**Implications of identifying the recently defined members of the** ***S. aureus* complex,** ***S. argenteus* and *S. schweitzeri*:**

**A position paper of members** **of the ESCMID Study Group for Staphylococci and Staphylococcal Diseases (ESGS)**

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**Running title:** Guidance for dealing with *S. argenteus* and *S. schweitzeri*

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**Abstract**

Background: *Staphylococcus argenteus* and *Staphylococcus schweitzeri,* previously known as divergent *Staphylococcus aureus* clonal lineages, have been recently established as novel, difficultly delimitable, coagulase-positive species within the *S.aureus* complex*.* Methicillin-resistant *S.argenteus* are known from Australia and U.K.. Knowledge on their epidemiology, medical significance and transmission risk is poor and partly contradictory, thus, not yet allowing for definite recommendations. There is mounting evidence that *S.argenteus´* pathogenicity is similar to “classical” *S.aureus* while hitherto no *S.schweitzeri* infections have been reported.

Aim: Provide decision support whether and how to distinguish and report both species.

Sources: PubMed search about *S.argenteus* and *S.schweitzeri*.

Content: This position paper reviews the main characteristics of both species and draws conclusions for microbiological diagnostics as well as infection prevention and control measures.

Implications: We propose not distinguishing within the *S.aureus* complex for routine reporting purposes until there is evidence that pathogenicity or clinical outcome differ markedly between the different species. Primarily for research purposes, suitably equipped laboratories are encouraged to differentiate between *S.argenteus* and *S.schweitzeri*. Caution is urged if these novel species are explicitly reported. In such cases, a specific comment should be added (i.e. “member of the *S.aureus* complex”) to prevent confusion with less or non-pathogenic staphylococci. Prioritizing aspects of patient safety, methicillin-resistant isolates should be handled as recommended for MRSA . In those cases, the responsible clinician should be directly contacted and informed by the diagnosing microbiological laboratory, as they would be for MRSA. Research is warranted to clarify their epidemiology, clinical impact and significance for infection control*.*

**Keywords:**

*Staphylococcus aureus; Staphylococcus argenteus*; *Staphylococcus schweitzeri*; diagnostics, prevention, epidemiology, pathogenicity, infection control, recommendation, MRSA

Methicillin-resistant (MR) *Staphylococcus aureus* (MRSA) isolates cause extensive morbidity, mortality and economic burden in human and veterinary medicine, and thus, remain a global major public health problem [1]. MRSA isolates have substantially limited treatment options and the laboratory report of a methicillin-resistant isolate of *S. aureus* in most institutions is associated with additional infection control measures. Therefore, the reliable detection and identification of MRSA as well as their unambiguous reporting are imperative for adequate patient management [2].

In 2015, whole genome sequencing confirmed that isolates from several specific *S. aureus* clonal lineages were sufficiently divergent from *S. aureus* to be designated as a separate coagulase-positive species, *Staphylococcus argenteus,* which includes also methicillin-resistant isolates [3]. Similarly, whole genome sequencing of isolates from other separate clonal lineages identified a different closely related, coagulase-positive species, designated *Staphylococcus schweitzeri* [3,4]. To date, classical routine diagnostics do not distinguish these species from *S. aureus*. Furthermore, it is still a matter of debate whether they differ significantly from *S. aureus* in clinical outcome and infection prevention relevance. Considering the recent description of a multitude of novel species especially belonging to coagulase-negative staphylococci (CoNS) [5,6], the report of *S. argenteus* and *S. schweitzeri* could be misinterpreted by clinicians as less or non-pathogenic staphylococcal species not belonging to the *S. aureus* complex with possible adverse consequences in terms of classification as causative agent and therapy requirements. Moreover, if tested methicillin-resistant, necessary prevention measures could be neglected. Thus, interpretation of laboratory reports as well as clinical and prevention management necessitate an update and adjustment.

Here, members of the Study Group for Staphylococci and Staphylococcal Diseases (ESGS) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) agreed on a common position (i) whether the *S. aureus* complex should be distinguished on a regular basis in microbiological routine diagnostics and how isolates of the *S. aureus* complex should be reported and (ii) which prevention measures should be initiated in the case of reporting MR-*S. argenteus*. For this purpose, the complete literature about *S. argenteus* and *S. schweitzeri* listed in PubMed has been reviewed. We also drew on the diverse experiences and unpublished findings of the ESGS Study Group members. The recommendations were drafted by the corresponding author as a basis for discussion within the ESGS Study Group, contributions of the co-authors and other ESGS Study Group members were included and the recommendations were finally approved by all co-authors.

**Phylogenetic and taxonomic background**

Today, the *S. aureus* complex consists of three species (*S. aureus*, *S. argenteus* and *S. schweitzeri*) with *S. aureus* divided into the two subspecies designated *aureus* and *anaerobius* [3,7,8].

**Delimitation of *S. argenteus***

In 2006, a divergent community associated (CA)-MRSA lineage was reported from Australia belonging to clonal complex (CC) CC75 [9], which was later delimited as *S. argenteus*. It has an average nucleotide identity of 87.4% corresponding to a DNA-DNA-hybridization value of 33.5% compared to *S. aureus*. *S. argenteus* possesses the peptidoglycan type L-Lys–L-Ala–(Gly)4-5, which is different from L-Lys–(Gly)4-5 formed by *S. aureus*. *S. argenteus* can be considered as an ancestral lineage of *S. aureus* due to the very small accessory genome and its lower genomic plasticity that is most likely due to the presence of a CRISPR/cas system, which is only very rarely found within *S. aureus* genomes (e.g. in some Canadian CC398 isolates) [10,11]. In contrast to the 16S rRNA gene, which is identical for *S. argenteus* and *S. aureus* [3,12], sequencing of *gap, rpoB, sodA, tuf*, and *hsp60* revealed a clear separation between the two species [12].

**Novel description of *S. schweitzeri***

A highly divergent *S. aureus* lineage based on MLST was detected in African bats and monkeys in 2011 [10,13]. These isolates had in common that PCR assays using species-specific primers targeting the *S. aureus* thermostable nuclease gene (*nuc1*) failed due to the possession of a thermostable nuclease homologue (NucM) with only 78–80% amino acid property similarities [4]. Whole genome sequencing (WGS) later revealed an average nucleotide identity (ANI) of 88.6% corresponding to a DNA-DNA-hybridization (DDH) value of 36.3% compared to *S. aureus* [3]. *S. schweitzeri* peptidoglycan type (L-Lys–L-Ala–(Gly)4-5 is the same as *S. argenteus* and differs from *S. aureus*.

**Epidemiology**

*S. argenteus* and *S. schweitzeri* differ in their epidemiology regarding dissemination, clonal complexity and host preferences. Like *S. aureus*, *S. argenteus* seems to be predominantly human-associated while the recovery of this species from specimens of diverse animals has been reported and animal-adapted STs/CCs may exist. In contrast, *S. schweitzeri* is predominantly wildlife-associated. *S. argenteus* has been detected in several geographic regions comprising several CCs with many sequence types (STs) and at least some CCs (e.g. CC75 and CC2250, Fig.1) show a widespread geographic distribution indicating an international spread [14]. In contrast, *S. schweitzeri*'sdistribution seems to be restricted to Sub-Saharan Africa.

***S. argenteus***

First descriptions of the phylogenetically distinct CC75 were from remote aboriginal communities in the Northern Territory of Australia in 2006 and 2009 [9,12] followed by reports from New Zealand and Fiji [9,12,15,16] and other regions: Africa [17,18], America (French Guiana and Trinidad and Tobago) [19,20], Asia (Cambodia, China, India, Israel, Japan, Laos, Malaysia, Myanmar, Singapore, Taiwan, and Thailand) [21-29], and Europe (Belgium, Denmark, France, and U.K.) [25,30-32].

At least three geographical “hot spots” of *S. argenteus* exist, Southeast Asia and remote human populations in Australia and the Amazon. *S. argenteus* is a predominant lineage in Australian Aboriginal communities accounting for 71% of community-associated MRSA [9]. Also in remotely living Wayampi Amerindians from the Amazonian forest, the prevalence of nasal carriage of a CC75-related lineage (ST1223) was 7.8% [20]. In a prospective multicentre observational study comparing community-onset *S. argenteus* and *S. aureus* sepsis in Thailand, 19% of the patients were infected with *S. argenteus,* mostly with ST2250 [33]. A possible reservoir in Thailand may be livestock (bovine) [27]. In contrast, the occurrence in Europe seems to be rare (<1%, Belgium) and an unknown number of these cases might be imported from endemic countries [31,32,34]. Animal-and food-associated isolates have been reported from Asia (rabbits, pork) and Africa (fruit bats, monkeys, and great apes) [13,17,35-38].

Of note, *S. argenteus* MLST profiles are not restricted to CC75. Current MLST data (assessed October 2018) subdivides the population structure into several CCs according to eBURST. The largest clonal complex is CC2196 (n=35 STs), followed by CC1594 (n=12 STs), CC2198 (n=7 STs), CC75 (n=5 STs), and CC2793 (n=2 STs). The estimate of average evolutionary divergence over all concatenated MLST sequence pairs is 0.02 base substitutions per site (Fig.1). This is a remarkable degree of divergence usually found in separate species and not in clonal complexes [10]. Phylogenetic analyses of *S. argenteus* strains revealed several clonal complexes with more than 60 STs described so far (Fig.1) [3,34].

***S. schweitzeri***

So far, *S. schweitzeri* seems to be restricted to the African continent, particularly occurring in West and Central Africa, mainly in Gabon and also in Côte d’Ivoire, DR Congo and Nigeria, but neither geographical nor species-related clusters were detected [13,37]. The vast majority of *S. schweitzeri* isolates have been isolated from animals, such as fruit bats [37], non-human primates [36,39] and in one case from a gorilla [36]. Hitherto only three isolates have been recovered from human samples in Africa, however, none of the human- and animal-associated cases were associated with infections until now [40,41].

Currently available *S. schweitzeri* isolates belong to 30 STs including CC2074 (ST2074 and ST4137) and CC2463 (ST2463, ST3962, and ST4316). The estimate of average evolutionary divergence over all concatenated MLST sequence pairs is 0.01 base substitutions per site (Fig.1).

**Pathogenicity and clinical relevance**

To assess their clinical significance, the question needs to be addressed whether both novel species differ from *S. aureus* in terms of their clinical manifestations, epidemiology (e.g. morbidity and mortality) and resistance pattern. Furthermore, their pathogenic capabilities and equipment with virulence factors should be taken into account.

***S. argenteus***

Its general clinical impact is difficult to assess due to the limited number of studies and datasets and divergent observations exist. However, a lower virulence and major clinical differences compared to *S. aureus* was assumed in early reports [10,33,42]. In contrast, recent studies suggest the frequency of health care-associated infections, morbidity and mortality that are comparable to *S. aureus* [21,22].

First observations already underlined the capacity of *S. argenteus* to cause skin and soft tissue infections such as impetigo, but even severe entities as necrotizing fasciitis [9,10,12]. Subsequently, bone and joint infections were reported [18,21,23,30]. Also, blood stream infections have been repeatedly described [10,18,21,33]. *S. argenteus* seems to be able to cause toxin-mediated syndromes similar to *S. aureus*. Several independent cases of food poisoning were reported from Asia [25,43,44].

Based on epidemiological studies and since *S. argenteus* lacks the *crtOPQMN* operon, which encodes the carotenoid pigment, staphyloxanthin, the question rose whether this species may be less virulent compared to *S. aureus* [10,42]. However, complementation experiments with a carotenoid operon-containing plasmid led to increased susceptibility to host defense peptides and reduced virulence in murine skin and sepsis models and a rabbit endocarditis model [42,45].

Preliminary comparative genomics of 15 *S. argenteus* genomes showed that of 111 virulence genes associated with *S. aureus,* 76.6% were detected also in *S. argenteus* [14]. Notably, the Panton-Valentine leucocidin (PVL) was found in *S. argenteus* isolates associated with skin and soft tissue infections [18,32]. However, none of the isolates including also those from pyoderma patients living in remote aboriginal communities in Australia were positive for PVL [9]. A variety of genes encoding hemolysins, capsule polysaccharides, adhesins, staphylococcal enterotoxins (SEs) and other leucocidins have also been reported in *S. argenteus* (Tab.1). Missing genes include variant types of staphylococcal enterotoxins (SEs) and SE-like toxins and other leucocidins occurring variably in *S. aureus* [14]. Genomic analyses revealed that *S. argenteus* lineages harbor pathogenicity islands (PIs) and genes mediating the PI transfer (*int*, *rep*, and *ter*) [34]. Moreover, the genomic islands νSaα and νSaβ were found in this species [14]. *S. argenteus* possesses CRISPR/cas system subtypes also found in some *S. aureus* lineages and an ancestral possession or bidirectional transfer of the CRISPR loci has been proposed [10,14]. It harbors also genes encoding integrases of temperate *Siphoviridae* bacteriophages, which indicates the presence of these prophages and their associated virulence genes in the genome [14]. At least eight bacteriophage sequences resembling those known for *S. aureus* have been detected [34].

In conclusion, *S. argenteus* carries virulence genes associated with *S. aureus´* pathogenicity and causes similar infections to *S. aureus*.

***S. schweitzeri***

While human infections have not been described so far, *S. schweitzeri* does not differ substantially from *S. aureus* in terms of the possession of virulence factors. Also *S. schweitzeri* harbors νSaα and νSaβ as well as many virulence genes being essentially for the pathogenicity of *S. aureus* (Tab.1) [14]. By genomic comparison analysis, 77.5% of virulence genes associated with *S. aureus* were also detected in *S. schweitzeri* [14]. Some classical SE and several SE-like encoding genes and the toxic shock syndrome toxin-1 gene (*tst*) were found [38,39]. However, the pathogenic capacity of this species cannot yet be determined at this time.

Similar to *S. argenteus*, *S. schweitzeri* also possesses genes of the integrase groups phi 1–3 indicating the presence of the prophages in its genome [14]. However, it is missing the individual genes carried on phi3 prophage that are associated with human adaptation and evasion of immune response, *scn*, *chp* and *sak* genes [36,37]. This is consistent with a lack of adaptation to the human host [46].

**Antimicrobial resistance**

The rates of resistance seem to be lower in *S. argenteus* than in *S. aureus* [33]. While penicillin-resistant (*blaZ*-positive) isolates are common, other resistance phenotypes are rare [33]. Some isolates were tested resistant towards tetracyclines, aminoglycosides, clindamycin and erythromycin [9,18,22,33,35]. Regarding methicillin resistance, there is an obvious discrepancy between the Australian and U.K. isolates, which are frequently or even in the majority methicillin-resistant [9,32,42], and those from other parts of the world (including the Pacific Islands) which tested methicillin-susceptible [15,16,18,21,22,33]. As far as analyzed, the methicillin resistance of the Australian and European *S. argenteus* isolates is *mecA*-based and they possess an SCC*mec* type IV element (rarely also SCC*mec* type V) [9,14,31,34].

*S. schweitzeri* isolates were tested susceptible to almost all antibiotics and no methicillin-resistant isolates were found so far. Even penicillin-and tetracycline-resistant isolates have been only exceptionally reported [37].

**Routine diagnostics and reporting**

Misinterpretation of microbiological reports may result in serious consequences for patient care. In this regard, species designation may cause confusion due to re-named or newly delimited species. As an example, this is the reason why *Shigella* species remain a separate nomenspecies [47] and respective isolates will not be reported as “*Escherichia coli* pathovar.” although they are considered one genomospecies [48]. Also, the mere mention of members of the *Mycobacterium tuberculosis* complex other than *Mycobacterium tuberculosis*, has been reported as source for putative misinterpretation [49].

A similar problem arose from the delimitation of *S. argenteus* and *S. schweitzeri* from *S. aureus* in 2015 [3]. Consequently, the questions remain whether a differentiation of the *S. aureus* complex will be necessarily or advantageous on a routine basis and how both delimited species should be reported if specifically detected. Possible principle threats by reporting these species comprise (i) misinterpretation of the clinical significance, e.g. by false classification as one of the many newly described CoNS species and (ii) underestimation of their relevance for infection prevention and control measures if tested methicillin-resistant (see below).

Concerning detection and confirmation of the methicillin resistance in *S. argenteus*, the routine pheno- and genotypic approaches are applicable.

**Methods for differentiation within the *S. aureus* complex**

Colony appearance and phenotypic tests including those usually applied as part of the classic Kloos-Schleifer scheme (e.g. clumping factor and tube coagulase tests) [50] or of the modern automated systems and agglutination tests fail to accurately distinct between both species (Tab.2). The eponymous creamy white colonies without pigmentation described for *S. argenteus* can be observed also in “classical” *S. aureus* isolates. Likewise ambiguously, *S. schweitzeri* usually produces a double zone of hemolysis on Columbia blood agar (F. Schaumburg, own observation).

Hitherto, no commercially available DNA-based assays exist that are able to differentiate within the *S. aureus* complex. For the thermostable nuclease gene (*nuc1*), sequence differences have been reported [3]. Of note, usage of the frequently applied PCR primers according to Brakstad et al. [51] results, at least to a certain extent, in amplification products for *S. argenteus* isolates despite some mismatches, but not for *S. schweitzeri* [3,4]. For *S. schweitzeri,* a thermostable nuclease homologue (NucM) has been detected, which can be used for the design of specific PCR primers [4].Nevertheless, since *nuc1* gene variations are more common than previously assumed [52], more specific PCR primers targeting the *nuc1* gene are also warranted for *S. argenteus*. Since both speciesdo not show a large deletion of a hypothetical nonribosomal peptide synthetase (NRPS) gene as found in *S. aureus,* a PCR assay has been developed that allows their differentiation from *S. aureus* [53].

Concerning universal („broad range“) DNA-based approaches, 16S rRNA gene sequencing fails in the case of delimiting *S. argenteus* due to complete sequence homology. An 1-bp-difference has been described for *S. schweitzeri* [3]. Besides WGS allowing a definitive allocation, MLST genotyping could help to identify those genotypes associated with either *S. argenteus* or *S. schweitzeri* (Fig.1).

The hitherto most practicable approach is based on the application of the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS). Meanwhile, several specific MALDI-TOF MS signals were identified allowing differentiation [17,54]. Notable, older database versions provided by the manufacturers did not include MS profiles data sufficient for separation of both species within the *S. aureus* complex and not all recently available database versions have been complemented. Even if both species have been already included, the strain coverage used for the establishment of these databases might be still limited.

**Recommendations**

**Is it necessary to differentiate within the *S. aureus* complex for routine purposes and how should *S. argenteus* and *S. schweitzeri* be reported?**

Since no significant differences concerning morbidity and mortality as well as transmission in health care facilities have been described to date for *S. argenteus* and no human *S. schweitzeri* infections are reported so far, it seems not to be necessary to distinguish within the *S. aureus* complex for routine diagnostics purposes at present. If genotyping is performed, e.g. for surveillance or outbreak analysis, respective MLST and *spa* types will give hints for the occurrence of *S. argenteus* and *S. schweitzeri*, respectively. In those cases, the species status can be validated by the methods mentioned above.

To avoid misinterpretation and underestimation of reporting “*S. argenteus”* and “*S. schweitzeri”*, ESGS members recommend adding the comment “member of the *S. aureus* complex” to the species designation. If identified and reported, the microbiological laboratory should immediately contact the attending clinicians as they would for S. aureus.

**Consequences for infection prevention and control measures**

Nasal carriage of *S. aureus* is source and risk factor for subsequent infections by the colonizing strain [55,56]. Persistent carriers that comprise about 20-40% of healthy humans are of particular risk for endogenous infection [57-60]. Infections by MRSA strains are characterized by increased morbidity and mortality [61,62]. While no methicillin-resistant *S. schweitzeri* isolates have been reported, *S. argenteus*-associated CCs comprising methicillin-susceptible and methicillin-resistant strains are endemic in some (remote) human populations [9,20]. In these and other regions, this species is a frequent cause of community-associated infections [9,18,33], however, health care-associated infections by *S. argenteus* cases exist [21,63].

Hitherto, no studies have been performed addressing the spread and transmission of both newly established species in the hospital environment. The few existing cases due to MR-*S. argenteus* attributed to health care-associated infections suggest that the same infection prevention and control measures can be applied as for MRSA [21,63]. Since *S. argenteus* infections may be associated with serious morbidity, mortality and nosocomial infection, the usual MRSA prevention measurements seem to be appropriate unless the contrary is proven. Until then, ESGS members recommend that microbiological reports should be accompanied by this or similar comment: “Should be dealt with in accordance to the MRSA guidelines!”. The attending clinicians should be contacted directly.

**Summary**

Recently, two novel members of the *S. aureus* complex have been established, designated *S. argenteus* and *S. schweitzeri.* Overall, data on their epidemiology, clinical significance and nosocomial impact are still poor and in part contradictory. For routine purposes, no additional efforts are necessary to distinguish within the *S. aureus* complex. However, to improve the evidence basis for future decisions for surveillance, clinical significance and infection control measurements, suitably equipped laboratories are encouraged to differ between *S. argenteus* and *S. schweitzeri*. Prioritizing aspects of patient safety, we suggest that these novel species are reported as “member of the *S. aureus* complex” in the case they have been specified. If tested methicillin-resistant, they should be reported and handled as MRSA until there is clear evidence for the contrary. In areas where these species are only rarely detected it is particularly necessary to ensure that these novel species cannot be mistaken for less or non-pathogenic staphylococcal species. For that purpose, immediate personal communication between the diagnosing microbiological laboratory and the responsible clinician should be taken.

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**Conflict of interest**

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**Figure legend**

Figure 1.

Phylogenetic tree of *Staphylococcus aureus*, *Staphylococcus schweitzeri* and *Staphylococcus argenteus*. A neighbor-joining tree was constructed using the concatenated sequences of the seven multilocus sequence typing (MLST) loci. The sequences of the most common *S. aureus* lineages (ST5, ST22, ST30, ST45 and ST398) were used for rooting. All published MLST sequence types (ST) of *S. schweitzeri* and *S. argenteus* were included. Additional related ST were identified in the MLST database (accessed 9 October 2018, https://pubmlst.org/saureus/) using eBURST.

Tab. 1. Genes encoding virulence factors (selection) detected so far in *S. argenteus* and *S. schweitzeri* isolates

|  |  |  |  |
| --- | --- | --- | --- |
| Group of virulence factors | Detection1 of genes in | | Reference |
| *S. argenteus* | *S. schweitzeri* |
| Staphylococcal enterotoxins and enterotoxin-like toxins | *seb, secbov, seg, seh, sei, selk, selm, seln, selo, selq, selu2, selx, sely, sel26, sel27* | *seb, sec, seg, sei, sell, selm, seln, selo, selu, selx, sely, sel26, sel27* | [24,27,31,34,38,39,43] |
| Toxic shock syndrome toxins |  | *tst* | [39] |
| Superantigen-like proteins | *ssl1, ssl2, ssl3, ssl4, ssl5, ssl7, ssl8, ssl9, ssl10, ssl11* | *ssl1, ssl2, ssl3, ssl4, ssl5, ssl6, ssl7, ssl9, ssl10, ssl11* | [14] |
| Exfoliative toxins | *eta, etc* | *eta, etc, etd* | [14] |
| Leucocidins | *lukS/F-PV, lukD, lukE* | *lukD, lukE* | [18,24,33-35] |
| Hemolysins | *hla, hlb, hld, hlgA-C* | *hla, hlb, hld, hlgA-C* | [14,35] |
| Adhesins | *clfA, clfB, cna, eap, ebh, epb, efb, fnbA/B, icaA-D, icaR, isdA, isdB, sdrC-E, spa, sasG* | *clfA, clfB, cna, ebh, ebp, efb, fnbA/B, icaA-D, icaR, isdA, isdB, sdrC-E, spa* | [14,34] |
| Autolysin | *atl* | *atl* | [14] |
| Immune response evasion factors | *chp, coa, adsA, esaC, essA, essC, esxA, flipr, sak, sbi, scn, VWbp* | *coa, adsA, esaC, essC, esxA, flipr, sbi, VWbp* | [14,31,34] |
| Heme uptake | *isdA-G, srtB* | *isdA-G, srtB* | [14] |
| Polysaccharide capsule | *cap5, cap8* | *cap5, cap8* | [14,34] |

1 Detection in at least one isolate.

Tab. 2. Usefulness of diagnostic approaches to differentiate within the *S. aureus* complex

|  |  |  |  |
| --- | --- | --- | --- |
| Diagnostic approach | Differentiation of *S. aureus* subsp. *aureus* from | | Reference |
| *S. argenteus* | *S. schweitzeri* |
| Microscopy | Not possible | Not possible | [3] |
| Colony morphology | Uncertain | Not possible | [3,10,12,44] |
| Chemotaxonomy  - Fatty acid composition  - Menaquinone composition  - Peptidoglycan composition | Not possible  Not possible  Possible | Not possible  Not possible  Possible | [3]  [3]  [3] |
| Tube coagulase assay | Not possible | Not possible | [3] |
| Biochemistry | Not definitively | Not definitively | [3] |
| DNA-based methods  - 16S rRNA gene targeting  - *nuc* gene targeting  - Whole genome sequencing | Not possible  Possible1  Possible | Possible (1 bp difference)  Possible1  Possible | [3]  [4]  [3] |
| Genotyping  - MLST  - *spa* typing | Indicative  Probably indicative (unstudied) | Indicative  Indicative | [3,9,36]  [36] |
| MALDI-TOF MS | Possible2 | Possible2 | [3,17,54] |

1 Dependent on the annealing sites and nucleotide composition of primers and probes used (widely used *nuc* PCR as described by Brakstad et al. [51] results in amplification products for *S. argenteus* isolates despite some mismatches, but not for *S. schweitzeri*);

2 Dependent on the database entries.

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