were found to become colonised with their original strain from the inoculation mixture and become carriers again, while the non-carriers quickly eliminated *S. aureus* cells from their nares (150).

The prevalence of nasal carriage with *S. aureus* varies between different groups, and is higher among men (32, 158), white people (32) and infants (162). The prevalence is also higher in hospitalised patients, persons with atopic dermatitis (240), HIV-infected patients (197), patients with diabetes mellitus (116), and in those undergoing haemodialysis (99) or in need of chronic ambulatory peritoneal dialysis (119). Protective factors for *S. aureus* nasal carriage include smoking (13, 22, 156) and a high

serum vitamin D level (156). The habit of nose-picking (235) as well as the use of oral contraceptives (22) have been found to be positively associated with the risk of *S. aureus* nasal carriage.

Mode of growth

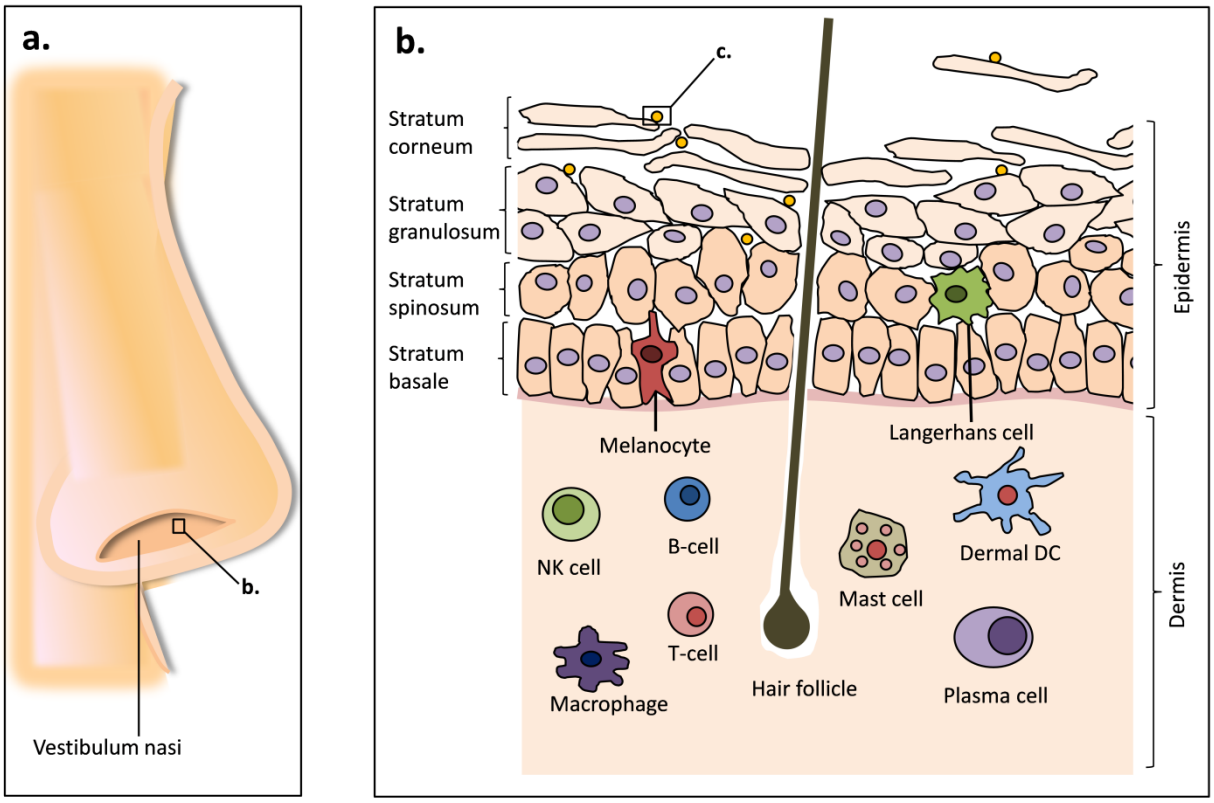
Most of the results from recent studies support a dispersed mode of growth, rather than growth in biofilms, during *S. aureus* nasal colonisation (Figure 3). The number *of S. aureus* colonies obtained from a nasal swab is relatively low, with a mean value of less than 100 CFU in intermittent carriers, and less than 10,000 CFU in persistent carriers (152). These numbers would be expected to be much higher if a biofilm was encountered (106). In addition *S. aureus* has been detected in the nose of cotton rats (15) and nasal tissues from human corpses (214) by microscopy, and in both cases, the bacteria were typically found as single cells or in small clusters, and no biofilm formation was observed.

Determinants of *S. aureus* nasal colonisation

Only a subset of the human population is persistently carrying *S. aureus*, indicating that human factors are involved in determining carriage status. In addition, some bacterial clones are observed more frequently than others, supporting the importance of bacterial factors involved in colonisation and carriage.

The relative importance of bacterial factors, host factors and environmental factors involved in *S. aureus* colonisation and carriage is largely unknown, but it has been suggested that host factors play a key role, whereas bacterial factors may decide which strain is carried rather than the carriage status (162). Mechanisms for establishment and maintenance of nasal colonisation need further elucidation (101, 234).

Shedding of squamous epithelial cells and mucus from the nose leads to constant mechanical clearance of *S. aureus* cells, and in this hostile environment the bacterium needs to proliferate to compensate for the removal (230). In addition, the host's immune defences must be evaded for *S. aureus* to become a persistent coloniser.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b.
c.
Stratum corneum
Stratum granulosum
Stratum spinosum
Stratum basale
Epidermis
Dermis
Melanocyte
Langerhans cell
NK cell
B-cell
Dermal DC
Mast cell
T-cell
Macrophage
Hair follicle
Plasma cell

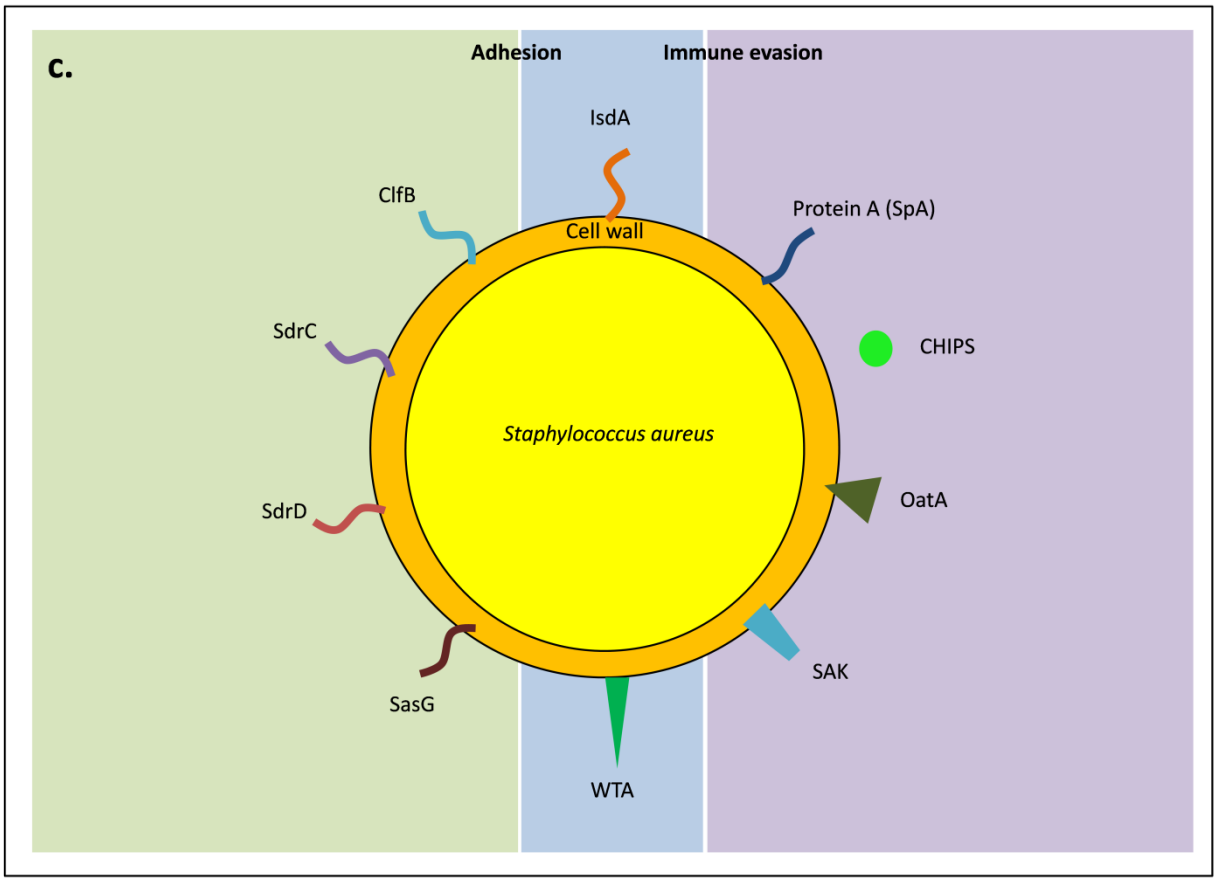
c.
Adhesion
Immune evasion
IsdA
ClfB
Cell wall
Protein A (SpA)
SdrC
CHIPS
Staphylococcus aureus
SdrD
OatA
SAK
SasG
WTA

Figure 3. Nasal colonisation with *S. aureus*. a. Vestibulum nasi, or the nasal vestibule, is thought to be the main niche for *S. aureus* in humans. The nasal vestibule is covered with keratinised epidermis (skin) (214). **b.** *S. aureus* can be found in the epidermis, which consists of several layers of keratinocytes; stratum corneum (the outermost layer), stratum granulosum, stratum spinosum and stratum basale. Langerhans cells are immune cells found in the epidermis, whereas natural killer (NK) cells, macrophages, T-cells, B-cells, mast cells, dermal dendritic cells (DC) and plasma cells are found in the dermis. Figure based on (105, 144). **c.** During nasal colonisation, several *S. aureus* several adhesion factors (green background) as well as factors involved in immune evasion (purple background) are involved, and some factors are shown to be important both in adhesion and immune evasion (blue background). CHIPS: chemotaxis inhibitory protein of *S. aureus*, OatA: O-acetyltransferase, SAK: staphylokinase, WTA: wall teichoic acid, SasG: *S. aureus* surface protein G, SdrD: serine-aspartic acid repeat protein D, SdrC: serine-aspartic acid repeat protein C, ClfB: clumping factor B, IsdA: iron-regulated surface determinant.

Bacterial factors

S. aureus lineages have individual combinations of surface proteins involved in adhesion as well as secreted proteins involved in immune response evasion (127) (Figure 3). In addition, the expression and secretion of proteins in *S. aureus* may vary with different modes of growth (142). During nasal colonisation, genes encoding adhesion and immune evasion determinants are typically expressed, whereas toxins are not (16). The individual combinations of adhesins and immune evasion factors as well as their expression levels may be important in determining the colonisation success of *S. aureus*.

Adhesion factors

For a bacterium to become a persistent coloniser of the human nasal epithelium it must be able to adhere to the skin surface by firm interactions with the human cell surfaces, simply to avoid being eliminated by physiochemical mechanisms (101). The adherence is a multifactorial process, involving different factors during different stages of the colonisation (232).

Wall teichoic acid (WTA) is thought to have an important role in attachment, both in the early stage of colonisation (232), as well as for continued colonisation (231). Using a cotton rat model, a WTA-deficient *S. aureus* mutant failed to colonise the cotton rat

nares, and the same WTA-deficient mutant was less efficient in adhering to human nasal epithelial cells (231).

Adhesive proteins belonging to a class of cell wall-associated proteins named microbial surface components recognising adhesive matrix molecules (MSCRAMMs), have been suggested to be important in the later stages of the colonisation (15), by promoting adhesion to epithelial cells (35). MSCRAMMs typically contain an N-terminal signal sequence and a C-terminal region with a LPXTG-motif, a hydrophobic domain and a charged tail, involved in covalent anchoring of the protein to the cell wall (188). *S. aureus* clumping factor B (ClfB) adheres to human cytokeratin 10 which is a component of squamous cells (155), and a mutant lacking ClfB did not survive in the human nose after 2 weeks (237). Iron-regulated surface determinant A (IsdA) has also been found to bind cytokeratin 10, in addition to loricrin and involucrin which are proteins found in the matrix surrounding the upper layers of epithelium in the nasal cavity (24). ClfB and IsdA have both been demonstrated to promote colonisation of the nares of rodents in *in vivo* models (25, 187), and were expressed during nasal colonisation in humans (16). *S. aureus* surface protein G (SasG) is another surface protein promoting adhesion to nasal epithelial cells (176). However, a mutant *S. aureus* strain defective in IsdA and ClfB and not expressing SasG, could still adhere to human squamous cells at approximately 40% of the level of the wildtype, indicating that other components of the cell surface are likely involved as well (25, 34). The bacterial surface serine-aspartic acid (SD) repeat proteins SdrC and SdrD were then demonstrated to contribute individually to *S. aureus* adherence to squamous cells, and a mutant defective in ClfB, IsdA, SdrC and SdrD was found not to adhere to desquamated nasal epithelial cells (35). Other adhesins interacting with host factors such as fibronectin, fibrinogen, elastin, collagen and von Willebrand factor have been identified (79), but their role in nasal colonisation is not clear (93).

Immune evasion factors

A large variety of secreted proteins involved in immune evasion can be produced by *S. aureus*. Several proteins target immunoglobulins, complement or neutrophil recruitment, others counteract the effects of antimicrobial molecules such as lysozyme

and defensins. McCarthy and Lindsay (127) found that when investigating 13 genes with a characterised or hypothesised role in immune evasion, most of these were present in all sequenced *S. aureus* genomes, indicating the important role of immune evasion for *S. aureus*.

The first line of defence against inhaled bacteria is nasal secretions, a complex mixture of proteins, sugars and salts, containing e.g. lysozyme and immunoglobulins (IgA and IgG) (95), as well as defensins (31) and complement proteins (18). *S. aureus* is resistant to lysozyme due to the cell wall modifying enzyme O-acetyltransferase (OatA) in combination with WTA (11). WTA (102) and IsdA (27) were demonstrated to make the *S. aureus* surface more hydrophilic, protecting against the innate host antimicrobial fatty acids requiring hydrophobic interaction to be active.

Expression of several factors, including staphylococcal protein A (SpA), staphylokinase (SAK) and chemotaxis inhibitory protein of *S. aureus* (CHIPS) has been shown in a study of mRNA levels in nose swabs from persistent carriers of *S. aureus* (16). SpA can limit opsonisation by binding to the Fc-region of IgG, rendering the bacterial cells coated with IgG in a conformation not recognised by neutrophils (137). Through this IgG-binding, SpA also interferes with binding of the complement system (177). SAK can inhibit the bactericidal activity of α-defensins (92), and can also convert surface-bound plasminogen into active plasmin which is capable of cleaving human IgG and the complement compound C3b, thereby preventing opsonisation and hence phagocytosis of the bacterial cell (178). CHIPS and the staphylococcal complement inhibitor (SCIN) are innate immune modulators, known to interfere with the human complement (221). Despite the presence of complement proteins in nasal secretions, SCIN was not found to be expressed in the nose during nasal colonisation of humans (16).

Host factors

It seems as multiple mechanisms are involved in *S. aureus* nasal colonisation and carriage, and that there is a fine-tuned match between the microbe and the host (218). Early studies demonstrated that the adherence of *S. aureus* to mucosal cells from the nose of carriers was significantly higher than adherence to cells from non-carriers (4). Nasal secretions are a part of the host defence against microbes, and it has been shown

that nasal fluids from non-carriers were bacteriostatic or bactericidal, whereas the nasal fluids from carriers allowed growth of *S. aureus* (31). It has been proposed that presence of haemoglobin in nasal secretions promotes *S. aureus* colonisation through inhibition of the *agr* system (166).

The role of host factors in nasal colonisation has been extensively studied. Although a recent Danish study on middle-aged and elderly twins concluded that host genetic factors only had a very limited influence on the *S. aureus* carrier state (5), host genetic factors have been suggested to be important determinants for persistent nasal carriage of *S. aureus* in humans, involving single nucleotide polymorphisms (SNPs) in several proteins. The first polymorphism found to be associated with persistent *S. aureus* nasal carriage was in the glucocorticoid receptor gene (220). Later, polymorphisms in the serine protease C1 inhibitor (C1INH) (43), mannose-binding lectin (MBL) genes (217), interleukin-4 (44, 180) and C-reactive protein (180), as well as the expression level of the antimicrobial peptide human-β-defensin 3 (HBD-3) (248), have all been found to be associated with nasal carriage status. These findings illustrate that there are several host genetic determinants involved in *S. aureus* nasal colonisation and carriage.

Environmental factors

An important determinant of intermittent *S. aureus* nasal carriage is exposure. *S. aureus* is acquired from sources in the environment, with human carriers as the most important source, but also animal carriers or *S. aureus* deposited on surfaces may serve as reservoirs for transmission (224).

The levels of crowding and hygiene in both hospital and household settings are important for the rate of transmission (241). Hospitalisation is known to be a risk factor for *S. aureus* nasal carriage (67). Healthcare workers have in some studies been reported to have rates of *S. aureus* nasal colonisation comparable to the general population (8, 94), however others have found a higher prevalence among healthcare workers than in the general population (42). A recent report also detected more frequent *S. aureus* nasal carriage in surgeons than in high-risk patients (190). Colonised healthcare workers can serve as important sources of *S. aureus* transmission, both as vectors and as reservoirs (12).

Furthermore, a family size of more than 5 people has been found to be correlated with a higher risk of *S. aureus* colonisation in children (13). It has been suggested that *S. aureus* nasal carriers may impose their carrier status upon other members of the household (138, 162). As most mothers carry the same strain as their infants, the mother is the probable source and the strain acquisition may thus be dictated by environmental factors (162). Pets can also be colonised with *S. aureus*, and may serve as vehicles for transmission to humans (195). Activities involving close physical contact and the risk of minor injuries, such as sports, are positively correlated with *S. aureus* acquisition and spread (13, 96).

Bacterial interference

Several factors determine whether bacteria can colonise a human nose or not, including the availability of resources (e.g. nutrients and attachment sites), the presence of harmful substances, and the host's immune responses. All these factors can be influenced by the presence of established bacterial communities in the nose (123).

The microbial ecology of the nasopharynx is complex. Most commonly found in the aerobic flora of the nasal vestibule are staphylococci and *Corynebacterium* spp, but streptococci, micrococci and some Gram-negative species can also be found (113). The nasal microbiota has by culture-independent approaches been shown to consist of a wide range of microbes, primarily from the phylum Actinobacteria (including *Propionibacterium* spp. and *Corynebacterium* spp.), but also other phyla, including Firmicutes (e.g. *Staphylococcus* spp.) and Proteobacteria (e.g. *Enterobacter* spp.) are found (55, 112).

Wos-Oxley *et al.* investigated the microbiota of the anterior nares, and found a significant negative correlation between the abundance of *S. aureus* and the anaerobic *Finegoldia magna*, and they found a positive correlation between *Corynebacterium pseudodiphteriticum* and *S. aureus*. The authors suggest that the potential interactions in the nasal microbiota need to be analysed with a higher level of resolution (245). The rate of *S. aureus* colonisation has previously been found to be lower among those colonised with corynebacteria, but the underlying mechanism is not known (113, 216).

Frank *et al.* reported negative associations between *S. aureus* and *S. epidermidis* suggesting microbial competition (55). The role for coagulase-negative staphylococci (CNS), and especially *S. epidermidis* in *S. aureus* colonisation has not been completely clear (113, 162). However, it has been reported that the serine protease Esp, secreted by a subset of *S. epidermidis*, is capable of inhibiting *S. aureus* nasal colonisation as well as biofilm formation (88). Although *S. aureus* does not typically form a biofilm during colonisation (106), the Esp protease may inhibit *S. aureus* nasal colonisation by removing *S. aureus* adhesins and/or immune evasion factors essential for colonisation (230). Other mechanisms of bacterial interference applied by *S. epidermidis* may involve phenol-soluble modulins (PSMs) (29, 30), lipoteichoic acid (LTA) (109), peptide pheromones (159), and induction of human beta-defensins (108).

Although colonisation with vaccine-type *Streptococcus pneumoniae* in the nasopharynx has been found to be inversely associated with *S. aureus* nasal carriage (13, 170), a study in children did not reveal an increase in prevalence of *S. aureus* colonisation after introduction of the 7-valent pneumococcal-conjugate vaccine (PCV7) (111). A study by Melles *et al.* investigated whether specific genotypes of *S. aureus* had a better capacity of competing with *S. pneumoniae* in co-colonisation of the nasopharynx in children. However, the results suggested that there were no such differences between the genotypes (129). It has been proposed that hydrogen peroxide produced by *S. pneumoniae* drives its bacterial interference activity (171), but this has not been confirmed in recent studies (122, 123).

A resident strain of *S. aureus* may resist replacement by another *S. aureus* strain, also known as colonisation resistance. This was exploited in the 1960s, when infants were inoculated with the supposedly non-pathogenic *S. aureus* isolate 502A, to avoid colonisation with pathogenic strains (193), but the practice was abandoned when it turned out that 502A was able to cause even fatal infections (85). It has also been shown that MSSA nasal carriage interferes with and hence may protect against MRSA acquisition (36). Colonisation resistance may be the result of a resident population producing harmful substances such as bacteriocins; antimicrobial molecules though to mediate population dynamics within a species (173). *S. aureus* is known to produce several types of bacteriocins with a broad-spectrum activity, targeting e.g. strains of *S. aureus*, CNS, corynebacteria and streptococci (242). Another suggested mechanism for

colonisation resistance is *agr* interference, based on polymorphisms in the regulatory *agr* locus resulting in inhibition of virulence gene expression and exclusion of heterologous strains (53, 61, 91). A recent study by Margolis *et al.* suggests that the mechanism behind *S. aureus* colonisation resistance may be a resource limitation on a "first come, first serve" basis, e.g. for attachment sites (123).

TOLL-LIKE RECEPTOR SIGNALLING IN HOST CELLS

The skin is both a physical and an immunological barrier that protects us from pathogens. The keratinocytes of the epidermis sense pathogens by expressing pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) (210). PAMPs are evolutionary conserved microbial components, including lipopolysaccharide (LPS), peptidoglycan and nucleic acids (144). The most extensively studied group of PRRs are the Toll-like receptors (TLRs), which are transmembrane glycoproteins with an extracellular domain recognizing PAMPs, a transmembrane domain, and an intracellular Toll/interleukin-1 receptor domain (TIR) domain responsible for initiation of intracellular signalling cascades (135) (Figure 4). This TIR-domain typically shows 20-30% conservation on the amino acid level (3) and contains 3 conserved boxes, two of which are crucial for signalling (199). When a ligand binds the extracellular domain, the TIR domain attracts adaptor proteins containing TIR-domains, such as the adaptor molecules myeloid differentiation primary response protein 88 (MyD88) or TIR-domain-containing adaptor protein (TIRAP) (210). This results in initiation of signalling cascades which activate transcription factors like nuclear factor κB (NFκB) and mitogen-activated kinases (MAPKs) (6), eventually leading to increased production of cytokines, chemokines and antimicrobial peptides and initiation of innate and adaptive immune responses which promote killing of *S. aureus* (3, 105).

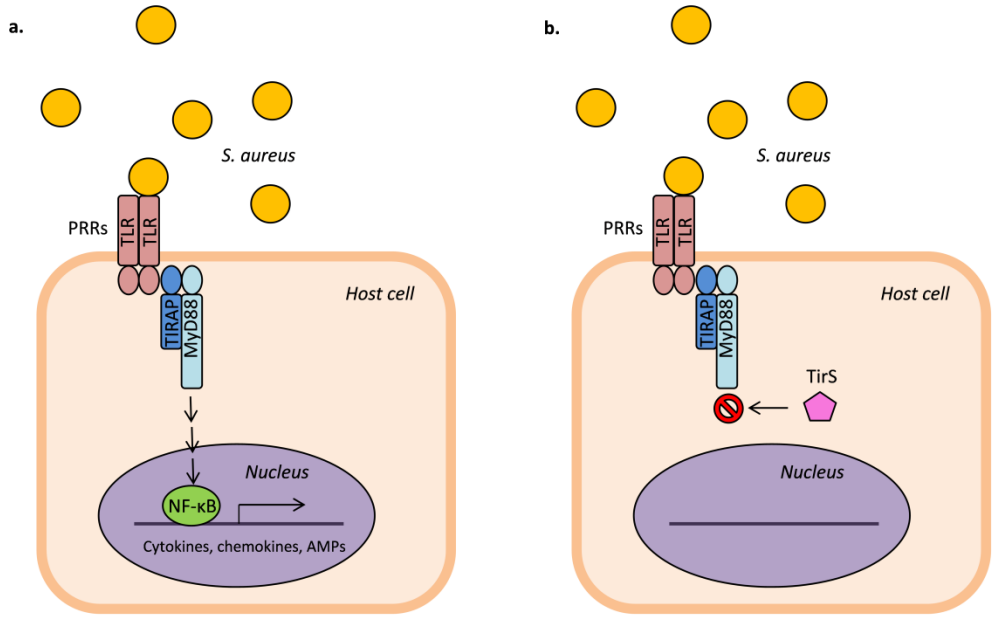

Figure 4. Simplified view of *S. aureus*-induced TLR-mediated NF-κB signalling. PAMPs from *S. aureus*, e.g. LTA or lipoproteins, activate the TLRs which recruit adaptors such as TIRAP and MyD88, initiating a signalling cascade. **a.** The signalling cascade activates the transcription factor NF-κB, resulting in transcription of cytokines, chemokines and AMPs. **b.** A TIR-containing protein (e.g. TirS) from bacteria can negatively interfere with the PAMP-induced signalling cascade, preventing the activation of NF-κB and hence the production of cytokines, chemokines and AMPs. Based on (93).

OBJECTIVES

The main objectives of this study were to investigate the population structure of *S. aureus* in colonised adults from the community and contribute to the knowledge on *S. aureus* interactions with the human host.

PAPER I

Although it is known that *S. aureus* nasal carriers are at risk of autoinfection, knowledge about the factors making specific strains successful colonisers is limited. This study aimed to identify the most successful *S. aureus* clones in nasal carriers from a general population and compare their distribution among host groups by using *spa* typing, MLST and statistical analyses.

PAPER II

Healthcare workers (HCWs) may serve as a reservoir for *S. aureus* transmission to patients. As studies of the healthcare setting often lack the perspective of how background prevalence in the general population and households may bias the result, we aimed to examine whether HCW status was associated with *S. aureus* nasal carriage and certain *spa* types in an unselected general population by the use of *spa* typing and multivariable logistic regression models.

PAPER III

Staphylococcal protein A (SpA) is a surface protein known to contribute to *S. aureus* pathogenesis by interference with the immune responses and activation of inflammation. Seven isolates with frameshift mutations in the *spa* repeat region were found among isolates from bacteraemia, MRSA-infection and one healthy nasal carrier.

The aim of this study was to investigate the molecular implications of the frameshift mutations by western blot and sequencing of the in-frame *spa* gene, and to find the relation between these isolates by running MLST.

PAPER IV

Bacterial proteins containing a Toll/Interleukin-1 receptor (TIR) domain have been found to interfere with the signalling of Toll-like receptors (TLRs) of human cells to suppress the innate immune response. Our aim was to confirm the presence of a putative TIR-domain containing protein in the *S. aureus* strain MSSA476 and to investigate its possible interference with TLR signalling and bacterial accumulation within human cells. We aimed to do this by cloning TirS into a eukaryotic expression vector and performing NF-κB reporter (luciferase) assays, western blot analyses, cytokine assays as well as intracellular survival assays in mammalian cells.

MAIN RESULTS

PAPER I

- In total, 1,981 isolates were included, 1,113 from baseline and 868 from the second screening. The isolates were assigned to 400 unique *spa* types, of which 91 were novel. The *spa* types grouped into 21 clusters and 16 singletons, with three *spa* clonal complexes (*spa* CCs) comprising 62.4% of the *S. aureus* isolates at baseline; *spa* CC012 (28.3%), *spa* CC065 (18.2%) and *spa* CC084 (15.9%).

- The most common *spa* types at the baseline of the study were t012 (8.4%), t084 (7.6%) and t065 (4.9%). 86.1% (317 of 368) of the *spa* types from baseline were found in less than four individuals, and 65.5% (241 of 368) were found only in single individuals, indicating large genetic diversity. 92.2% (671 of 728) of the persistent nasal carriers had identical *spa* types in both samples.

- MLST analysis of 176 consecutive isolates revealed 49 STs, 23 of which were not previously reported. Twenty-four new allele types were designated. The STs were grouped into 16 different clonal complexes (CCs), and four were singletons. CC30 (34.1%), CC45 (25.0%) and CC15 (13.1%) were the three largest CCs.

- The concordance between *spa* CCs (as defined by BURP clustering) and CCs (as defined by eBURST) was 0.76 by Adjusted Rand evaluation, while the Wallace coefficient indicated a 90% probability that two isolates belonging to the same *spa* CC will also share CC.

- In the colonised population, the prevalence of *spa* type t012 decreased significantly with increasing age and was almost twice as high in the youngest group compared to the oldest group (P = 0.03). The *spa* types t012 and t084 demonstrated significant gender associations, with t012 being more prevalent in females (P = 0.03) and t084 in males (P = 0.03). *spa* type t084 had a twofold

higher prevalence among intermittent carriers than among persistent carriers (*P* = 0.04).

- The *spa* types from bacteraemia isolates coincided with carrier strain *spa* types, with some exceptions.

PAPER II

- HCWs comprised 334 of 1,302 women and 71 of 977 men included in the study. The overall prevalence of *S. aureus* nasal carriage was 26.2% in HCWs and 26.0 in non-HCWs. The gender-specific rates in HCWs and non-HCWs were 22.5% and 18.4% in women (*P* = 0.11) and 43.7% and 34.1% in men (*P* = 0.10), respectively.

- Although HCW status was not associated with *S. aureus* nasal carriage in multivariable analysis of the total population, female HCWs had a 54% increased risk of *S. aureus* nasal carriage compared to female non-HCWs (odds ratio [OR] 1.54, 95% confidence interval [CI] 1.09-2.19). In men, no such differences were observed.

- Among women residing with children, there was an 86% increased risk of *S. aureus* nasal carriage in HCWs compared to non-HCWs (multivariable analysis: OR 1.86, 95% CI 1.14-3.04), while for men, there was no significant effect of family status.

- The majority of *spa* types were observed in both HCWs and non-HCWs.

- Among *S. aureus* nasal carriers, it was observed that HCWs had a higher risk of carrying *spa* types t012 and t015 (multivariable analysis: OR 2.17, 95% CI 1.16-4.08 and OR 3.16, 95% CI 1.13-8.87, respectively).

- For nasal carriers residing with children, the age- and gender-adjusted risk of carrying *spa* type t012 was higher in HCWs than in non-HCWs (age- and gender-adjusted analysis: OR 2.42, 95% CI 1.03-5.70), and this association was

particularly strong in male nasal carriers (age-adjusted analysis: OR 4.61, 95% CI 1.36-15.61).

- Among *S. aureus* nasal carriers not living with children, HCWs were found to have a fourfold increased risk of *spa* type t015 compared to non-HCWs (age- and gender-adjusted analysis: OR 4.28, 95% CI 0.99-18.43).

PAPER III

- Sequencing of the complete SpA encoding region revealed that none of the seven isolates had identical *spa* repeat successions, although the same deviant repeat was found in three of the isolates.

- For six isolates, the deviation was associated with the span of adenines in the 5th and 6th codons of a regular 24 or 27 bp repeat, with one base inserted or deleted, resulting in repeats of 23, 25 or 28 bp.

- The seventh isolate exhibited either a short *spa* repeat of 14 bp followed by a regular 24 bp repeat (r93), or possibly one 38 bp repeat resulting from the fusion of two repeats.

- All seven deviating *spa* repeats caused frameshifts in the SpA coding sequence, leading to premature translational stops upstream of the cell wall binding recognition sequence LPXTG, suggesting that the final gene product would lack cell wall binding ability.

- The size of each truncated product was predicted and varied between 32 and 47 kDa, including the signal sequence of 3.6 kDa.

- Five isolates displayed a SpA sequence of five IgG-binding domains each, whereas the last two isolates only had four IgG binding domains, with domain C or domain A absent.

- The seven isolates were assigned to 6 different MLST types; ST8, 15, 45, 58, 228 and the novel ST2834 due to the novel *glpF* allele type 292, and belonged to four different clonal complexes (CC5, CC8, CC15 and CC45).

- Western blots revealed that for six out of the seven isolates SpA was mainly present in the supernatant, and the size of the truncated proteins corresponded well with the predicted sizes from sequence analyses. For the seventh isolate no SpA was detected by western blot, neither in the bacterial pellet nor in the supernatant.

PAPER IV

- An ORF encoding a protein of 280 amino acids with a TIR domain was located in *S. aureus* MSSA476 and named *tirS*. The *tirS* gene was located on the SCC_{476} element. The TIR domain of TirS was 62% similar on the amino acid level to the TIR domain from TIR-containing protein C (TcpC) found in *Escherichia coli*.

- By luciferase assays detecting the activity of the transcription factor NF-κB, the presence of TirS was shown to significantly inhibit *S. aureus*-, synthetic triacylated lipoprotein (Pam3CSK4)- or lipoteichoic acid (LTA)-induced NF-κB activation in human embryonic kidney 293 (HEK293) cells. These results were confirmed in mouse leukaemic monocyte macrophage RAW264.7 cells stimulated with Pam3CSK4 or LTA. TirS was also shown to significantly inhibit *S. aureus*-induced NF-κB response in the HaCaT keratinocyte cell line after overexpression of TLR2. The presence of TirS in all these experiments was confirmed by western blot.

- Western blot analyses revealed that the presence of TirS negatively interfered with MAPK phosphorylation in HEK293 cells stimulated with *S. aureus*.

- The presence of TirS was shown to inhibit MyD88- and TIRAP- induced NF-κB activation in HEK293 cells, using luciferase assays for detection of NF-κB-activity.

- Intracellular survival assays revealed that the expression of TirS in HEK293 cells gave an increased intracellular accumulation of *S. aureus*.

- Results from the Milliplex analysis of secreted cytokines demonstrated that the presence of TirS negatively interfered with the level of secreted cytokines monocyte chemoattractant protein 1 (MCP-1) and granulocyte colony-stimulating factor (G-CSF).

- PCR was performed on DNA from 554 *S. aureus* isolates from nasal carriers in a general population, however, *tirS* was not detected in this material.

GENERAL DISCUSSION

Breakage of the skin barrier may lead to the transformation of *S. aureus* from a commensal coloniser to an invading pathogen, and its multitude of virulence factors enables it to adhere to and survive on and in the host cells (65). In most categories of hospitalised patients, *S. aureus* nasal carriage has been identified as a major risk factor for developing subsequent infections (234). Asserting that *S. aureus* infections are of endogenous origin is supported by studies revealing that isolates from nosocomial *S. aureus* infections were identical to nasal carrier isolates in 80% or more of the patients (119, 226, 236). In non-surgical patients who were *S. aureus* nasal carriers, the risk of acquiring a nosocomial *S. aureus* bacteraemia was three times higher than in non-carriers (236).

To gain an understanding of *S. aureus* nasal carriage and the connection with subsequent infection, the *S. aureus* population structure needs to be defined (234). In our studies, we investigated the population structure of *S. aureus* in adult nasal carriers from a general population (**paper I**) and among healthcare workers (**paper II**). Moreover, we have had a closer look at proteins involved in *S. aureus* immune evasion by investigating naturally occurring SpA mutants with frameshift mutations (**paper III**) and the bacterial TIR-domain containing protein TirS (**paper IV**).

NASAL COLONISATION BY *S. AUREUS*

For a commensal to live and prosper in a human host one can envision two possible strategies. Either, it can stay hidden from the human immune system, hence not provoking an immune response, or the other option is to be detected and then manipulate the immune response to be able to survive. For *S. aureus*, both strategies seem to be important for survival in a human host, involving immune evasion factors such as SpA and TirS (**paper III and IV**).

To investigate the population structure of *S. aureus*, we wanted to use an unselected collection of nasal isolates originating from a general population, thought to represent

the natural habitat for *S. aureus*. From this material, the bacterial population structure was elucidated. The diversity was striking; in a material from 1,113 participants, 368 unique *spa* types were observed, and the majority of these (65.5%) were observed in single individuals only. As many as 86.1% of the *spa* types were found in less than four individuals. This diversity is in line with findings from previous studies for both community and clinical strains (69, 132, 185, 203).

In **paper I**, the most commonly observed *spa* types from blood culture isolates belonged to the most widespread lineages among carriers, implying that the ability of the strain to adhere to host cells and evade the immune response may also be beneficial when invading the host. It has been suggested that the gene combinations important for invasive disease may be the same as those involved in nasal colonisation (115). In fact, the evolution and maintenance of virulence genes in *S. aureus* may be a consequence of the complex host-to-host transmission pathway of this bacterium (125). An alternative view is that *S. aureus* during colonisation represses its virulence to prevent causing infection in a healthy host. However, by scanning for weak points in the host defence and exploiting them when they arise, *S. aureus* can be the first to the table in a competitive environment (17).

Closer investigations of the bacterial population structure combined with host attributes revealed that there are seemingly more to the relationship between host and microbe, than just a developing bacterial population. Variation in *S. aureus* nasal carriage rates with gender and age, with lower carriage rates among women and the elderly, is a well-known phenomenon (66, 156, 158, 234). We observed intriguing gender and age preferences among certain *spa* types, suggesting matching between host and microbe. In this close interplay, the phenotypes of both host and microbe seem to be relevant for successful colonisation. The associations between *spa* type type t012 and women, as well as *spa* type t084 and men, could possibly be related to reproductive hormones or indirect effects of such hormones. Reproductive hormones have been shown to have immunomodulatory effects (100, 163). In fact, it has been observed that isolates of *S. aureus* displayed increased attachment to cultured HeLa cells stimulated with estrogens (208), and that use of hormonal contraceptives are positively associated with *S. aureus* nasal carriage (22, 247). One could speculate that *spa* type t012 holds a factor that is advantageous for colonisation of hosts with high levels of female reproductive

hormones, whereas for t084, male reproductive hormones or indirect effects of these may be involved. The t012 association with younger age of carriers was gender-independent, and hence could not be directly explained by reproductive hormones. However, associations of nasal carriage with factors such as glucocorticoid receptor gene polymorphisms (220) and 25-hydroxyvitamin D serum levels (156) indicate a possible role of steroid hormones in colonisation. Steroid hormones and hormone receptors regulate the expression of antimicrobial peptides (1, 192). A decrease in the number of glucocorticoid receptors in mononuclear leukocytes from elderly subjects compared with subjects younger than 20 years has previously been suggested (213). In addition, a positive association between age and 25-hydroxyvitamin D serum levels was found among both genders in the TSSS study (156). Possibly, t012 may be more sensitive to killing by antimicrobial peptides than other *spa* types, however this hypothesis has not been investigated further.

Our findings (**paper I**) are supported by the results from two artificial human colonisation studies (150, 219). In both studies, decolonised volunteers were artificially recolonized with a mixture of strains, including their former resident strain. Most non-carriers and almost all persistent carriers resumed their original carrier state, and most persistent carriers were recolonised with their original resident strain. These studies suggest the importance of an optimal fit between *S. aureus* and the human host, and highly specific host-microbe interactions. Host genotype may define whether one is a nasal carrier or not, through the expression level and polymorphisms of receptors for bacterial adherence in the nares and the immune response resulting in either tolerance or eradication of *S. aureus*, whereas the bacterial factors may define which strain is carried (161).

Sakwinska *et al.* found that when studying hospital personnel over a period of nine months, 8% acquired a strain entirely different from the original one (183). In comparison, our study (**paper I**) revealed that 7.8% (57/728) of those defined as persistent carriers did not have identical *spa* types in both samples, but as 19 of these 57 isolates (33.3%) belonged to the same *spa* CC, only 5.2% displayed entirely different *spa* types. The change in *spa* type from one sampling to the next may be due to the presence of several *spa* types simultaneously in human hosts (co-colonisation) (19, 185), strain replacement (183) or possibly *spa* type alterations such as repeat deletions, duplications

or point mutations (14). With a median time of 28 days between the two samples in our study, the rates of strain replacement or co-colonisation are in line with previous findings for MSSA in nasal carriers (183). As only one isolate per sample was selected in our study, we cannot rule out the possibility of several *spa* types present in the carriers. In fact, a recent study found that in 5.7% (5/88) of colonised volunteers, *S. aureus* isolates from the right and left nostrils had less than 50% PFGE identity (98). However, previous studies have found low rates of co-colonisation, with only 2.3% (3/130) and 2.2% (2/89) of healthy colonised individuals having multiple *S. aureus* strains in the nose (183, 185).

Interestingly, our results also revealed an association between *spa* type t084 and intermittent carriage. Previously, it has been reported that the number of *S. aureus* colony forming units (CFU) in the nose is higher among persistent carriers (median 3 x 10^5 CFU/swab) than the CFU among intermittent carriers (median 40 CFU/swab) (225). It has been shown that the *in vivo* abundance of bacteria differ between genotypes (185), and that lower bacterial counts are associated with an increased probability of eliminating colonisation (183). However, the fact that the most commonly observed genotypes in nasal carriers did not show increased CFU numbers compared to others, indicate that *in vivo* abundance cannot explain their success (185). It is possible that the association between *spa* type t084 and intermittent carriage has to do with an efficient transmission of this *spa* type between hosts, but this remains to be elucidated.

Households have been suggested to serve as a reservoir for *S. aureus* in the community (136), and children have been found to have a higher prevalence of colonisation with *S. aureus* than adults (38, 66, 73). An *S. aureus* nasal carrier may impose his or her carrier status upon other family members (138, 162, 228), and in households with more than one person colonised, 50% were found to carry the same strain (136). For MRSA, it has been reported that family members may serve as a reservoir for *S. aureus* to be re-introduced into the hospital via intrafamilial transmission to and from healthcare workers (118, 228). Several studies have investigated whether working in healthcare services may be an environmental risk factor for MSSA colonisation, but the results are not consistent (42, 94, 190, 207). In general, many of the studies in healthcare settings may have been biased by a lack of information on background prevalence in the relevant general population as well as in households. Hence, we wanted to explore the

epidemiology of *S. aureus* carriage among healthcare workers in an unselected population (**paper II**). Results from **paper II** demonstrated that working in healthcare services was associated with a 54% increased risk of *S. aureus* nasal carriage in women, and the risk was even more increased (86%) in women residing with children. Among men working in healthcare services and living with children, we found associations with increased prevalence of certain *spa* types.

Recently, niche-adaption in CC30 was described, suggesting that *S. aureus* through gain, loss and change of genes can adapt to niches such as the hospital environment (128). In fact, the associations between health care workers and the increased risk of *spa* types t012 and t015 may be hypothesised to be due to certain *spa* types being adapted to specific ecological niches. However, these associations between certain *spa* types and healthcare workers may also possibly result from an increased potential for transmission of these *spa* types in healthcare- and/or family settings. However, the degree of uncertainty in these estimates is considerable, and no firm conclusions should be drawn. The main weakness of **paper II** was the lack of information on profession, workplace and patient contact among the healthcare workers. Information on factors such as family size and age of children were also not available. Future studies including information on work and home exposures are therefore needed to improve our knowledge on the associations between health care workers and *S. aureus* nasal carriage.

Despite of screening the nasal samples from all 4,026 participants, no MRSA isolates were found (**paper I and II**). MRSA is not considered to be endemic in Norway, and the prevalence of MRSA in clinical *S. aureus* samples from surveillance in 2007-2008 was 0.2-0.7% (149). As the surveillance data do not reflect a healthy population they cannot be directly compared to the MRSA/MSSA colonisation rates in our study.

The typing method we selected for our studies was *spa* typing (**paper I, II and III**). *spa* typing has been thoroughly evaluated and is a rapid and informative method that is easy to use and interpret and hence enables high throughput at low costs, and the standardised nomenclature and interlaboratory reproducibility enables global comparisons (202). The discriminatory power has been reported to be higher than for MLST/eBURST in several studies (133, 203). We evaluated the concordance between *spa* typing and MLST in our material to verify the appropriateness of *spa* typing MSSA

isolates (**paper I**). The good concordance supports our hypothesis of the clonal dispersion of isolates, with *spa* CC012 corresponding to CC30, *spa* CC065 corresponding to CC45 and *spa* CC084 corresponding to CC15. However, one should be aware of the limitations of *spa* typing. Discrepancies may occur between *spa* typing and other methods, due to *spa* homoplasies or possibly chromosomal replacements involving the *spa* locus, resulting in identical *spa* types among non-related isolates (9, 74, 133, 154). This may result in e.g. misleading suggestions of global clonal spread (154).

HOST-MICROBE INTERACTIONS

There is a redundancy of surface proteins in *S. aureus*, where many of the surface proteins are able to bind to several host proteins and several surface proteins may bind to the same host protein (26). It is therefore challenging to prove the importance of individual proteins in virulence, and it is not unlikely that a combination of *S. aureus* proteins may be essential for virulence (127). The redundancy of surface proteins implies the importance of the functions of these proteins for *S. aureus* survival. In many cases, the bacterium will have a backup if there is a failure in the function of one of these proteins. For the seven isolates in **paper III** which secrete SpA to the extracellular environment, such backup mechanisms may come into play, leaving the bacteria capable of causing infections and surviving in carriers.

SpA is a highly conserved virulence factor that is present in virtually all *S. aureus* isolates. In addition to being a target for typing of *S. aureus* isolates, SpA has been suggested to have important roles in invasive infections (117), arthritis (160) and staphylococcal pneumonia (62). SpA exhibits an Immunoglobulin G (IgG) binding region of four or five repeats, binding tightly to the Fc region of IgG (137, 198). The polymorphic X_R-region follows the IgG binding region, and contains a variable number of repeats that are the target for *spa* typing (194) (Figure 2). An N-terminal signal sequence leads SpA into a protein export pathway, while three C-terminal elements; an LPXTG-motif, a hydrophobic domain and a charged tail are involved in the covalent anchoring of SpA to the cell wall. In a large material of more than 14,000 *S. aureus* isolates, only 7 isolates with deviating *spa* repeat lengths were found; 5 from bacteraemia, one from an MRSA infection and one from a healthy nasal carrier (**paper**

III). We found that SpA from all seven isolates were truncated in the C-terminal end, and hence was not attached to the cell wall. However, with an intact IgG binding region, SpA in the secreted form could still be beneficial for *S. aureus*. In addition to limiting opsonisation and phagocytosis by binding the Fc region of IgG, the IgG binding domains can bind the Fab region of the VH3 subclass immunoglobulins (90), inducing apoptosis of B lymphocytes (64). The IgG binding domains are also known to activate tumor necrosis factor receptor 1 (TNFR1) signalling, activating inflammation through chemokine expression (63). Other studies have found that SpA interacts with von Willebrand factor (vWF) (78), platelet gC1qR (146) as well as osteoblasts (28). In **paper III**, five of the SpA mutants were from blood cultures and one was isolated from an MRSA infection, suggesting that the isolates were still virulent. In fact, it was recently demonstrated that several host chemokines stimulate release of SpA from the cell wall, and the authors suggest that this release of SpA may serve as a potential immune evasion strategy (246). It has also been shown that SpA can induce biofilm formation without being anchored to the cell wall (134).

Structural mimicry is a mechanism utilised by bacteria to manipulate the innate immune response by mimicking host proteins (201). TLRs are key factors in the upregulation of the innate immune response, as they by sensing PAMPs start a signalling cascade that results in the initiation of immune responses (169). Bacterial proteins containing TIR domains were shown to interfere with host TLR signalling and decreasing the inflammatory response via structural mimicry (21). TIR proteins have been observed in several human pathogens, such as *Salmonella enterica*, *Escherichia coli* and *Brucella melitensis*, and TIR-containing proteins have been found to contribute to virulence by suppressing the innate immunity (23, 145). In *S. aureus*, a TIR protein was identified in strain MSSA476 via a database search (23). This TIR-containing protein, named TirS, was investigated further in **paper IV**. The results show that TirS negatively interferes with innate immunity by preventing cytokine production via TLR2-mediated signalling. MSSA476 belongs to a major global lineage associated with invasive community-acquired disease (82), but whether the presence of TirS increases the virulence of *S. aureus* strains requires further investigation. Other studies have demonstrated the importance of TLR2 and MyD88 in clearance of *S. aureus* infections (164, 211), and hence we propose that interfering with TLR2 may be beneficial for *S. aureus* by contributing to immune evasion. We hypothesised that TirS could be involved in nasal

colonisation. Although TirS was not detected among the 554 tested *S. aureus* isolates from nasal carriers, we did observe inhibition of *S. aureus*-induced NF-κB response in a keratinocyte cell line, and the putative role of TirS in colonisation remains to be elucidated.

S. aureus has classically been regarded as an extracellular bacterium, but its ability to internalise in a range of cell types, including endothelial cells, epithelial cells, fibroblasts, osteoblasts and keratinocytes, as well as professional phagocytes, has been demonstrated during the last decades (10, 57, 76, 87, 167). The ability of *S. aureus* to invade and survive within mammalian cells may contribute to chronic carriage and/or chronic or frequent relapse of staphylococcal infections such as osteomyelitis and mastitis (57, 196). It is not yet known whether *S. aureus* is found intracellularly during nasal colonisation, but intracellular persistence is thought to be a strategy for immune evasion where the bacteria are protected against professional phagocytes and extracellular antimicrobials (57). In **paper IV**, it was shown that the presence of TirS increased the intracellular accumulation of *S. aureus* in HEK cells, possibly contributing to bacterial survival during infection. This observed effect of TirS is in line with results from previous studies of TIR-containing proteins in intracellular survival (23, 145).

The ectopic expression of TirS in eukaryotic cells is a limitation of **paper IV**, as TirS then is constitutively expressed by a strong promoter, possibly yielding higher concentrations of TirS in the cytosol than bacterial expression of the protein would give. The TIR-domain containing protein TcpC from *E. coli* has been found to be secreted and to be taken up by host cells (23), but it is not yet known whether bacterial TirS is secreted, and how it may enter human cells during colonisation or infection.

The role of SCC elements in the spread of putative virulence factors, antimicrobial resistance determinants and genes involved in bacterial fitness has been proposed, and as *tirS* is located on the SCC_{476} element, spread by horizontal gene transfer to other members of the *S. aureus* population may occur (82).

CONCLUDING REMARKS

Even though the intimate relationship between the human host and *S. aureus* was recognised already during the late nineteenth century, the knowledge on the molecular interactions contributing to colonisation and carriage is still limited today.

A match between the microbe and the host seems to be essential for the interplay during colonisation and carriage. Our results support the view of a close relationship where the genotype/phenotype of both host and microbe are important, as we found associations between certain *spa* types and host characteristics such as age, gender and working in healthcare services. As for the future, we will continue our search for determinants among hosts and microbes that are involved in *S. aureus* colonisation of healthy individuals. WGS studies may help us obtain a broader understanding of the bacterial factors involved in colonisation. Also, *S. aureus* colonisation of other body niches is in our field of interest.

An extensive number of surface proteins and virulence factors can be found in *S. aureus*. SpA is reported to have several important functions for *S. aureus* in a host. We identified isolates from carriage and disease in which the SpA proteins were truncated and secreted into the extracellular environment, indicating that cell wall-attached SpA may not be essential for *S. aureus* virulence and colonisation.

The putative virulence factor TirS was found to negatively interfere with innate immunity and contribute to intracellular accumulation of *S. aureus* in a human cell line. Its location on a SCC element may enable horizontal transfer. Future studies will include analyses of bacterial TirS expression and localisation. Also, the influence of TirS on *S. aureus* colonisation, invasion and intracellular survival will be investigated.

In summary, our results have increased the knowledge on the *S. aureus* population structure in an unselected human population and have added some pieces to the puzzle on the relationship between *S. aureus* and its human host. However, further studies on determinants involved in *S. aureus* colonisation and disease are needed to fully understand this host-microbe relationship.

REFERENCES

1. **Adams, J. S., and M. Hewison.** 2008. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nat. Clin. Pract. Endocrinol. Metab. **4:**80-90.

2. **Aires de Sousa, M., and H. de Lencastre.** 2004. Bridges from hospitals to the laboratory: genetic portraits of methicillin-resistant *Staphylococcus aureus* clones. FEMS Immunol. Med. Microbiol. **40:**101-111.

3. **Akira, S., and K. Takeda.** 2004. Toll-like receptor signalling. Nat. Rev. Immunol. **4:**499-511.

4. **Aly, R., H. I. Shinefield, W. G. Strauss, and H. I. Maibach.** 1977. Bacterial adherence to nasal mucosal cells. Infect.Immun. **17:**546-549.

5. **Andersen, P. S., J. K. Pedersen, P. Fode, R. L. Skov, V. G. Fowler, Jr., M. Stegger, and K. Christensen.** 2012. Influence of host genetics and environment on nasal carriage of *Staphylococcus aureus* in Danish middle-aged and elderly twins. J. Infect. Dis. **206:**1178-1184.

6. **Baba, T., T. Bae, O. Schneewind, F. Takeuchi, and K. Hiramatsu.** 2008. Genome sequence of *Staphylococcus aureus* strain Newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands. J. Bacteriol. **190:**300-310.

7. **Baba, T., F. Takeuchi, M. Kuroda, H. Yuzawa, K. Aoki, A. Oguchi, Y. Nagai, N. Iwama, K. Asano, T. Naimi, H. Kuroda, L. Cui, K. Yamamoto, and K. Hiramatsu.** 2002. Genome and virulence determinants of high virulence community-acquired MRSA. Lancet **359:**1819-1827.

8. **Bannerman, T. L., G. A. Hancock, F. C. Tenover, and J. M. Miller.** 1995. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J. Clin. Microbiol. **33:**551-555.

9. **Basset, P., U. Nubel, W. Witte, and D. S. Blanc.** 2012. Evaluation of adding a second marker to overcome *Staphylococcus aureus spa* typing homoplasies. J. Clin. Microbiol. **50:**1475-1477.

10. **Bayles, K. W., C. A. Wesson, L. E. Liou, L. K. Fox, G. A. Bohach, and W. R. Trumble.** 1998. Intracellular *Staphylococcus aureus* escapes the endosome and induces apoptosis in epithelial cells. Infect. Immun. **66:**336-342.

11. **Bera, A., R. Biswas, S. Herbert, E. Kulauzovic, C. Weidenmaier, A. Peschel, and F. Gotz.** 2007. Influence of wall teichoic acid on lysozyme resistance in *Staphylococcus aureus*. J. Bacteriol. **189:**280-283.

12. **Blok, H. E., A. Troelstra, T. E. Kamp-Hopmans, A. C. Gigengack-Baars, C. M. Vandenbroucke-Grauls, A. J. Weersink, J. Verhoef, and E. M. Mascini.** 2003. Role of healthcare workers in outbreaks of methicillin-resistant *Staphylococcus aureus*: a 10-year evaluation from a Dutch university hospital. Infect. Control Hosp. Epidemiol. **24:**679-685.

13. **Bogaert, D., A. van Belkum, M. Sluijter, A. Luijendijk, R. de Groot, H. C. Rumke, H. A. Verbrugh, and P. W. Hermans.** 2004. Colonisation by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. Lancet **363:**1871-1872.

14. **Boye, K., and H. Westh.** 2011. Variations in *spa* types found in consecutive MRSA isolates from the same patients. FEMS Microbiol. Lett. **314:**101-105.

15. **Burian, M., M. Rautenberg, T. Kohler, M. Fritz, B. Krismer, C. Unger, W. H. Hoffmann, A. Peschel, C. Wolz, and C. Goerke.** 2010. Temporal expression of adhesion factors and activity of global regulators during establishment of *Staphylococcus aureus* nasal colonization. J. Infect. Dis. **201:**1414-1421.

16. **Burian, M., C. Wolz, and C. Goerke.** 2010. Regulatory adaptation of *Staphylococcus aureus* during nasal colonization of humans. Plos One **5:**e10040.

17. **Camargo, I. L., and M. S. Gilmore.** 2008. *Staphylococcus aureus*--probing for host weakness? J. Bacteriol. **190:**2253-2256.

18. **Casado, B., L. K. Pannell, P. Iadarola, and J. N. Baraniuk.** 2005. Identification of human nasal mucous proteins using proteomics. Proteomics **5:**2949-2959.

19. **Cespedes, C., B. Said-Salim, M. Miller, S. H. Lo, B. N. Kreiswirth, R. J. Gordon, P. Vavagiakis, R. S. Klein, and F. D. Lowy.** 2005. The clonality of *Staphylococcus aureus* nasal carriage. J. Infect. Dis. **191:**444-452.

20. **Chain, P. S., D. V. Grafham, R. S. Fulton, M. G. Fitzgerald, J. Hostetler, D. Muzny, J. Ali, B. Birren, D. C. Bruce, C. Buhay, J. R. Cole, Y. Ding, S. Dugan, D. Field, G. M. Garrity, R. Gibbs, T. Graves, C. S. Han, S. H. Harrison, S. Highlander, P. Hugenholtz, H. M. Khouri, C. D. Kodira, E. Kolker, N. C. Kyrpides, D. Lang, A. Lapidus, S. A. Malfatti, V. Markowitz, T. Metha, K. E. Nelson, J. Parkhill, S. Pitluck, X. Qin, T. D. Read, J. Schmutz, S. Sozhamannan, P. Sterk, R. L. Strausberg, G. Sutton, N. R. Thomson, J. M. Tiedje, G. Weinstock, A. Wollam, and J. C. Detter.** 2009. Genomics. Genome project standards in a new era of sequencing. Science **326:**236-237.

21. **Chan, S. L., L. Y. Low, S. Hsu, S. Li, T. Liu, E. Santelli, G. Le Negrate, J. C. Reed, V. L. Woods, Jr., and J. Pascual.** 2009. Molecular mimicry in innate immunity: crystal structure of a bacterial TIR domain. J. Biol. Chem. **284:**21386-21392.

22. **Choi, C. S., C. S. Yin, A. A. Bakar, Z. Sakewi, N. N. Naing, F. Jamal, and N. Othman.** 2006. Nasal carriage of *Staphylococcus aureus* among healthy adults. J. Microbiol. Immunol. Infect. **39:**458-464.

23. **Cirl, C., A. Wieser, M. Yadav, S. Duerr, S. Schubert, H. Fischer, D. Stappert, N. Wantia, N. Rodriguez, H. Wagner, C. Svanborg, and T. Miethke.** 2008. Subversion of Toll-like receptor signaling by a unique family of bacterial Toll/interleukin-1 receptor domain-containing proteins. Nat. Med. **14:**399-406.

24. **Clarke, S. R., G. Andre, E. J. Walsh, Y. F. Dufrene, T. J. Foster, and S. J. Foster.** 2009. Iron-regulated surface determinant protein A mediates adhesion of *Staphylococcus aureus* to human corneocyte envelope proteins. Infect. Immun. **77:**2408-2416.

25. **Clarke, S. R., K. J. Brummell, M. J. Horsburgh, P. W. McDowell, S. A. Mohamad, M. R. Stapleton, J. Acevedo, R. C. Read, N. P. Day, S. J. Peacock, J. J. Mond, J. F. Kokai-Kun, and S. J. Foster.** 2006. Identification of *in vivo*-expressed antigens of *Staphylococcus aureus* and their use in vaccinations for protection against nasal carriage. J. Infect. Dis. **193:**1098-1108.

26. **Clarke, S. R., and S. J. Foster.** 2006. Surface adhesins of *Staphylococcus aureus*. Adv. Microb. Physiol. **51:**187-224.

27. **Clarke, S. R., R. Mohamed, L. Bian, A. F. Routh, J. F. Kokai-Kun, J. J. Mond, A. Tarkowski, and S. J. Foster.** 2007. The *Staphylococcus aureus* surface protein IsdA mediates resistance to innate defenses of human skin. Cell host & microbe **1:**199-212.

28. **Claro, T., A. Widaa, M. O'Seaghdha, H. Miajlovic, T. J. Foster, F. J. O'Brien, and S. W. Kerrigan.** 2011. *Staphylococcus aureus* protein A binds to osteoblasts and triggers signals that weaken bone in osteomyelitis. Plos One **6:**e18748.

29. **Cogen, A. L., K. Yamasaki, J. Muto, K. M. Sanchez, L. Crotty Alexander, J. Tanios, Y. Lai, J. E. Kim, V. Nizet, and R. L. Gallo.** 2010. *Staphylococcus epidermidis* antimicrobial delta-toxin (phenol-soluble modulin-gamma) cooperates with host antimicrobial peptides to kill group A *Streptococcus*. Plos One **5:**e8557.

30. **Cogen, A. L., K. Yamasaki, K. M. Sanchez, R. A. Dorschner, Y. Lai, D. T. MacLeod, J. W. Torpey, M. Otto, V. Nizet, J. E. Kim, and R. L. Gallo.** 2010. Selective antimicrobial action is provided by phenol-soluble modulins derived from *Staphylococcus epidermidis*, a normal resident of the skin. J. Invest. Dermatol. **130:**192-200.

31. **Cole, A. M., P. Dewan, and T. Ganz.** 1999. Innate antimicrobial activity of nasal secretions. Infect. Immun. **67:**3267-3275.

32. **Cole, A. M., S. Tahk, A. Oren, D. Yoshioka, Y. H. Kim, A. Park, and T. Ganz.** 2001. Determinants of *Staphylococcus aureus* nasal carriage. Clin. Diagn. Lab Immunol. **8:**1064-1069.

33. **Cooper, J. E., and E. J. Feil.** 2006. The phylogeny of *Staphylococcus aureus* - which genes make the best intra-species markers? Microbiology **152:**1297-1305.

34. **Corrigan, R. M., and T. J. Foster.** 2009. An improved tetracycline-inducible expression vector for *Staphylococcus aureus*. Plasmid **61:**126-129.

35. **Corrigan, R. M., H. Miajlovic, and T. J. Foster.** 2009. Surface proteins that promote adherence of *Staphylococcus aureus* to human desquamated nasal epithelial cells. BMC Microbiol. **9:**22.

36. **Dall'Antonia, M., P. G. Coen, M. Wilks, A. Whiley, and M. Millar.** 2005. Competition between methicillin-sensitive and -resistant *Staphylococcus aureus* in the anterior nares. J. Hosp. Infect. **61:**62-67.

37. **Danbolt, N.** 1931. Undersøkelser over stafylokokker med særlig henlikk på furunkulosens epidemiologi. Skr Norske Vidensk Akad I Mat-Nat KI **10**.

38. **Davis, M. F., S. A. Iverson, P. Baron, A. Vasse, E. K. Silbergeld, E. Lautenbach, and D. O. Morris.** 2012. Household transmission of meticillin-resistant *Staphylococcus aureus* and other staphylococci. Lancet Infect. Dis. **12:**703-716.

39. **Diekema, D. J., M. A. Pfaller, F. J. Schmitz, J. Smayevsky, J. Bell, R. N. Jones, and M. Beach.** 2001. Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin. Infect. Dis. **32 Suppl 2:**S114-S132.

40. **Diep, B. A., S. R. Gill, R. F. Chang, T. H. Phan, J. H. Chen, M. G. Davidson, F. Lin, J. Lin, H. A. Carleton, E. F. Mongodin, G. F. Sensabaugh, and F. Perdreau-Remington.** 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. Lancet **367:**731-739.

41. **El Garch, F., M. Hallin, R. De Mendonca, O. Denis, A. Lefort, and M. J. Struelens.** 2009. StaphVar-DNA microarray analysis of accessory genome elements of community-acquired methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. **63:**877-885.

42. **Elie-Turenne, M. C., H. Fernandes, J. R. Mediavilla, M. Rosenthal, B. Mathema, A. Singh, T. R. Cohen, K. A. Pawar, H. Shahidi, B. N. Kreiswirth, and E. A. Deitch.** 2010. Prevalence and characteristics of *Staphylococcus aureus* colonization among healthcare professionals in an urban teaching hospital. Infect. Control Hosp. Epidemiol. **31:**574-580.

43. **Emonts, M., C. E. de Jongh, J. J. Houwing-Duistermaat, W. B. van Leeuwen, R. de Groot, H. A. Verbrugh, P. W. Hermans, and A. van Belkum.** 2007. Association between nasal carriage of *Staphylococcus aureus* and the human complement cascade activator serine protease C1 inhibitor (C1INH) valine vs. methionine polymorphism at amino acid position 480. FEMS Immunol. Med. Microbiol. **50:**330-332.

44. **Emonts, M., A. G. Uitterlinden, J. L. Nouwen, I. Kardys, M. P. Maat, D. C. Melles, J. Witteman, P. T. Jong, H. A. Verbrugh, A. Hofman, P. W. Hermans,**

and A. van Belkum. 2008. Host polymorphisms in interleukin 4, complement factor H, and C-reactive protein associated with nasal carriage of *Staphylococcus aureus* and occurrence of boils. J. Infect. Dis. **197:**1244-1253.

45. **Enright, M. C.** 2008. The Population Structure of *Staphylococcus aureus*. *In* J. A. Lindsay (ed.), *Staphylococcus* - Molecular genetics. Caister Academic Press, Norfolk, UK.

46. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008-1015.

47. **Eriksen, N. H., F. Espersen, V. T. Rosdahl, and K. Jensen.** 1995. Carriage of *Staphylococcus aureus* among 104 healthy persons during a 19-month period. Epidemiol. Infect. **115:**51-60.

48. **Eyre, D. W., T. Golubchik, N. C. Gordon, R. Bowden, P. Piazza, E. M. Batty, C. L. Ip, D. J. Wilson, X. Didelot, L. O'Connor, R. Lay, D. Buck, A. M. Kearns, A. Shaw, J. Paul, M. H. Wilcox, P. J. Donnelly, T. E. Peto, A. S. Walker, and D. W. Crook.** 2012. A pilot study of rapid benchtop sequencing of *Staphylococcus aureus* and *Clostridium difficile* for outbreak detection and surveillance. BMJ open **2:**10.1136/bmjopen-2012-001124.

49. **Fan, J., M. Shu, G. Zhang, W. Zhou, Y. Jiang, Y. Zhu, G. Chen, S. J. Peacock, C. Wan, W. Pan, and E. J. Feil.** 2009. Biogeography and virulence of *Staphylococcus aureus*. Plos One **4:**e6216.

50. **Feil, E. J., J. E. Cooper, H. Grundmann, D. A. Robinson, M. C. Enright, T. Berendt, S. J. Peacock, J. M. Smith, M. Murphy, B. G. Spratt, C. E. Moore, and N. P. Day.** 2003. How clonal is *Staphylococcus aureus*? J. Bacteriol. **185:**3307-3316.

51. **Feil, E. J., B. C. Li, D. M. Aanensen, W. P. Hanage, and B. G. Spratt.** 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J. Bacteriol. **186:**1518-1530.

52. **Feil, E. J., J. M. Smith, M. C. Enright, and B. G. Spratt.** 2000. Estimating recombinational parameters in *Streptococcus pneumoniae* from multilocus sequence typing data. Genetics **154:**1439-1450.

53. **Fleming, V., E. Feil, A. K. Sewell, N. Day, A. Buckling, and R. C. Massey.** 2006. Agr interference between clinical *Staphylococcus aureus* strains in an insect model of virulence. J. Bacteriol. **188:**7686-7688.

54. **Francois, P., A. Huyghe, Y. Charbonnier, M. Bento, S. Herzig, I. Topolski, B. Fleury, D. Lew, P. Vaudaux, S. Harbarth, W. van Leeuwen, A. van Belkum, D. S. Blanc, D. Pittet, and J. Schrenzel.** 2005. Use of an automated multiple-locus, variable-number tandem repeat-based method for rapid and high-throughput genotyping of *Staphylococcus aureus* isolates. J. Clin. Microbiol. **43:**3346-3355.

55. **Frank, D. N., L. M. Feazel, M. T. Bessesen, C. S. Price, E. N. Janoff, and N. R. Pace.** 2010. The human nasal microbiota and *Staphylococcus aureus* carriage. Plos One **5:**e10598.

56. **Gagliotti, C., A. Balode, F. Baquero, J. Degener, H. Grundmann, D. Gur, V. Jarlier, G. Kahlmeter, J. Monen, D. L. Monnet, G. M. Rossolini, C. Suetens, K. Weist, and O. Heuer.** 2011. *Escherichia coli* and *Staphylococcus aureus*: bad news and good news from the European Antimicrobial Resistance Surveillance Network (EARS-Net, formerly EARSS), 2002 to 2009. Euro. Surveill. **16**.

57. **Garzoni, C., and W. L. Kelley.** 2009. *Staphylococcus aureus*: new evidence for intracellular persistence. Trends Microbiol. **17:**59-65.

58. **Gill, S. R.** 2009. Genomics of the Staphylococci. *In* K. B. J. Crossley, K.K.; Archer, G.L.; Fowler, V.G. (ed.), Staphylococci in Human Disease. Wiley-Blackwell, Chichester, UK.

59. **Gill, S. R., D. E. Fouts, G. L. Archer, E. F. Mongodin, R. T. Deboy, J. Ravel, I. T. Paulsen, J. F. Kolonay, L. Brinkac, M. Beanan, R. J. Dodson, S. C. Daugherty, R. Madupu, S. V. Angiuoli, A. S. Durkin, D. H. Haft, J. Vamathevan, H. Khouri, T. Utterback, C. Lee, G. Dimitrov, L. Jiang, H. Qin, J. Weidman, K. Tran, K. Kang, I. R. Hance, K. E. Nelson, and C. M. Fraser.** 2005. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. J. Bacteriol. **187:**2426-2438.

60. **Gillaspy, A. F. W., V.; Orvis, J.; Roe, B.A.; Dyer, D.W; Iandolo, J.J.** 2006. The *Staphylococcus aureus* NCTC 8325 Genome. *In* V. A. N. Fischetti, R.P; Ferretti, J.J; Portnoy, D.A.; Rood, J.I. (ed.), Gram-Positive Pathogens, Second ed. ASM Press, Washington, DC.

61. **Goerke, C., M. Kummel, K. Dietz, and C. Wolz.** 2003. Evaluation of intraspecies interference due to *agr* polymorphism in *Staphylococcus aureus during* infection and colonization. J. Infect. Dis. **188:**250-256.

62. **Gomez, M. I., A. Lee, B. Reddy, A. Muir, G. Soong, A. Pitt, A. Cheung, and A. Prince.** 2004. *Staphylococcus aureus* protein A induces airway epithelial inflammatory responses by activating TNFR1. Nat. Med. **10:**842-848.

63. **Gomez, M. I., M. O'Seaghdha, M. Magargee, T. J. Foster, and A. S. Prince.** 2006. *Staphylococcus aureus* protein A activates TNFR1 signaling through conserved IgG binding domains. J. Biol. Chem. **281:**20190-20196.

64. **Goodyear, C. S., and G. J. Silverman.** 2003. Death by a B cell superantigen: In vivo VH-targeted apoptotic supraclonal B cell deletion by a Staphylococcal Toxin. J. Exp. Med. **197:**1125-1139.

65. **Gordon, R. J., and F. D. Lowy.** 2008. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin. Infect. Dis. **46:**S350-S359.

66. **Gorwitz, R. J., D. Kruszon-Moran, S. K. McAllister, G. McQuillan, L. K. McDougal, G. E. Fosheim, B. J. Jensen, G. Killgore, F. C. Tenover, and M. J. Kuehnert.** 2008. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. J. Infect. Dis. **197:**1226-1234.

67. **Goslings, W. R., and K. Buchli.** 1958. Nasal carrier rate of antibiotic-resistant staphylococci; influence of hospitalization on carrier rate in patients, and their household contacts. AMA Arch. Intern. Med. **102:**691-715.

68. **Gould, I. M.** 2006. Costs of hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and its control. Int. J. Antimicrob. Agents. **28:**379-384.

69. **Grundmann, H., D. M. Aanensen, C. C. van den Wijngaard, B. G. Spratt, D. Harmsen, and A. W. Friedrich.** 2010. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. PLoS Med. **7:**e1000215.

70. **Grundmann, H., S. Hori, M. C. Enright, C. Webster, A. Tami, E. J. Feil, and T. Pitt.** 2002. Determining the genetic structure of the natural population of *Staphylococcus aureus*: a comparison of multilocus sequence typing with pulsed-field gel electrophoresis, randomly amplified polymorphic DNA analysis, and phage typing. J. Clin. Microbiol. **40:**4544-4546.

71. **Guinan, M. E., B. B. Dan, R. J. Guidotti, A. L. Reingold, G. P. Schmid, E. J. Bettoli, J. G. Lossick, K. N. Shands, M. A. Kramer, N. T. Hargrett, R. L. Anderson, and C. V. Broome.** 1982. Vaginal colonization with *Staphylococcus aureus* in healthy women: a review of four studies. Ann. Intern. Med. **96:**944-947.

72. **Guttman, D. S., and J. Stavrinides.** 2010. Population Genomics of Bacteria, p. 121-151. *In* D. A. Robinson, D. Falush, and E. J. Feil (ed.), Bacterial Population Genetics in Infectious Disease. John Wiley & Sons, Hoboken, New Jersey.

73. **Halablab, M. A., S. M. Hijazi, M. A. Fawzi, and G. F. Araj.** 2010. *Staphylococcus aureus* nasal carriage rate and associated risk factors in individuals in the community. Epidemiol. Infect. **138:**702-706.

74. **Hallin, M., A. Deplano, O. Denis, M. R. de, R. R. De, and M. J. Struelens.** 2007. Validation of pulsed-field gel electrophoresis and *spa* typing for long-term, nationwide epidemiological surveillance studies of *Staphylococcus aureus* infections. J. Clin. Microbiol. **45:**127-133.

75. **Hamdan-Partida, A., T. Sainz-Espunes, and J. Bustos-Martinez.** 2010. Characterization and persistence of *Staphylococcus aureus* strains isolated from the anterior nares and throats of healthy carriers in a Mexican community. J. Clin. Microbiol. **48:**1701-1705.

76. **Hamill, R. J., J. M. Vann, and R. A. Proctor.** 1986. Phagocytosis of *Staphylococcus aureus* by cultured bovine aortic endothelial cells: model for postadherence events in endovascular infections. Infect. Immun. **54:**833-836.

77. **Harris, S. R., E. J. Feil, M. T. Holden, M. A. Quail, E. K. Nickerson, N. Chantratita, S. Gardete, A. Tavares, N. Day, J. A. Lindsay, J. D. Edgeworth, H. de Lencastre, J. Parkhill, S. J. Peacock, and S. D. Bentley.** 2010. Evolution of MRSA during hospital transmission and intercontinental spread. Science **327:**469-474.

78. **Hartleib, J., N. Kohler, R. B. Dickinson, G. S. Chhatwal, J. J. Sixma, O. M. Hartford, T. J. Foster, G. Peters, B. E. Kehrel, and M. Herrmann.** 2000. Protein A is the von Willebrand factor binding protein on *Staphylococcus aureus*. Blood **96:**2149-2156.

79. **Heilmann, C.** 2011. Adhesion mechanisms of staphylococci. Adv. Exp. Med. Biol. **715:**105-123.

80. **Herron-Olson, L., J. R. Fitzgerald, J. M. Musser, and V. Kapur.** 2007. Molecular correlates of host specialization in *Staphylococcus aureus*. Plos One **2:**e1120.

81. **Highlander, S. K., K. G. Hulten, X. Qin, H. Jiang, S. Yerrapragada, E. O. Mason, Jr., Y. Shang, T. M. Williams, R. M. Fortunov, Y. Liu, O. Igboeli, J. Petrosino, M. Tirumalai, A. Uzman, G. E. Fox, A. M. Cardenas, D. M. Muzny, L. Hemphill, Y. Ding, S. Dugan, P. R. Blyth, C. J. Buhay, H. H. Dinh, A. C. Hawes, M. Holder, C. L. Kovar, S. L. Lee, W. Liu, L. V. Nazareth, Q. Wang, J. Zhou, S. L. Kaplan, and G. M. Weinstock.** 2007. Subtle genetic changes enhance virulence of methicillin resistant and sensitive *Staphylococcus aureus*. BMC Microbiol. **7:**99.

82. **Holden, M. T., E. J. Feil, J. A. Lindsay, S. J. Peacock, N. P. Day, M. C. Enright, T. J. Foster, C. E. Moore, L. Hurst, R. Atkin, A. Barron, N. Bason, S. D. Bentley, C. Chillingworth, T. Chillingworth, C. Churcher, L. Clark, C. Corton, A. Cronin, J. Doggett, L. Dowd, T. Feltwell, Z. Hance, B. Harris, H. Hauser, S. Holroyd, K. Jagels, K. D. James, N. Lennard, A. Line, R. Mayes, S. Moule, K. Mungall, D. Ormond, M. A. Quail, E. Rabbinowitsch, K. Rutherford, M. Sanders, S. Sharp, M. Simmonds, K. Stevens, S. Whitehead, B. G. Barrell, B. G. Spratt, and J. Parkhill.** 2004. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc. Natl. Acad. Sci. U. S. A. **101:**9786-9791.

83. **Holden, M. T., J. A. Lindsay, C. Corton, M. A. Quail, J. D. Cockfield, S. Pathak, R. Batra, J. Parkhill, S. D. Bentley, and J. D. Edgeworth.** 2010. Genome sequence of a recently emerged, highly transmissible, multi-antibiotic- and antiseptic-resistant variant of methicillin-resistant *Staphylococcus aureus*, sequence type 239 (TW). J. Bacteriol. **192:**888-892.

84. **Horsburgh, M. J.** 2008. The response of *S. aureus* to environmental stimuli. *In* J. A. Lindsay (ed.), *Staphylococcus* - Molecular genetics. Caister Academic Press, Norfolk, UK.

85. **Houck, P. W., J. D. Nelson, and J. L. Kay.** 1972. Fatal septicemia due to *Staphylococcus aureus* 502A. Report of a case and review of the infectious complications of bacterial interference programs. Am. J. Dis. Child. **123:**45-48.

86. **Hu, L., A. Umeda, S. Kondo, and K. Amako.** 1995. Typing of *Staphylococcus aureus* colonising human nasal carriers by pulsed-field gel electrophoresis. J. Med. Microbiol. **42:**127-132.

87. **Hudson, M. C., W. K. Ramp, N. C. Nicholson, A. S. Williams, and M. T. Nousiainen.** 1995. Internalization of *Staphylococcus aureus* by cultured osteoblasts. Microb. Pathog. **19:**409-419.

88. **Iwase, T., Y. Uehara, H. Shinji, A. Tajima, H. Seo, K. Takada, T. Agata, and Y. Mizunoe.** 2010. *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. Nature **465:**346-349.

89. **Jacobsen, B. K., A. E. Eggen, E. B. Mathiesen, T. Wilsgaard, and I. Njolstad.** 2012. Cohort profile: The Tromso Study. Int. J. Epidemiol. **41:**961-967.

90. **Jansson, B., M. Uhlen, and P. A. Nygren.** 1998. All individual domains of staphylococcal protein A show Fab binding. FEMS Immunol. Med. Microbiol. **20:**69-78.

91. **Ji, G., R. Beavis, and R. P. Novick.** 1997. Bacterial interference caused by autoinducing peptide variants. Science **276:**2027-2030.

92. **Jin, T., M. Bokarewa, T. Foster, J. Mitchell, J. Higgins, and A. Tarkowski.** 2004. *Staphylococcus aureus* resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. J. Immunol. **172:**1169-1176.

93. **Johannessen, M., J. E. Sollid, and A. M. Hanssen.** 2012. Host- and microbe determinants that may influence the success of *S. aureus* colonization. Front. Cell. Infect. Microbiol. **2:**56.

94. **Johnston, C. P., A. K. Stokes, T. Ross, M. Cai, K. C. Carroll, S. E. Cosgrove, and T. M. Perl.** 2007. *Staphylococcus aureus* colonization among healthcare workers at a tertiary care hospital. Infect. Control Hosp. Epidemiol. **28:**1404-1407.

95. **Kaliner, M. A.** 1991. Human nasal respiratory secretions and host defense. Am. Rev. Respir. Dis. **144:**S52-56.

96. **Kazakova, S. V., J. C. Hageman, M. Matava, A. Srinivasan, L. Phelan, B. Garfinkel, T. Boo, S. McAllister, J. Anderson, B. Jensen, D. Dodson, D. Lonsway, L. K. McDougal, M. Arduino, V. J. Fraser, G. Killgore, F. C. Tenover, S. Cody, and D. B. Jernigan.** 2005. A clone of methicillin-resistant *Staphylococcus aureus* among professional football players. N. Engl. J. Med. **352:**468-475.

97. **Kennedy, A. D., M. Otto, K. R. Braughton, A. R. Whitney, L. Chen, B. Mathema, J. R. Mediavilla, K. A. Byrne, L. D. Parkins, F. C. Tenover, B. N. Kreiswirth, J. M. Musser, and F. R. DeLeo.** 2008. Epidemic community-associated methicillin-resistant *Staphylococcus aureus*: recent clonal expansion and diversification. Proc. Natl. Acad. Sci. U. S. A. **105:**1327-1332.

98. **Kildow, B. J., J. P. Conradie, and R. L. Robson.** 2012. Nostrils of healthy volunteers are independent with regard to *Staphylococcus aureus* carriage. J. Clin. Microbiol. **50:**3744-3746.

99. **Kirmani, N., C. U. Tuazon, H. W. Murray, A. E. Parrish, and J. N. Sheagren.** 1978. *Staphylococcus aureus* carriage rate of patients receiving long-term hemodialysis. Arch. Intern. Med. **138:**1657-1659.

100. **Klein, S. L.** 2000. The effects of hormones on sex differences in infection: from genes to behavior. Neurosci. Biobehav. Rev. **24:**627-638.

101. **Kluytmans, J., B. A. van, and H. Verbrugh.** 1997. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin. Microbiol. Rev. **10:**505-520.

102. **Kohler, T., C. Weidenmaier, and A. Peschel.** 2009. Wall teichoic acid protects *Staphylococcus aureus* against antimicrobial fatty acids from human skin. J. Bacteriol. **191:**4482-4484.

103. **Koreen, L., S. V. Ramaswamy, E. A. Graviss, S. Naidich, J. M. Musser, and B. N. Kreiswirth.** 2004. *spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J. Clin. Microbiol. **42:**792-799.

104. **Koser, C. U., M. T. Holden, M. J. Ellington, E. J. Cartwright, N. M. Brown, A. L. Ogilvy-Stuart, L. Y. Hsu, C. Chewapreecha, N. J. Croucher, S. R. Harris, M. Sanders, M. C. Enright, G. Dougan, S. D. Bentley, J. Parkhill, L. J. Fraser, J. R. Betley, O. B. Schulz-Trieglaff, G. P. Smith, and S. J. Peacock.** 2012. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. N. Engl. J. Med. **366:**2267-2275.

105. **Krishna, S., and L. S. Miller.** 2012. Host-pathogen interactions between the skin and *Staphylococcus aureus*. Curr. Opin. Microbiol. **15:**28-35.

106. **Krismer, B., and A. Peschel.** 2011. Does *Staphylococcus aureus* nasal colonization involve biofilm formation? Future Microbiol. **6:**489-493.

107. **Kuroda, M., T. Ohta, I. Uchiyama, T. Baba, H. Yuzawa, I. Kobayashi, L. Cui, A. Oguchi, K. Aoki, Y. Nagai, J. Lian, T. Ito, M. Kanamori, H. Matsumaru, A. Maruyama, H. Murakami, A. Hosoyama, Y. Mizutani-Ui, N. K. Takahashi, T. Sawano, R. Inoue, C. Kaito, K. Sekimizu, H. Hirakawa, S. Kuhara, S. Goto, J. Yabuzaki, M. Kanehisa, A. Yamashita, K. Oshima, K. Furuya, C. Yoshino, T. Shiba, M. Hattori, N. Ogasawara, H. Hayashi, and K. Hiramatsu.** 2001. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet **357:**1225-1240.

108. **Lai, Y., A. L. Cogen, K. A. Radek, H. J. Park, D. T. Macleod, A. Leichtle, A. F. Ryan, A. Di Nardo, and R. L. Gallo.** 2010. Activation of TLR2 by a small molecule produced by *Staphylococcus epidermidis* increases antimicrobial defense against bacterial skin infections. J. Invest. Dermatol. **130:**2211-2221.

109. **Lai, Y., N. A. Di, T. Nakatsuji, A. Leichtle, Y. Yang, A. L. Cogen, Z. R. Wu, L. V. Hooper, R. R. Schmidt, A. S. von, K. A. Radek, C. M. Huang, A. F. Ryan, and R. L. Gallo.** 2009. Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. Nat. Med. **15:**1377-1382.

110. **Lee, C. J., S. Sankaran, D. V. Mukherjee, Z. L. Apa, C. A. Hafer, L. Wright, E. L. Larson, and F. D. Lowy.** 2011. *Staphylococcus aureus* oropharyngeal carriage in a prison population. Clin. Infect. Dis. **52:**775-778.

111. **Lee, G. M., S. S. Huang, S. L. Rifas-Shiman, V. L. Hinrichsen, S. I. Pelton, K. Kleinman, W. P. Hanage, M. Lipsitch, A. J. McAdam, and J. A. Finkelstein.** 2009. Epidemiology and risk factors for *Staphylococcus aureus* colonization in children in the post-PCV7 era. BMC Infect. Dis. **9:**110.

112. **Lemon, K. P., V. Klepac-Ceraj, H. K. Schiffer, E. L. Brodie, S. V. Lynch, and R. Kolter.** 2010. Comparative analyses of the bacterial microbiota of the human nostril and oropharynx. mBio **1:**e00129-00110.

113. **Lina, G., F. Boutite, A. Tristan, M. Bes, J. Etienne, and F. Vandenesch.** 2003. Bacterial competition for human nasal cavity colonization: role of Staphylococcal *agr* alleles. Appl. Environ. Microbiol. **69:**18-23.

114. **Lindsay, J. A.** 2010. Genomic variation and evolution of *Staphylococcus aureus*. Int. J. Med. Microbiol. **300:**98-103.

115. **Lindsay, J. A., C. E. Moore, N. P. Day, S. J. Peacock, A. A. Witney, R. A. Stabler, S. E. Husain, P. D. Butcher, and J. Hinds.** 2006. Microarrays reveal that each of the ten dominant lineages of *Staphylococcus aureus* has a unique combination of surface-associated and regulatory genes. J. Bacteriol. **188:**669-676.

116. **Lipsky, B. A., R. E. Pecoraro, M. S. Chen, and T. D. Koepsell.** 1987. Factors affecting staphylococcal colonization among NIDDM outpatients. Diabetes care **10:**483-486.

117. **Loughman, J. A., S. A. Fritz, G. A. Storch, and D. A. Hunstad.** 2009. Virulence gene expression in human community-acquired *Staphylococcus aureus* infection. J. Infect. Dis. **199:**294-301.

118. **Lu, P. L., J. C. Tsai, Y. W. Chiu, F. Y. Chang, Y. W. Chen, C. F. Hsiao, and L. K. Siu.** 2008. Methicillin-resistant *Staphylococcus aureus* carriage, infection and transmission in dialysis patients, healthcare workers and their family members. Nephrol. Dial. Transplant. **23:**1659-1665.

119. **Luzar, M. A., G. A. Coles, B. Faller, A. Slingeneyer, G. D. Dah, C. Briat, C. Wone, Y. Knefati, M. Kessler, and F. Peluso.** 1990. *Staphylococcus aureus* nasal carriage and infection in patients on continuous ambulatory peritoneal dialysis. N. Engl. J. Med. **322:**505-509.

120. **Maiden, M. C., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt.** 1998. Multilocus sequence typing: a portable approach to the

identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. U. S. A. **95:**3140-3145.

121. **Malachowa, N., and F. R. DeLeo.** 2010. Mobile genetic elements of *Staphylococcus aureus*. Cell Mol. Life Sci. **67:**3057-3071.

122. **Margolis, E.** 2009. Hydrogen peroxide-mediated interference competition by *Streptococcus pneumoniae* has no significant effect on *Staphylococcus aureus* nasal colonization of neonatal rats. J. Bacteriol. **191:**571-575.

123. **Margolis, E., A. Yates, and B. R. Levin.** 2010. The ecology of nasal colonization of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus*: the role of competition and interactions with host's immune response. BMC Microbiol. **10:**59.

124. **Marshall, C., and D. Spelman.** 2007. Re: is throat screening necessary to detect methicillin-resistant *Staphylococcus aureus* colonization in patients upon admission to an intensive care unit? J. Clin. Microbiol. **45:**3855.

125. **Massey, R. C., M. J. Horsburgh, G. Lina, M. Hook, and M. Recker.** 2006. The evolution and maintenance of virulence in *Staphylococcus aureus*: a role for host-to-host transmission? Nat. Rev. Microbiol. **4:**953-958.

126. **Mathema, B. M., J.R.; Chen, L.; Kreiswirth, B.N.** 2009. Evolution and taxonomy of Staphylococci. *In* K. B. J. Crossley, K.K.; Archer, G.L.; Fowler, V.G. (ed.), Staphylococci in Human Disease, 2 ed. Wiley-Blackwell, Chichester, UK.

127. **McCarthy, A. J., and J. A. Lindsay.** 2010. Genetic variation in *Staphylococcus aureus* surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. BMC Microbiol. **10:**173.

128. **McGavin, M. J., B. Arsic, and N. N. Nickerson.** 2012. Evolutionary blueprint for host- and niche-adaptation in *Staphylococcus aureus* clonal complex CC30. Front. Cell. Infect. Microbiol. **2:**48.

129. **Melles, D. C., D. Bogaert, R. F. Gorkink, J. K. Peeters, M. J. Moorhouse, A. Ott, W. B. van Leeuwen, G. Simons, H. A. Verbrugh, P. W. Hermans, and A. van Belkum.** 2007. Nasopharyngeal co-colonization with *Staphylococcus aureus* and *Streptococcus pneumoniae* in children is bacterial genotype independent. Microbiology **153:**686-692.

130. **Melles, D. C., R. F. Gorkink, H. A. Boelens, S. V. Snijders, J. K. Peeters, M. J. Moorhouse, P. J. van der Spek, W. B. van Leeuwen, G. Simons, H. A. Verbrugh, and A. van Belkum.** 2004. Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. J. Clin. Invest **114:**1732-1740.

131. **Melles, D. C., W. B. van Leeuwen, S. V. Snijders, D. Horst-Kreft, J. K. Peeters, H. A. Verbrugh, and A. van Belkum.** 2007. Comparison of multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), and amplified fragment length polymorphism (AFLP) for genetic typing of *Staphylococcus aureus*. J. Microbiol. Methods **69:**371-375.

132. **Mellmann, A., T. Weniger, C. Berssenbrugge, U. Keckevoet, A. W. Friedrich, D. Harmsen, and H. Grundmann.** 2008. Characterization of clonal relatedness among the natural population of *Staphylococcus aureus* strains by using *spa* sequence typing and the BURP (based upon repeat patterns) algorithm. J. Clin. Microbiol. **46:**2805-2808.

133. **Mellmann, A., T. Weniger, C. Berssenbrugge, J. Rothganger, M. Sammeth, J. Stoye, and D. Harmsen.** 2007. Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term evolution of *Staphylococcus aureus* populations based on *spa* polymorphisms. BMC. Microbiol. **7:**98.

134. **Merino, N., A. Toledo-Arana, M. Vergara-Irigaray, J. Valle, C. Solano, E. Calvo, J. A. Lopez, T. J. Foster, J. R. Penades, and I. Lasa.** 2009. Protein A-mediated multicellular behavior in *Staphylococcus aureus*. J. Bacteriol. **191:**832-843.

135. **Miller, L. S.** 2008. Toll-like receptors in skin. Adv. Dermatol. **24:**71-87.

136. **Miller, M., H. A. Cook, E. Y. Furuya, M. Bhat, M. H. Lee, P. Vavagiakis, P. Visintainer, G. Vasquez, E. Larson, and F. D. Lowy.** 2009. *Staphylococcus aureus* in the community: colonization versus infection. Plos One **4:**e6708.

137. **Moks, T., L. Abrahmsen, B. Nilsson, U. Hellman, J. Sjoquist, and M. Uhlen.** 1986. Staphylococcal protein A consists of five IgG-binding domains. Eur. J. Biochem. **156:**637-643.

138. **Mollema, F. P., J. H. Richardus, M. Behrendt, N. Vaessen, W. Lodder, W. Hendriks, H. A. Verbrugh, and M. C. Vos.** 2010. Transmission of methicillin-resistant *Staphylococcus aureus* to household contacts. J. Clin. Microbiol. **48:**202-207.

139. **Monecke, S., L. Jatzwauk, S. Weber, P. Slickers, and R. Ehricht.** 2008. DNA microarray-based genotyping of methicillin-resistant *Staphylococcus aureus* strains from Eastern Saxony. Clin. Microbiol. Infect. **14:**534-545.

140. **Morgan, M.** 2008. Methicillin-resistant *Staphylococcus aureus* and animals: zoonosis or humanosis? J. Antimicrob. Chemother. **62:**1181-1187.

141. **Murchan, S., M. E. Kaufmann, A. Deplano, R. de Ryck, M. Struelens, C. E. Zinn, V. Fussing, S. Salmenlinna, J. Vuopio-Varkila, N. El Solh, C. Cuny, W. Witte, P. T. Tassios, N. Legakis, W. van Leeuwen, A. van Belkum, A. Vindel, I. Laconcha, J. Garaizar, S. Haeggman, B. Olsson-Liljequist, U. Ransjo, G. Coombes, and B. Cookson.** 2003. Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J. Clin. Microbiol. **41:**1574-1585.

142. **Muthukrishnan, G., G. A. Quinn, R. P. Lamers, C. Diaz, A. L. Cole, S. Chen, and A. M. Cole.** 2011. Exoproteome of *Staphylococcus aureus* reveals putative determinants of nasal carriage. J. Proteome. Res. **10:**2064-2078.

143. **Mwangi, M. M., S. W. Wu, Y. Zhou, K. Sieradzki, H. de Lencastre, P. Richardson, D. Bruce, E. Rubin, E. Myers, E. D. Siggia, and A. Tomasz.** 2007. Tracking the in vivo evolution of multidrug resistance in *Staphylococcus aureus* by whole-genome sequencing. Proc. Natl. Acad. Sci. U. S. A. **104:**9451-9456.

144. **Nestle, F. O., M. P. Di, J. Z. Qin, and B. J. Nickoloff.** 2009. Skin immune sentinels in health and disease. Nat. Rev. Immunol. **9:**679-691.

145. **Newman, R. M., P. Salunkhe, A. Godzik, and J. C. Reed.** 2006. Identification and characterization of a novel bacterial virulence factor that shares homology with mammalian Toll/interleukin-1 receptor family proteins. Infect. Immun. **74:**594-601.

146. **Nguyen, T., B. Ghebrehiwet, and E. I. B. Peerschke.** 2000. *Staphylococcus aureus* protein A recognizes platelet gC1qR/p33: a novel mechanism for staphylococcal interactions with platelets. Infect. Immun. **68:**2061-2068.

147. **Nilsson, P., and T. Ripa.** 2006. *Staphylococcus aureus* throat colonization is more frequent than colonization in the anterior nares. J. Clin. Microbiol. **44:**3334-3339.

148. **NORM/NORM-VET.** 2010. Usage of antimicrobial agents and occurence of antimicrobial resistance in Norway. Tromsø/Oslo 2011. ISSN:1502-2307 (print)/1890-9965 (electronic).

149. **NORM/NORM-VET.** 2008. Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. Tromsø/Oslo 2009. ISSN:1502-2307 (print)/1890-9965 (electronic).

150. **Nouwen, J., H. Boelens, A. van Belkum, and H. Verbrugh.** 2004. Human factor in *Staphylococcus aureus* nasal carriage. Infect. Immun. **72:**6685-6688.

151. **Nouwen, J. L., M. W. Fieren, S. Snijders, H. A. Verbrugh, and A. van Belkum.** 2005. Persistent (not intermittent) nasal carriage of *Staphylococcus aureus* is the determinant of CPD-related infections. Kidney Int. **67:**1084-1092.

152. **Nouwen, J. L., A. Ott, M. F. Kluytmans-Vandenbergh, H. A. Boelens, A. Hofman, A. van Belkum, and H. A. Verbrugh.** 2004. Predicting the *Staphylococcus aureus* nasal carrier state: derivation and validation of a "culture rule". Clin. Infect. Dis. **39:**806-811.

153. **Novick, R. P.** 2006. Staphylococcal pathogenesis and pathogenicity factors: Genetics and regulation, p. 496-516. *In* V. A. N. Fischetti, R.P; Ferretti, J.J; Portnoy, D.A.; Rood, J.I. (ed.), Gram-Positive Pathogens. ASM Press, Washington, D.C.

154. **Nubel, U., P. Roumagnac, M. Feldkamp, J. H. Song, K. S. Ko, Y. C. Huang, G. Coombs, M. Ip, H. Westh, R. Skov, M. J. Struelens, R. V. Goering, B. Strommenger, A. Weller, W. Witte, and M. Achtman.** 2008. Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*. Proc. Natl. Acad. Sci. U. S. A. **105:**14130-14135.

155. **O'Brien, L. M., E. J. Walsh, R. C. Massey, S. J. Peacock, and T. J. Foster.** 2002. *Staphylococcus aureus* clumping factor B (ClfB) promotes adherence to human type I cytokeratin 10: implications for nasal colonization. Cell Microbiol. **4:**759-770.

156. **Olsen, K., B. M. Falch, K. Danielsen, M. Johannessen, J. U. Ericson Sollid, I. Thune, G. Grimnes, R. Jorde, G. S. Simonsen, and A. S. Furberg.** 2012. *Staphylococcus aureus* nasal carriage is associated with serum 25-hydroxyvitamin D levels, gender and smoking status. The Tromso Staph and Skin Study. Eur. J. Clin. Microbiol. Infect. Dis. **31:**465-473.

157. **Olsen, K., M. Sangvik, G. S. Simonsen, J. U. Sollid, A. Sundsfjord, I. Thune, and A. S. Furberg.** 2012. Prevalence and population structure of *Staphylococcus aureus* nasal carriage in healthcare workers in a general population. The Tromso Staph and Skin Study. Epidemiol. Infect.**:**doi:10.1017/S0950268812000465.

158. **Olsen, K., G. S. Simonsen, A. Sundsfjord, H. H. Haukland, J. E. Sollid, K. Danielsen, B. Haldorsen, A. E. Eggen, and A.-S. Furberg.** 2009. The epidemiology of *Staphylococcus aureus* nasal and throat carriage in a large community-based population in North Norway. The Tromsø Staph and Skin Study, abstr. 1678. Abstr. 19th Eur. Congr. Clin. Microbiol. Infect. Dis.

159. **Otto, M.** 2001. *Staphylococcus aureus* and *Staphylococcus epidermidis* peptide pheromones produced by the accessory gene regulator *agr* system. Peptides **22:**1603-1608.

160. **Palmqvist, N., T. Foster, A. Tarkowski, and E. Josefsson.** 2002. Protein A is a virulence factor in *Staphylococcus aureus* arthritis and septic death. Microb. Pathog. **33:**239-249.

161. **Peacock, S. J., I. de Silva, and F. D. Lowy.** 2001. What determines nasal carriage of *Staphylococcus aureus*? Trends Microbiol. **9:**605-610.

162. **Peacock, S. J., A. Justice, D. Griffiths, G. D. de Silva, M. N. Kantzanou, D. Crook, K. Sleeman, and N. P. Day.** 2003. Determinants of acquisition and carriage of *Staphylococcus aureus* in infancy. J. Clin. Microbiol. **41:**5718-5725.

163. **Pennell, L. M., C. L. Galligan, and E. N. Fish.** 2012. Sex affects immunity. J. Autoimmun. **38:**J282-291.

164. **Picard, C., H. von Bernuth, P. Ghandil, M. Chrabieh, O. Levy, P. D. Arkwright, D. McDonald, R. S. Geha, H. Takada, J. C. Krause, C. B. Creech, C. L. Ku, S. Ehl, L. Marodi, S. Al-Muhsen, S. Al-Hajjar, A. Al-Ghonaium, N. K. Day-Good, S. M. Holland, J. I. Gallin, H. Chapel, D. P. Speert, C. Rodriguez-Gallego, E. Colino, B. Z. Garty, C. Roifman, T. Hara, H. Yoshikawa, S. Nonoyama, J. Domachowske, A. C. Issekutz, M. Tang, J. Smart, S. E. Zitnik, C. Hoarau, D. S. Kumararatne, A. J. Thrasher, E. G. Davies, C. Bethune, N. Sirvent, D. de Ricaud, Y. Camcioglu, J. Vasconcelos, M. Guedes, A. B. Vitor, C. Rodrigo, F. Almazan, M. Mendez, J. I. Arostegui, L. Alsina, C. Fortuny, J. Reichenbach, J. W. Verbsky, X. Bossuyt, R. Doffinger, L. Abel, A. Puel, and J. L. Casanova.** 2010. Clinical features and outcome of patients with IRAK-4 and MyD88 deficiency. Medicine **89:**403-425.

165. **Projan, S. J., Ruzin, A.** 2006. Antibiotic Resistance in the Staphylococci, p. 587-597. *In* V. A. N. Fischetti, R.P; Ferretti, J.J; Portnoy, D.A.; Rood, J.I. (ed.), Gram-Positive Pathogens. ASM Press, Washington, D.C.

166. **Pynnonen, M., R. E. Stephenson, K. Schwartz, M. Hernandez, and B. R. Boles.** 2011. Hemoglobin promotes *Staphylococcus aureus* nasal colonization. PLoS Pathog. **7:**e1002104.

167. **Qazi, S. N., S. E. Harrison, T. Self, P. Williams, and P. J. Hill.** 2004. Real-time monitoring of intracellular *Staphylococcus aureus* replication. J. Bacteriol. **186:**1065-1077.

168. **Rajakaruna, L., G. Hallas, L. Molenaar, D. Dare, H. Sutton, V. Encheva, R. Culak, I. Innes, G. Ball, A. M. Sefton, M. Eydmann, A. M. Kearns, and H. N. Shah.** 2009. High throughput identification of clinical isolates of *Staphylococcus aureus* using MALDI-TOF-MS of intact cells. Infect. Genet. Evol. **9:**507-513.

169. **Rana, R. R., M. Zhang, A. M. Spear, H. S. Atkins, and B. Byrne.** 2012. Bacterial TIR-containing proteins and host innate immune system evasion. Med. Microbiol. Immunol.**:**doi:10.1007/s00430-00012-00253-00432.

170. **Regev-Yochay, G., R. Dagan, M. Raz, Y. Carmeli, B. Shainberg, E. Derazne, G. Rahav, and E. Rubinstein.** 2004. Association between carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in Children. J. Amer. Med. Assoc. **292:**716-720.

171. **Regev-Yochay, G., K. Trzcinski, C. M. Thompson, R. Malley, and M. Lipsitch.** 2006. Interference between *Streptococcus pneumoniae* and *Staphylococcus aureus*: In vitro hydrogen peroxide-mediated killing by *Streptococcus pneumoniae*. J. Bacteriol. **188:**4996-5001.

172. **Ridley, M.** 1959. Perineal carriage of *Staph. aureus*. Br. Med. J. **1:**270-273.

173. **Riley, M. A.** 1998. Molecular mechanisms of bacteriocin evolution. Annu. Rev. Genet. **32:**255-278.

174. **Robinson, D. A., and M. C. Enright.** 2004. Evolution of *Staphylococcus aureus* by large chromosomal replacements. J. Bacteriol. **186:**1060-1064.

175. **Robinson, D. A., and M. C. Enright.** 2004. Multilocus sequence typing and the evolution of methicillin-resistant *Staphylococcus aureus*. Clin. Microbiol. Infect. **10:**92-97.

176. **Roche, F. M., M. Meehan, and T. J. Foster.** 2003. The *Staphylococcus aureus* surface protein SasG and its homologues promote bacterial adherence to human desquamated nasal epithelial cells. Microbiology **149:**2759-2767.

177. **Rooijakkers, S. H., and J. A. van Strijp.** 2007. Bacterial complement evasion. Mol. Immunol. **44:**23-32.

178. **Rooijakkers, S. H., W. J. van Wamel, M. Ruyken, K. P. van Kessel, and J. A. van Strijp.** 2005. Anti-opsonic properties of staphylokinase. Microbes. Infect. **7:**476-484.

179. **Ross, T. L., W. G. Merz, M. Farkosh, and K. C. Carroll.** 2005. Comparison of an automated repetitive sequence-based PCR microbial typing system to pulsed-field gel electrophoresis for analysis of outbreaks of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **43:**5642-5647.

180. **Ruimy, R., C. Angebault, F. Djossou, C. Dupont, L. Epelboin, S. Jarraud, L. A. Lefevre, M. Bes, B. E. Lixandru, M. Bertine, M. A. El, M. Renard, R. M. Bettinger, M. Lescat, O. Clermont, G. Peroz, G. Lina, M. Tavakol, F. Vandenesch, A. van Belkum, F. Rousset, and A. Andremont.** 2010. Are host genetics the predominant determinant of persistent nasal *Staphylococcus aureus* carriage in humans? J. Infect. Dis. **202:**924-934.

181. **Ruimy, R., A. Maiga, L. Armand-Lefevre, I. Maiga, A. Diallo, A. K. Koumare, K. Ouattara, S. Soumare, K. Gaillard, J. C. Lucet, A. Andremont, and E. J. Feil.** 2008. The carriage population of *Staphylococcus aureus* from Mali is composed of a combination of pandemic clones and the divergent Panton-Valentine leukocidin-positive genotype ST152. J. Bacteriol. **190:**3962-3968.

182. **Sabat, A., J. Krzyszton-Russjan, W. Strzalka, R. Filipek, K. Kosowska, W. Hryniewicz, J. Travis, and J. Potempa.** 2003. New method for typing *Staphylococcus aureus* strains: multiple-locus variable-number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. J. Clin. Microbiol. **41:**1801-1804.

183. **Sakwinska, O., D. S. Blanc, C. Lazor-Blanchet, M. Moreillon, M. Giddey, and P. Moreillon.** 2010. Ecological temporal stability of *Staphylococcus aureus* nasal carriage. J. Clin. Microbiol. **48:**2724-2728.

184. **Sakwinska, O., M. Giddey, M. Moreillon, D. Morisset, A. Waldvogel, and P. Moreillon.** 2011. *Staphylococcus aureus* host range and human-bovine host shift. Appl. Environ. Microbiol. **77:**5908-5915.

185. **Sakwinska, O., G. Kuhn, C. Balmelli, P. Francioli, M. Giddey, V. Perreten, A. Riesen, F. Zysset, D. S. Blanc, and P. Moreillon.** 2009. Genetic diversity and ecological success of *Staphylococcus aureus* strains colonizing humans. Appl. Environ. Microbiol. **75:**175-183.

186. **Saunders, N. A., A. Underwood, A. M. Kearns, and G. Hallas.** 2004. A virulence-associated gene microarray: a tool for investigation of the evolution and pathogenic potential of *Staphylococcus aureus*. Microbiology **150:**3763-3771.

187. **Schaffer, A. C., R. M. Solinga, J. Cocchiaro, M. Portoles, K. B. Kiser, A. Risley, S. M. Randall, V. Valtulina, P. Speziale, E. Walsh, T. Foster, and J. C. Lee.** 2006. Immunization with *Staphylococcus aureus* clumping factor B, a major determinant in nasal carriage, reduces nasal colonization in a murine model. Infect. Immun. **74:**2145-2153.

188. **Schneewind, O., D. Mihaylova-Petkov, and P. Model.** 1993. Cell wall sorting signals in surface proteins of gram-positive bacteria. EMBO J. **12:**4803-4811.

189. **Schouls, L. M., E. C. Spalburg, M. van Luit, X. W. Huijsdens, G. N. Pluister, M. G. van Santen-Verheuvel, H. G. van der Heide, H. Grundmann, M. E. Heck, and A. J. de Neeling.** 2009. Multiple-locus variable number tandem repeat analysis of *Staphylococcus aureus*: comparison with pulsed-field gel electrophoresis and *spa*-typing. Plos One **4:**e5082.

190. **Schwarzkopf, R., R. C. Takemoto, I. Immerman, J. D. Slover, and J. A. Bosco.** 2010. Prevalence of *Staphylococcus aureus* colonization in orthopaedic surgeons and their patients: a prospective cohort controlled study. J. Bone Joint Surg. Am. **92:**1815-1819.

191. **Selander, R. K., D. A. Caugant, H. Ochman, J. M. Musser, M. N. Gilmour, and T. S. Whittam.** 1986. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl. Environ. Microbiol. **51:**873-884.

192. **Sevilla, L. M., P. Bayo, V. Latorre, A. Sanchis, and P. Perez.** 2010. Glucocorticoid receptor regulates overlapping and differential gene subsets in developing and adult skin. Mol. Endocrinol. **24:**2166-2178.

193. **Shinefield, H. R., J. D. Wilsey, J. C. Ribble, M. Boris, H. F. Eichenwald, and C. I. Dittmar.** 1966. Interactions of staphylococcal colonization. Influence of normal nasal flora and antimicrobials on inoculated *Staphylococcus aureus* strain 502A. Am. J. Dis. Child. **111:**11-21.

194. **Shopsin, B., M. Gomez, S. O. Montgomery, D. H. Smith, M. Waddington, D. E. Dodge, D. A. Bost, M. Riehman, S. Naidich, and B. N. Kreiswirth.** 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J. Clin. Microbiol. **37:**3556-3563.

195. **Simoons-Smit, A. M., P. H. Savelkoul, J. Stoof, T. M. Starink, and C. M. Vandenbroucke-Grauls.** 2000. Transmission of *Staphylococcus aureus* between humans and domestic animals in a household. Eur. J. Clin. Microbiol. Infect. Dis. **19:**150-152.

196. **Sinha, B., and M. Fraunholz.** 2010. *Staphylococcus aureus* host cell invasion and post-invasion events. Int. J. Med. Microbiol. **300:**170-175.

197. **Sissolak, D., A. Geusau, G. Heinze, W. Witte, and M. L. Rotter.** 2002. Risk factors for nasal carriage of *Staphylococcus aureus* in infectious disease patients, including patients infected with HIV, and molecular typing of colonizing strains. Eur. J. Clin. Microbiol. Infect. Dis. **21:**88-96.

198. **Sjodahl, J.** 1977. Repetitive sequences in protein A from *Staphylococcus aureus*. Arrangement of five regions within the protein, four being highly homologous and Fc-binding. Eur. J. Biochem. **73:**343-351.

199. **Slack, J. L., K. Schooley, T. P. Bonnert, J. L. Mitcham, E. E. Qwarnstrom, J. E. Sims, and S. K. Dower.** 2000. Identification of two major sites in the type I

interleukin-1 receptor cytoplasmic region responsible for coupling to pro-inflammatory signaling pathways. J. Biol. Chem. **275:**4670-4678.

200. **Smyth, D. S., and D. A. Robinson.** 2010. Population genetics of *Staphylococcus*, p. 321-343. *In* D. A. Robinson, D. Falush, and E. J. Feil (ed.), Bacterial population genetics in infectious disease. John Wiley & Sons, Inc., Hoboken, NJ.

201. **Stebbins, C. E., and J. E. Galan.** 2001. Structural mimicry in bacterial virulence. Nature **412:**701-705.

202. **Stefani, S., D. R. Chung, J. A. Lindsay, A. W. Friedrich, A. M. Kearns, H. Westh, and F. M. Mackenzie.** 2012. Meticillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. Int. J. Antimicrob. Agents **39:**273-282.

203. **Strommenger, B., C. Kettlitz, T. Weniger, D. Harmsen, A. W. Friedrich, and W. Witte.** 2006. Assignment of *Staphylococcus* isolates to groups by *spa* typing, SmaI macrorestriction analysis, and multilocus sequence typing. J. Clin. Microbiol. **44:**2533-2540.

204. **Struelens, M. J.** 1996. Consensus guidelines for appropriate use and evaluation of microbial epidemiologic typing systems. Clin. Microbiol. Infect. **2:**2-11.

205. **Struelens, M. J.** 1998. Molecular epidemiologic typing systems of bacterial pathogens: current issues and perspectives. Mem. Inst. Oswaldo Cruz **93:**581-585.

206. **Struelens, M. J., P. M. Hawkey, G. L. French, W. Witte, and E. Tacconelli.** 2009. Laboratory tools and strategies for methicillin-resistant *Staphylococcus aureus* screening, surveillance and typing: state of the art and unmet needs. Clin. Microbiol. Infect. **15:**112-119.

207. **Suffoletto, B. P., E. H. Cannon, K. Ilkhanipour, and D. M. Yealy.** 2008. Prevalence of *Staphylococcus aureus* nasal colonization in emergency department personnel. Ann. Emerg. Med. **52:**529-533.

208. **Sugarman, B., and L. R. Epps.** 1982. Effect of estrogens on bacterial adherence to HeLa cells. Infect. Immun. **35:**633-638.

209. **Sung, J. M., D. H. Lloyd, and J. A. Lindsay.** 2008. *Staphylococcus aureus* host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. Microbiology **154:**1949-1959.

210. **Takeuchi, O., and S. Akira.** 2010. Pattern recognition receptors and inflammation. Cell **140:**805-820.

211. **Takeuchi, O., K. Hoshino, and S. Akira.** 2000. Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to *Staphylococcus aureus* infection. J. Immunol. **165:**5392-5396.

212. **Takuno, S., T. Kado, R. P. Sugino, L. Nakhleh, and H. Innan.** 2012. Population genomics in bacteria: a case study of *Staphylococcus aureus*. Mol. Biol. Evol. **29:**797-809.

213. **Tanaka, H., H. Akama, Y. Ichikawa, M. Homma, and H. Oshima.** 1991. Glucocorticoid receptors in normal leukocytes: effects of age, gender, season, and plasma cortisol concentrations. Clin. Chem. **37:**1715-1719.

214. **Ten Broeke-Smits, N. J., J. A. Kummer, R. L. Bleys, A. C. Fluit, and C. H. Boel.** 2010. Hair follicles as a niche of *Staphylococcus aureus* in the nose; is a more effective decolonisation strategy needed? J. Hosp. Infect. **76:**211-214.

215. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233-2239.

216. **Uehara, Y., H. Nakama, K. Agematsu, M. Uchida, Y. Kawakami, A. S. Abdul Fattah, and N. Maruchi.** 2000. Bacterial interference among nasal inhabitants: eradication of *Staphylococcus aureus* from nasal cavities by artificial implantation of *Corynebacterium sp*. J. Hosp. Infect. **44:**127-133.

217. **van Belkum, A., M. Emonts, H. Wertheim, C. de Jongh, J. Nouwen, H. Bartels, A. Cole, A. Cole, P. Hermans, H. Boelens, N. L. Toom, S. Snijders, H. Verbrugh, and W. van Leeuwen.** 2007. The role of human innate immune factors in nasal colonization by *Staphylococcus aureus*. Microbes. Infect. **9:**1471-1477.

218. **van Belkum, A., D. C. Melles, J. Nouwen, W. B. van Leeuwen, W. van Wamel, M. C. Vos, H. F. Wertheim, and H. A. Verbrugh.** 2009. Co-evolutionary aspects of human colonisation and infection by *Staphylococcus aureus*. Infect. Genet. Evol. **9:**32-47.

219. **van Belkum, A., N. J. Verkaik, C. P. de Vogel, H. A. Boelens, J. Verveer, J. L. Nouwen, H. A. Verbrugh, and H. F. Wertheim.** 2009. Reclassification of *Staphylococcus aureus* nasal carriage types. J. Infect. Dis. **199:**1820-1826.

220. **van den Akker, E. L., J. L. Nouwen, D. C. Melles, E. F. van Rossum, J. W. Koper, A. G. Uitterlinden, A. Hofman, H. A. Verbrugh, H. A. Pols, S. W. Lamberts, and A. van Belkum.** 2006. *Staphylococcus aureus* nasal carriage is associated with glucocorticoid receptor gene polymorphisms. J. Infect. Dis. **194:**814-818.

221. **van Wamel, W. J., S. H. Rooijakkers, M. Ruyken, K. P. van Kessel, and J. A. van Strijp.** 2006. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. J. Bacteriol. **188:**1310-1315.

222. **Vandenbergh, M. F., and H. A. Verbrugh.** 1999. Carriage of *Staphylococcus aureus*: epidemiology and clinical relevance. J. Lab Clin. Med. **133:**525-534.

223. **Vandenbergh, M. F., E. P. Yzerman, A. van Belkum, H. A. Boelens, M. Sijmons, and H. A. Verbrugh.** 1999. Follow-up of *Staphylococcus aureus* nasal carriage

after 8 years: redefining the persistent carrier state. J. Clin. Microbiol. **37:**3133-3140.

224. **Verbrugh, H. A.** 2009. Colonization with *Staphylococcus aureus* and the role of colonization in causing infection. *In* K. B. J. Crossley, K.K.; Archer, G.L.; Fowler, V.G. (ed.), Staphylococci in human disease, 2nd ed. Wiley-Blackwell, Chichester, UK.

225. **Verhoeven, P. O., F. Grattard, A. Carricajo, F. Lucht, C. Cazorla, O. Garraud, B. Pozzetto, and P. Berthelot.** 2012. An algorithm based on one or two nasal samples is accurate to identify persistent nasal carriers of *Staphylococcus aureus*. Clin. Microbiol. Infect. **18:**551-557.

226. **von Eiff, C., K. Becker, K. Machka, H. Stammer, and G. Peters.** 2001. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. N. Engl. J. Med. **344:**11-16.

227. **Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau.** 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23:**4407-4414.

228. **Wagenvoort, J. H., E. I. De Brauwer, M. L. Sijstermans, and H. M. Toenbreker.** 2005. Risk of re-introduction of methicillin-resistant *Staphylococcus aureus* into the hospital by intrafamilial spread from and to healthcare workers. J. Hosp. Infect. **59:**67-68.

229. **Weese, J. S.** 2010. Methicillin-resistant *Staphylococcus aureus* in animals. ILAR J. **51:**233-244.

230. **Weidenmaier, C., C. Goerke, and C. Wolz.** 2012. *Staphylococcus aureus* determinants for nasal colonization. Trends Microbiol. **20:**243-250.

231. **Weidenmaier, C., J. F. Kokai-Kun, S. A. Kristian, T. Chanturiya, H. Kalbacher, M. Gross, G. Nicholson, B. Neumeister, J. J. Mond, and A. Peschel.** 2004. Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. Nat. Med. **10:**243-245.

232. **Weidenmaier, C., J. F. Kokai-Kun, E. Kulauzovic, T. Kohler, G. Thumm, H. Stoll, F. Gotz, and A. Peschel.** 2008. Differential roles of sortase-anchored surface proteins and wall teichoic acid in *Staphylococcus aureus* nasal colonization. Int. J. Med. Microbiol. **298:**505-513.

233. **Wentworth, B. B.** 1963. Bacteriophage typing of the Staphylococci. Bacteriol. Rev. **27:**253-272.

234. **Wertheim, H. F., D. C. Melles, M. C. Vos, W. van Leeuwen, A. van Belkum, H. A. Verbrugh, and J. L. Nouwen.** 2005. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect. Dis. **5:**751-762.

235. **Wertheim, H. F., M. van Kleef, M. C. Vos, A. Ott, H. A. Verbrugh, and W. Fokkens.** 2006. Nose picking and nasal carriage of *Staphylococcus aureus*. Infect. Control Hosp. Epidemiol. **27:**863-867.

236. **Wertheim, H. F., M. C. Vos, A. Ott, A. van Belkum, A. Voss, J. A. Kluytmans, P. H. van Keulen, C. M. Vandenbroucke-Grauls, M. H. Meester, and H. A. Verbrugh.** 2004. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet **364:**703-705.

237. **Wertheim, H. F., E. Walsh, R. Choudhurry, D. C. Melles, H. A. Boelens, H. Miajlovic, H. A. Verbrugh, T. Foster, and B. A. van.** 2008. Key role for clumping factor B in *Staphylococcus aureus* nasal colonization of humans. PLoS Med. **5:**e17.

238. **White, A.** 1961. Quantitative studies of nasal carriers of staphylococci among hospitalized patients. J. Clin. Invest. **40:**23-30.

239. **White, A.** 1961. Relation between quantitative nasal cultures and dissemination of staphylococci. J. Lab. Clin. Med. **58:**273-277.

240. **Williams, J. V., B. R. Vowels, P. J. Honig, and J. J. Leyden.** 1998. *S. aureus* isolation from the lesions, the hands, and the anterior nares of patients with atopic dermatitis. Pediatr. Dermatol. **15:**194-198.

241. **Williams, R. E.** 1963. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. Bacteriol. Rev. **27:**56-71.

242. **Wilson, M.** 2008. Bacteriology of humans : an ecological perspective. Blackwell Pub., Malden, MA.

243. **Witte, W., Strommenger, B., Werner, G. .** 2006. Diagnostics, typing and taxonomy, p. 371-380. *In* V. A. N. Fischetti, R.P; Ferretti, J.J; Portnoy, D.A.; Rood, J.I. (ed.), Gram-positive pathogens, 2nd ed. ASM Press, Washington, D.C.

244. **Wolters, M., H. Rohde, T. Maier, C. Belmar-Campos, G. Franke, S. Scherpe, M. Aepfelbacher, and M. Christner.** 2011. MALDI-TOF MS fingerprinting allows for discrimination of major methicillin-resistant *Staphylococcus aureus* lineages. Int. J. Med. Microbiol. **301:**64-68.

245. **Wos-Oxley, M. L., I. Plumeier, C. von Eiff, S. Taudien, M. Platzer, R. Vilchez-Vargas, K. Becker, and D. H. Pieper.** 2010. A poke into the diversity and associations within human anterior nare microbial communities. ISME J. **4:**839-851.

246. **Yung, S. C., D. Parenti, and P. M. Murphy.** 2011. Host chemokines bind to *Staphylococcus aureus* and stimulate protein A release. J. Biol. Chem. **286:**5069-5077.

247. **Zanger, P., D. Nurjadi, M. Gaile, S. Gabrysch, and P. G. Kremsner.** 2012. Hormonal contraceptive use and persistent *Staphylococcus aureus* nasal carriage. Clin. Infect. Dis.**:**doi:10.1093/cid/cis1778.

248. **Zanger, P., D. Nurjadi, B. Vath, and P. G. Kremsner.** 2011. Persistent nasal carriage of *Staphylococcus aureus* is associated with deficient induction of human beta-defensin 3 after sterile wounding of healthy skin in vivo. Infect. Immun. **79:**2658-2662.

Paper I

Age- and gender-associated *Staphylococcus aureus* *spa* types found among nasal carriers in a general population: The Tromsø Staph and Skin Study.

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Paper II

Prevalence and population structure of *Staphylococcus aureus* nasal carriage in healthcare workers in a general population. The Tromsø Staph and Skin Study

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Paper III

Staphylococcus aureus mutants lacking cell-wall bound protein A found in isolates from bacteraemia, MRSA-infection and a healthy nasal carrier

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Submitted

Staphylococcus aureus mutants lacking cell-wall bound protein A found in isolates from bacteraemia, MRSA-infection and a healthy nasal carrier

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Running title: MRSA and MSSA protein A mutants

Paper IV

Staphylococcus aureus TIR-domain protein, TirS, negatively interferes with TLR2-, MyD88- and TIRAP-mediated NF-κB signaling and increases intracellular bacterial accumulation

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