

# Molecular epidemiology of *Staphylococcus aureus* among asymptomatic carriers from Saxony

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**Objectives:** The objective was to characterise the colonising *Staphylococcus aureus* population among asymptomatic carriers from Saxony to provide data for comparisons to isolates from defined clinical conditions.

**Methods:** Diagnostic microarrays were used in order to extensively characterise 155 *S. aureus* isolates obtained from asymptomatic carriers (admission screening of trauma and neurosurgical patients, nasal swabs from junior medical students and from workers of a biomedical facility).

**Results:** Some superantigens proved to be very common. Toxic shock syndrome toxin (*tsl*) was detected in 14.8% of these 155 isolates. The enterotoxin cluster *egc* comprising of *seg*, *sei*, *sem*, *sen*, *seo*, and *seu* was very common (45.8%). Enterotoxin A (*sea*) was found in 17.4%. Enterotoxins D, J and R (*sed*, *sej*, *ser*) were always detected together in 15.5%. Enterotoxin genes C (*sec*) and L (*sel*) also occurred together in 12.3%. The genes encoding Panton-Valentine leukocidin (*lukS-PV* and *lukF-PV*) were found only once, in a CC30 MSSA isolate. This virtual absence of PVL in asymptomatic carriers emphasises its pathogenetic significance in patients with skin and soft tissue infections.

Three isolates (1.9%) were MRSA. Most isolates (71.0%) harboured the beta lactamase gene *blaZ*, while other resistance genes were found only sporadically. The 155 isolates typed in this study belonged to twenty different clonal complexes (CC). The most common CC was CC8 (18.7%). It was followed by CC15 (16.8%), CC30 (16.1%) and CC45 (9.0%).

**Discussion:** These data might provide an insight into pathogenesis, especially with regard to the different epidemiology of superantigens and PVL. In a previous study (Monecke & Ehricht, 2007) PVL was found in 30% of abscess isolates, but it was present in only 0.6% of asymptomatic carriers. This emphasises its pathogenetic significance in patients with skin and soft tissue infections. Contrastingly, there was virtually no difference between abscess and carrier isolates with regard to abundances of superantigens including *tsl*. Prevalence data on surface antigens, such as capsules, could be helpful for the design of a future vaccine.

**Introduction:** *Staphylococcus aureus* can be found in the anterior nares of a great proportion – ca. 30% – of a healthy human population. Since *S. aureus* is also able to cause a variety of infections, it is a challenge to define which genetic factors determine whether an encounter between a human and *S. aureus* results in asymptomatic carriage or in clinical disease.

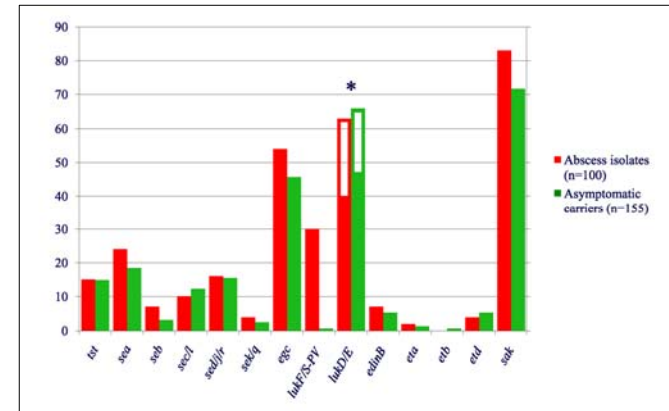
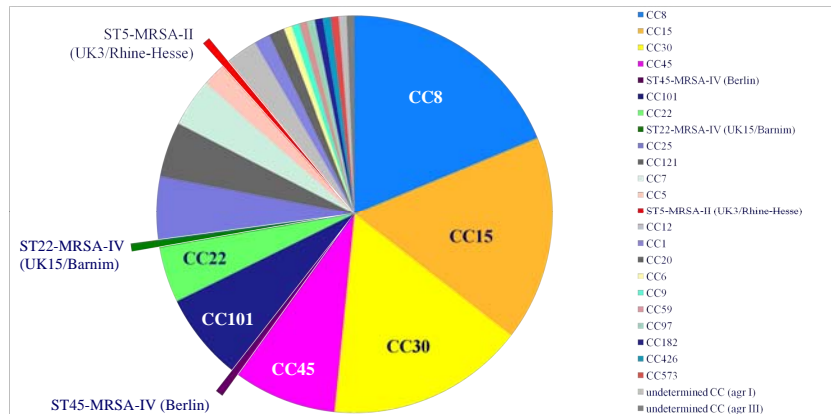
Recent studies [e.g., 1] indicated that there was no fundamental difference between infecting and colonising *S. aureus* populations. In order to obtain data on the “normal” carriage population, we characterised isolates from healthy carriers (or patients with surgical conditions unrelated to *S. aureus*) using microarrays. This allowed us to describe the presence of virulence factors, as well as the affiliation to clonal complexes.

**Methods:** 155 isolates of *S. aureus* were collected between 2005 and 2008 in Dresden in Saxony. They were obtained from three different groups of carriers were characterised. The first group were junior medical students, who did not yet have worked in hospital (77 isolates from nasal swabs). The second group (67 isolates) were patients with conditions unrelated to *S. aureus* infection. Samples included nasal swabs of patients suffering from stroke, intracerebral haemorrhage or trauma, taken on admission as part of a MRSA screening program, as well as conjunctival swabs taken as pre-operative screening prior to cataract surgery. The third group comprised employees of a biomedical facility (11 isolates from nasal swabs). Samples were taken as part of a quality control scheme to guarantee absence of pathogenic bacteria from products of that company.

All relevant exotoxin genes including *ssl/set* genes and resistance genes were covered. Additionally, species specific probes as well as probes for determination of *agr* groups, capsule types, and SCCmec types were included. Probe as well as primer sequences were derived from consensus regions of all published target gene sequences. Details are given in [2]. 3'-amino-modified oligonucleotides were synthesised by Metabion and spotted on arrays (ArrayTube™ system, by CLONDIAG). DNA was prepared using enzymatic lysis (lyso-staphin+lysozym) followed by purification using the Qiagen device EZ1. A multiplex primer elongation reaction was used to incorporate biotin-16-dUTP into the amplicons. The labelled sample was hybridised against the DNA array and horseradish peroxidase coupled streptavidin was added. Then, the ArrayTube was placed into a reading device (ATR 01™, CLONDIAG). Serumun Green substrate was added for staining. Scans were recorded and analysed. Details have been published previously [2]. Analysis of hybridisation pattern also allowed assignment to clonal complexes [2].

**Figure 1:** Affiliation of carrier isolates to clonal complexes as determined by comparison of hybridisation patterns to previously typed reference strains [2].

Three MRSA were found in subjects with a history of previous hospitalisations. These isolates belonged to the locally most abundant strains.



**Figure 2:** Comparison of the pre-valence rates of virulence factors found in this study (green) to abscess isolates from a previous study ([3], red).

(\*): for *lukD/E*, the percentage of isolates being positive for both components is indicated (solid bar) as well as the proportion of isolates in which only one out of the two components was detectable

**Results:** Isolates belonged to 20 different clonal complexes (CC) as shown in Figure 1. The most common CC was CC8 (18.7%), followed by CCs 15, 30 and 45. Three isolates (1.9%) were MRSA (see Figure 1). Beta-lactamase was common (71%), but other resistance genes were found only sporadically. Genes encoding superantigens were abundant. The enterotoxin cluster *egc* (*seg*, *sei*, *sem*, *sen*, *seo* and *seu*) was found in 45.8%. In another two isolates (1.3%), that locus was truncated (*seo*, respectively *sem+seo*). The toxic shock syndrome toxin gene *tsl* was detected in 14.8%, and 17.4% harboured enterotoxin A alleles (*sea*, *sea*-N315). Contrarily, Panton-Valentine leukocidin (*lukS-F-PV*) was rare, being found only in a single methicillin-susceptible CC30 isolate. Its low prevalence in asymptomatic carriers might emphasise a pathogenetic significance in patients with skin and soft tissue infections. Most MSCRAMM (microbial surface components recognising adhesive matrix molecules of the host) genes were nearly ubiquitously present. However, two MSCRAMM genes, *cna* (collagen adhaesin) and *sasG* (surface protein G), were detected only in some CCs. Fifty-five isolates (35.5%) carried capsule type 5 genes (*capH/I/J/K-5*). This included all isolates of CCs 5, 8, 9, 20, 22, 25, 97, 182 and 573. Another 100 isolates (64.5%) were positive for capsule type 8 (*capH/I/J/K-8*).

**Discussion:** These data provide an insight into pathogenesis, especially when compared to isolates from patients with defined clinical conditions. For instance (Figure 2), abundances of most virulence markers in carrier isolates were virtually identical to the frequencies of these markers in previously characterised abscess isolates [3]. PVL was the only exception, being common in patient isolates (30%), but very rare in carriers (0.65%). This emphasises the pathogenetic significance of PVL in patients with skin and soft tissue infections. The characterisation of a “normal” carriage population of *S. aureus* also might be helpful for the design of a future vaccine.

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