Genotypic and phenotypic variation among *Staphylococcus saprophyticus* from human and animal isolates

Britta Kleine, Sören Gatermann and Türkan Sakinc*

Abstract

**Background:** The main aim of this study was to examine the genotypic and phenotypic diversity of *Staphylococcus saprophyticus* isolates from human and animal origin.

**Findings:** In total, 236 clinical isolates and 15 animal isolates of *S. saprophyticus* were characterized in respect of the occurrence of 9 potential virulence genes and four surface properties. All strains were PCR positive for the regulatory genes *agr*, *sar*-$\text{it}$-A and *rot* as well as for the surface proteins UafA and Aas. Nearly 90% of the clinical isolates were found to possess the gene for the surface-associated lipase Ssp and 10% for the collagen binding MSCRAMM SdrI. All animal isolates were negative for *sdrI*. Lipolytic activity could be detected in 66% of the clinical and 46% of the animal isolates. Adherence to collagen type I was shown of 20% of the clinical strains and 6% of the strains of animal origin. Most *S. saprophyticus* strains showed hydrophobic properties and only few could agglutinate sheep erythrocytes.

**Conclusions:** We described a broad analysis of animal and human *S. saprophyticus* isolates regarding virulence genes and phenotypic properties such as lipase activity, hydrophobicity, and adherence. While *S. saprophyticus* strains from animal sources have prerequisites for colonization of the urinary tract like the D-serine-deaminase, our findings suggested that they need to acquire new genes e.g. MSCRAMMS for adherence like sdrI and to modulate their existing properties e.g. increasing the lipase activity or reducing hydrophobicity. These apparently important new genes or properties for virulence have to be further analyzed.

Introduction

Many genes and characteristics were investigated for the staphylococcal species *S. aureus* and *S. epidermidis*, and the distribution of potential virulence factors among infectious isolates have been studied extensively. In contrast, such study does not exist for the pathogenic *S. saprophyticus*, which is an important cause of urinary tract infections especially in young women [1]. Previously it has been shown that this bacterium is a contaminant of food of animal origin [2]. It was found in 7.1% of rectal swabs from cattle carcasses and 7.3% of rectal swabs from slaughtered pigs.

The urease was the first virulence factor characterized [3] and was found in all *S. saprophyticus* strains. Only four surface proteins have been characterized so far: the collagen-binding serine-aspartat-repeat protein SdrI [4], the uro-adherence factor UafA [5], the fibronectin-binding autolysin Aas [6] and the surface-associated lipase Ssp [7]. *S. saprophyticus* binds different extracellular matrix proteins like collagen [8,4], fibronectin [9,10] and laminin [8] and exhibits different surface properties like hydrophobicity and hemagglutination [11]. According to other staphylococci, an agr-like system was identified [12].

At present, virtually nothing has been reported about the occurrence of all these putative virulence factors among *S. saprophyticus*. Here 236 clinical isolates of *S. saprophyticus* and 15 isolates of animal origin were characterized regarding the existence of these genes and surface properties.

Methods

**Bacterial strains**

A total of 236 *S. saprophyticus* isolates from patients with clinically relevant symptoms and 15 isolates from differ-
ent animal sources were used in this study. The species were verified using biochemical techniques [13] and equivocal results were resolved by sequencing of the sodA gene [14]. The type strain S. saprophyticus ATCC 15305 and the already characterized clinical isolate 7108 were used as controls.

Hemagglutination
For the hemagglutination assay the method described by Gatermann et al. [15] was used with slight modifications. In brief, bacteria were grown in 10 ml peptone yeast extract broth (PY) for 16 h (130 rpm, 37°C), cells were harvested (2000 g, 10 min), washed twice with PBS (140 mM NaCl, 8 mM Na₂HPO₄, 2 mM KH₂PO₄, pH 7.4) and adjusted at an optical density of 6.0 at 600 nm. The erythrocyte suspension (25 μl, 1 v/v% in PBS) was added to the same volume of serial dilutions of bacteria. Results were read after 2 h incubation at room temperature.

Lipase activity assay
Lipolytic activity was determined by an agar plate assay containing tributyrylglycerol as described elsewhere [16]. A cleared halo around the colonies smaller than 1 mm after 48 h was considered as negative, whereas a halo greater than 3 mm was considered as highly lipolytic active.

Collagen adherence assay
Binding of S. saprophyticus to immobilized collagen was done as described before [4] with the modification that we adjusted the bacterial suspension to an optical density of 3.0 instead of 6.0 at 600 nm. Collagen adherence was considered as weak if the optical density after staining of the bound bacteria was above 0.15 and as high above 0.25.

Hydrophobicity
For testing of hydrophobicity bacteria were cultured for 16 h in PH broth (100 rpm, 37°C). Cells were washed twice with PBS (pH 7.2) and adjusted to an optical density of 0.3 in 0.9% NaCl. To 1 ml of this suspension 0.5 ml Xylene was added. After gently mixing for 10 min and an incubation of 15 min for phase separation, the optical density of the lower phase was measured. The hydrophobicity index (HPBI) was calculated as: 1-(OD_{final}/OD_{initial}) \times 100. A HPBI above 10% was regarded as weak hydrophobicity, above 40% as high hydrophobicity.

PCR analysis
Genomic DNA of the S. saprophyticus strains was extracted using the QIAamp DNA Mini Kit (Qiagen) suspending the bacteria in the recommended buffer for gram-positive bacteria and addition of 100 μg lysostaphin at the lysis step. For the amplification of the genes three different PCR-programs were used with an initial denaturation at 94°C for 5 min and final extension at 72°C for 7 min. and 35 cycles. Program 1 (sdrI, dsdA): 94°C 30 s, 50°C 30 s, 72°C 1 min; program 2 (ssp, uafA): 94°C 30 s, 55°C 30 s, 72°C 2 min; program 3: 94°C 30 s, 50°C 30 s, 72°C 2 s. The following primers were used: sdrI fwd-GGATAAAAAATAGCAATCGGAA/rev-CAAGGCTATATTATGCTTT, 1624 bp; ssp fwd-AAATTCAGAAATTAAGTACCC/rev-ATGAAGAGT-TAGTTCACAC, 3164 bp; uafA fwd-CGGGATCT-CCCAACATCAGAATGATATGG/rev-GCGAAGCTTTGTCGAAAGAAACCTAAACCAGC, 2267 bp; dsdA fwd-AACGATTGCAACACTTC/rev-CTATAACGAAGTTTACC, 1299 bp; capD fwd-CGGTTGAAGATAAAGACCG/rev-TTACCAAGATCTAATGCC, 604 bp; aas fwd-CAGGTACCGTATTAAAGTAC/rev-GATACAACTATTTACGTTCACAC, 3164 bp; agr fwd- AATGCGAACCAAAAAATGCAATCGGAC/rev-GTTAGCT-TCTTTAATGCCG, 236 bp; rot fwd-TGGTGGAAAGATACTCGAG/rev-AATGGATAATAACTGTACG, 2267 bp; sodA fwd-GGATAANAATAGCAATCGGAA/rev-CAAGGCTATATTATGCTTT, 1624 bp; ssp fwd-AAATTCAGAAATTAAGTACCC/rev-ATGAAGAGT-TAGTTCACAC, 3164 bp; uafA fwd-CGGGATCT-CCCAACATCAGAATGATATGG/rev-GCGAAGCTTTGTCGAAAGAAACCTAAACCAGC, 2267 bp; dsdA fwd-AACGATTGCAACACTTC/rev-CTATAACGAAGTTTACC, 1299 bp; capD fwd-CGGTTGAAGATAAAGACCG/rev-TTACCAAGATCTAATGCC, 604 bp; aas fwd-CAGGTACCGTATTAAAGTAC/rev-GATACAACTATTTACGTTCACAC, 3164 bp; agr fwd- AATGCGAACCAAAAAATGCAATCGGAC/rev-GTTAGCT-TCTTTAATGCCG, 236 bp; rot fwd-TGGTGGAAAGATACTCGAG/rev-AATGGATAATAACTGTACG, 2267 bp.

Statement of ethical approval
All procedures performed on the animals were in strict accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the local Animal Care and Use Committee.

Results
Distribution of genes
The staphylococcus regulatory genes agr, sarA and rot were present in all tested S. saprophyticus strains independent of their origin as was the gene for the D-serine deaminase (dsdA). The capsular gene (capD) from the type strain ATCC 15305 could only be found in three clinical isolates. In all S. saprophyticus strains the gene for the major autolysin Aas and the surface-protein uro- adherence-factor A (UafA) were detectable. In all animal isolates as well as in 86.9% of the clinical isolates the presence of lipase gene ssp could be shown. Another surface protein SdrI was present in 10.2% of the clinical isolates but not in any animal isolate. All results are summarized in Table 1.

Lipolytic activity
Sixty-six per cent of the clinical isolates showed lipolytic activity on tributyrin agar plates, 15.9% of them a high lipolytic activity. Of the clinical isolates containing the gene for the lipase Ssp 59.3% possessed detectable lipolytic activity. Of the clinical isolates containing the gene for the lipase Ssp 59.3% possessed detectable lipolytic activity. The results are shown in Table 2.

Hydrophobicity
Seventy-five per cent of the clinical isolates were hydrophobic whereas all of the animal isolates showed this
property (Table 2). The animal isolates were generally more hydrophobic. Although SdrI seems to be involved in hydrophobicity in strain 7108 no correlation between hydrophobicity and the presence of the sdrI gene could be detected. Only 9% of the hydrophobic clinical isolates were sdrI positive.

**Collagen adherence**

Adherence to immobilized collagen could be observed in 19.9% of the clinical isolates and in 6.7% of the animal isolates. The intensity of collagen binding in human isolates was much higher than in animal isolates. 30% of the sdrI positive strains and 18% of the sdrI negative strains showed binding to collagen (Table 2.).

**Hemagglutination**

Hemagglutination of sheep erythrocytes was shown by 15.7% of the clinical isolates and 6.7% of the animal isolates. No correlation with a known protein could be identified because both proteins that are thought to be responsible for hemagglutination, UafA and Aas were found to be present in all tested *S. saprophyticus* isolates.

### Discussion

In the present study 236 *S. saprophyticus* strains isolated from patients and 15 strains of animal origin were characterized for several putative virulence genes by PCR and for phenotypic characteristics often associated with virulence. In all strains analyzed in this study the regulatory genes agr, sarA and rot could be detected even though a regulatory function of these genes in *S. saprophyticus* has not been shown yet. Moreover, all strains were found to possess the gene for the D-serine deaminase (dsdA) which is also characteristic for uropathogenic *E. coli*. The high D-serine concentration in urine is toxic to bacteria unless they possess dsdA. Even though *S. saprophyticus* is considered as a natural colonist of the animal skin it seems to have already the basic prerequisite for colonisation of the urinal tract.

Lipases are thought to be involved in pathogenesis whether in nutrition [17] or adherence [18,19]. *S. saprophyticus* living on the skin which is low in nutrition but rich in lipids may use their lipase for this purpose. But Ssp is also an important factor during urinary tract infections (data not published) and this may be reflected in the generally higher lipase activity we could observe. Over 60%
of the clinical isolates showed lipolytic activity and most of them possessed the lipase gene ssp. Because S. aureus or S. epidermidis have more than one lipase gene [17-20] the lipolytic ssp-negative S. saprophyticus strains may possess a second lipase. Adherence to extracellular matrix proteins or epithelial cells is a crucial step in bacterial colonization. S. saprophyticus binds to different proteins of the extracellular matrix and we could show that in S. saprophyticus strain 7108 the surface protein SdrI is responsible for binding to collagen type I [4]. Nearly 20% of the clinical isolates showed adherence to collagen but only about 15% of these strains contained the sdrI gene. The second surface protein UafA is not involved in adherence to collagen (data not shown) so a further unknown MSCRAMM can be postulated which is not present in S. saprophyticus strains 7108 and the sequenced strain ATTC 15305. In animal isolates collagen binding is less prevalent and less intense and sequenced strain ATTC 15305. In animal isolates collagen-binding is less prevalent and less intense and sequenced strain ATTC 15305.

Strains showed hemagglutination despite the fact that all strains characterized are hydrophobic but only very few strains showed hemagglutination. Hemagglutination and hydrophobicity are also adherence properties. Most strains characterized are hydrophobic but only very few strains showed hemagglutination despite the fact that all possessed Aas as well as UafA both proteins that are reputedly responsible for hemagglutination [15,5]. Often a capsule is thought to mask such effects of surface proteins but the capsular gene capD of the strain ATCC 15305 could only be detected in 3 strains. One positive strain, S. saprophyticus 9325, is hemagglutination-negative and hydrophilic but degradation of the capsule turned this strain hydrophobic (not shown). At least in this case the capsule seems to mask hydrophobicity. Further capsular genes or regulation mechanisms are possible.

Phenotypic and genotypic characterization of S. saprophyticus strains was done for various genes and properties considered to be involved in pathogenesis. The findings suggested that S. saprophyticus possesses more surface proteins especially for adherence to extracellular matrix proteins and eukaryotic cells than the four proteins that have been identified.

Conclusions
We described a broad analysis of animal and human S. saprophyticus isolates regarding virulence genes and phenotypic properties such as lipase activity, hydrophobicity, and adherence. While S. saprophyticus strains from animal sources have prerequisites for colonization of the urinary tract like the D-serine-deaminase, out findings suggested that they need to acquire new genes e.g. MSCRAMMS for adherence like sdrI and to modulate their existing properties e.g. increasing the lipase activity or reducing hydrophobicity. These apparently important new genes or properties for virulence have to be further analyzed.

Competing interests
None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Authors’ contributions
BK performed the experimental work and wrote the manuscript. ST wrote and edited the manuscript and coordinated the study and SG helped to edit the manuscript. All authors read and approved the final manuscript.

Author Details
Institut für Hygiene und Mikrobiologie, Abteilung für Medizinische Mikrobiologie, Ruhr-Universität Bochum, D-44780 Bochum, Germany

Received: 12 February 2010 Accepted: 10 June 2010

References
1. Wallmark G, Anremark I, Telander B: Staphylococcus saprophyticus: a frequent cause of acute urinary tract infection among female outpatients. The Infect Diseases 1978, 138:791-797.
2. Hedman P, Ringertz O, Lindström M, Olsson K: The origin of Staphylococcus saprophyticus from cattle and pigs. Scand J Infect Dis 1993, 25(1):57-60.
3. Gatermann S, John J, Marre R: Staphylococcus saprophyticus urease: characterization and contribution to uropathogenicity in unobstructed urinary tract infection of rats. Infect Immun 1989, 57:110-116.
4. Sakinc T, Kleine B, Gatermann SG: SdrI, a serine-aspartate repeat protein identified in Staphylococcus saprophyticus strain 7108, is a collagen-binding protein. Infect Immun 2006, 74:4615-4623.
5. Kuroda M, Yamashita A, Hirakawa H, Kumano M, Morikawa K, Higashide M, Kuroda M, Yamashita A, Hirakawa H, Kumano M, Morikawa K, Higashide M, Maruyama A, Inose Y, Matoba K, Toh H, Kuhara S, Hatton M, Ohta T: Whole genome sequence of Staphylococcus saprophyticus reveals the pathogenesis of uncomplicated urinary tract infection. Proc Natl Acad Sci USA 2005, 102:13272-13277.
6. Hell W, Meyer HG, Gatermann SG: Cloning of a gene encoding a surface protein with adhesive and autolytic properties. Mol Microbiol 1998, 9:781-881.
7. Sakinc T, Woznowski M, Ebsen M, Gatermann SG: The surface-associated protein of Staphylococcus saprophyticus is a lipase. Infect Immun 2005, 73:6419-6428.
8. Paulsson M, Ljungah L, Wadström T: Rapid identification of fibronectin vitronectin, laminin and collagen cell surface binding proteins on coagulase-negative staphylococci by particle agglutination assays. J Clin Microbiol 1992, 30:2006-2012.
9. Gatermann G, Meyer HG: Staphylococcus saprophyticus hemagglutinin binds fibrinectin. Infect Immun 1994, 62:4556-4563.
10. Svitlakos LM, Rydén C, Rubin K, Ljungah L, Hook M, Wadström T: Binding of fibronectin to Staphylococcus strains. Infect Immun 1983, 42:628-633.
11. Meyer HG, Gatermann SG: Surface properties of Staphylococcus saprophyticus:hydrophobicity haemagglutination and Staphylococcus saprophyticus surface-associated protein (Sp) represent distinct entities. J Appl Microbiol 1993, 105:538-544.
12. Sakinc T, Kulczak P, Henne K, Gatermann SG: Cloning of an agr homologue of Staphylococcus saprophyticus. FEMS Microbiol Lett 2004, 237:157-161.
13. de Paula AN, Predari SC, Chazarreta CD, Santoanni JI: Five-test simple scheme for species-level identification of clinically significant coagulase-negative staphylococci. J Clin Microbiol 2003, 41:1219-1224.
14. Poyart C, Quepine Q, Boumaila C, Tristan-Coupet P: Rapid and accurate species-level identification of coagulase-negative staphylococci by using the sodA gene as a target. J Clin Microbiol 2001, 39:4296-4301.
15. Gatermann SG, Meyer HG, Wanner G: Staphylococcus saprophyticus hemagglutinin is a 160-dalton surface polypeptide. Infect Immun 1992, 60:4127-4132.
16. Nikolet K, Rosenztein R, Verheji HM, Götz C: Comparative biochemical and molecular analysis of the Staphylococcus hyicus Staphylococcus
 aureus and a hybrid lipase. Indication for a C-terminal phospholipase
domain. EurJ Biochem 1995, 228:732-738.
17. Longshaw CM, Farrell AM, Wright JD, Holland KT. Identification of a
second lipase gene gehD, in Staphylococcus epidermidis comparison
of sequence with those of other staphylococcal lipases. Microbial 2000,
146:1419-1427.
18. Gribbon EM, Cunliffe WJ, Holland KT. Interaction of Propionibacterium
acnes with skin lipids in vitro. J Gen Microbial 1993, 139:1745-1751.
19. Bowden MG, Visai L, Longshaw CM, Holland KT, Speziale P, Hook M: Is the
GehD lipase from Staphylococcus epidermidis a collagen binding
adhesin? J Biol Chem 2002, 277:43017-43023.
20. Rosenstein R, Gotz F. Staphylococcal lipases: biochemical and
molecular characterization. Biochimie 2000, 82:1005-1014.