Infection and Drug Resistance

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ORIGINAL RESEARCH

Which methods are appropriate for the detection of *Staphylococcus argenteus* and is it worthwhile to distinguish *S. argenteus* from *S. aureus*?

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Purpose: To further analyze a clinical isolate originally identified as methicillin-resistant *Staphylococcus aureus* (MRSA) using whole-genome sequencing and comparative genomics.

Materials and methods: Classical diagnostic methods such as cultivation, biochemical tests, and PCR were supplemented with whole-genome sequencing and comparative genomics, to identify the isolate.

Results: The isolate was phenotypically similar to MRSA. However, the presence of the *nuc* gene could not be confirmed using PCR, while it was positive for the *mecA* gene. Whole-genome sequencing correctly identified the isolate as *Staphylococcus argenteus*. The isolate possessed several resistance genes, such as *mecA*, *blaZ* (β-lactam antibiotics) and *dfrG* (trimethoprim). The *nuc* gene differed from that of MRSA. Six phylogenetic distinct clusters were identified by average nucleotide identity (ANI) analysis of all available *S. argenteus* whole-genome sequences. Our isolate, RK308, clustered with those isolated in Europe and Asia.

Conclusion: Due to the invasive potential, the multi-drug resistance and the similarity to MRSA, *S. argenteus* should be included in the MRSA screening. Due to the divergent genome compared to MRSA, new PCR approaches have to be developed to avoid an unnoticed spreading of *S. argenteus*.

Keywords: *Staphylococcus argenteus*, *Staphylococcus aureus*, MRSA, whole-genome sequencing, clinical diagnostics

Introduction

The first outbreaks of methicillin-resistant *Staphylococcus aureus* (MRSA) were reported from European hospitals in the early 1960s.[1] Gradually, MRSA turned out to be one of the main causes of nosocomial infections worldwide but also community-acquired infections are reported frequently.

S. aureus clonal complex 75 (CC75) was described as the distinct species *Staphylococcus argenteus* in 2015.[2] *S. argenteus* usually have the same virulence factors[3] and antibiotic resistance genes as *S. aureus*. The lack of genes for staphyloxanthin production first led to the conclusion that *S. argenteus* might be less virulent[4] since this carotenoid pigment protects against oxidative stress and impairs neutrophil killing of *S. aureus*.[5] This hypothesis could then be refuted through studies showing comparable morbidity and health care-associated infection rates for both species[6,7] and furthermore, a recent study reported even increased mortality rates compared to *S. aureus*.[8]

Due to the close relationship between *S. aureus* and *S. argenteus*, it is difficult to distinguish the species with common diagnostic methods,[9] as both species have

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identical 16S rRNA genes[10,11] and also harbor thermostable nuclease genes, such as *nuc*. Positive *mecA* assays commonly lead to initial characterization of *S. argenteus* as MRSA.[12]

Here, we report how complementation of the classical diagnostic methods for *S. aureus* and MRSA screening with whole-genome sequencing correctly identified *S. argenteus* and also provided clues on virulence and antibiotic resistance.

Materials and methods

Bacterial isolates

The bacterial isolate RK308 was originally isolated from a clinical sample sent to the Clinical Microbiology Laboratory at Uppsala University Hospital and was thereafter anonymously analyzed in this study. The type strain of the species *S. aureus* 1800^T and the MRSA CCUG 35601 reference strain were also included for comparison.

Phenotypical analyses

The original clinical sample was selectively enriched overnight at 37°C in MRSA broth (Iso-Sensitest broth, Oxoid, Basingstoke, United Kingdom) with 4 mg/L cefoxitin (Sigma-Aldrich, Steinheim, Germany) and 60 mg/L aztreonam (Bristol-Myers Squibb, Solna, Sweden). A broth volume of 5 µL was transferred to blood and *S. aureus* ID (SAID) agar plates (bioMérieux, La Balme Les Grottes, France) with a cefoxitin disc (30 µg; Oxoid, Basingstoke, United Kingdom) placed on the agar surface. Bacterial colonies that grew into the antibiotic zone that represented the chromogenic effect of SAID agar, and that had a phenotypical occurrence like that of *S. aureus* were chosen for further analysis. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF, Bruker Daltonik GmbH, Leipzig, Germany) was used to determine the bacterial species.[13] Separate colonies were transferred to analysis plates, and 1 µL HCCA (α-Cyano-4-hydroxycinnamic acid) matrix was added to each sample spot to ionize peptides, smaller proteins, triacylglycerols and many other compounds in the initial phase of the analysis. Bruker's default settings were used for analysis of the obtained spectra. The StaphAurex test (Remel, Lenexa, USA) was applied to confirm suspected *S. aureus* colonies. Furthermore, heat-inactivated colonies (100°C, 15 minutes) were placed on DNase Agar (Thermo Fisher Scientific, Basingstoke, United Kingdom) plates for confirming extracellular heat-stable DNase activity. The ID32 Staph system (bioMérieux, SA, Marcy l'Etoile, France), commonly used for phenotypical differentiation of 27 staphylococcal species,[14] was also applied.

PCR analyses

The DNA from one pure colony was extracted using the Amplicor Respiratory Specimen Preparation Kit (Roche Diagnostics, Mannheim, Germany). The presence or absence of the thermostable nuclease gene (*nuc*) and the *mecA* gene was tested by PCR with the primers 5′-TCA GCA AAT GCA TCA CAA ACA G-3′ and 5′-CGT AAA TGC ACT TGC TTC AGG-3′ specific for the *nuc* gene and 5′-GGG ATC ATA GCG TCA TTA TTC-3′ and 5′-AAC GAT TGT GAC ACG ATA GCC-3′ specific for the *mecA* gene.[15] MRSA broth without bacteria was used as the negative control while the positive control was the MRSA strain CCUG 35601. PCR was performed using a GeneAmp PCR System 9700 (Applied Biosystems, Houston, USA) with initial denaturation at 95°C for 15 minutes and 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds.

Whole genome sequencing

Pure colonies from blood agar plates were transferred to MRSA broth (Oxoid) containing cefoxitin (4 mg/L) and aztreonam (60 mg/L) and incubated overnight at 37°C. DNA extraction was performed from 400 µL of broth with MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche, Mannheim, Germany) according to the manufacturer's protocol version 12. The libraries for WGS were prepared with a Nextera XT sample preparation kit (Illumina, San Diego, USA). An Illumina HiSeq platform with a 2 × 100 paired end run was used for WGS. The single reads were assembled to contigs with Velvet.[16] The paired reads and merging contigs were assembled by Geneious version 8.1.5.[17] The average nucleotide identity (ANI), which is based on base by base comparison, to closely related taxa was calculated using the Gegenees software version 2.2.1 with a threshold of 20%.[18] The ANI-based phylogenetic tree was constructed in Geneious. The PathoFinder 1.1 database of the Center for Genomic Epidemiology Denmark[19] was used to predict the pathogenicity.

Ethical considerations

The isolate studied was obtained as part of the routine hospital laboratory procedure in the identification of infectious agents for the patient. Patient data were kept anonymous for analysis in the research project. As the study only focused on a bacterial isolate, neither written informed consent nor

ethical approval was necessary for the study, according to the Swedish act concerning the ethical review of research involving humans, Etikprövningslagen (2003:460).

Results

Identification of RK308 with classical methods

The analyzed isolate, RK308 grew on blood agar plates like *S. aureus* with beta hemolysis but as whitish colonies, similar to MRSA CCUG 35601 (Figure 1A). On SAID agar plates, the RK308 colonies were clear blue while *S. aureus* was green (Figure 1). The DNase reaction, as well as the StaphAurex test, were positive for RK308 (Table 1). The analyzed isolate was identified as *S. aureus* with MALDI-TOF with a score value of 1.916 in the corresponding database (Bruker). ID32 Staph analysis also resulted in determination as *S. aureus* according to the ID32 Staph manual. To determine if the isolate was an MRSA, a multiplex PCR for amplification of a *nuc* gene fragment and a *mecA* gene fragment was applied. The PCR gave a negative signal for *nuc* but a positive signal for *mecA* (Table 1), ruling out other nuc-negative Staphylococci.

Genomic characterization

The RK308 isolate was whole-genome sequenced. Gap closing was not performed for the RK308 genome since it is not applicable to clinical diagnostic approaches and not essential for the current analysis.[20] The final assemblies are available from the NCBI database (BioProject number PRJNA310972, GenBank/EMBL/DDBJ accession number LSFQ00000000).

The *S. aureus* strain Newman (NCBI accession AP009351), which is a representative strain of *S. aureus*,[21] shares 93% identity of the whole genome with *S. aureus* subspecies *anaerobius*. These strains showed 74% and 73% identical bases with RK308 in ANI-analysis, which is based on base by base comparison, respectively, while the whole genomes of *S. argenteus* MSHR1132^T and RK308 had 97.2% identical bases.

All available *S. argenteus* whole genome sequences (n=116, May 2018, Table S1) were compared using ANI-analysis. The diversity of the analyzed genomes ranged from 0% to 4.2% (data not shown). Phylogenetic analysis identified six clusters (I-VI, Figure 2), where RK308 clustered with strains mainly isolated in Asia (cluster VI), although its closest relatives seemed to be from Europe (Figure 2).

The *spa* gene was extracted from the whole genome. The *spa*-type was t6675 (299-25-17-17-16-16-16). However, the *spa* gene contained 3 variations in the binding site of the forward primer compared to *S. aureus*. Furthermore, a low frequent G → A variation was observed in the *spa* gene.

Both thermonucleases of strain Newman were found in RK308. For one of them, amino acid alignment showed high variation in the N-terminal part of the protein (Figure 3A), while the other variant was very similar (Figure 3B).

All existing 16S rRNA gene sequences of the whole genome were blasted[22] to ensure that there was no contamination within the culturing and sequencing procedure. All sequences were determined as *S. aureus* with an identity of 99%.

Presence of virulence and antibiotic resistance genes

Resistance genes for β-lactams (*mecA*), penicillins (*blaZ*) and trimethoprim (*dfrG*) were detected in the whole genome of RK308. The *mecA* gene had three-point mutations located outside of the first open reading frame but was otherwise completely functional in vitro. There was no variation within the *mecA* primer binding sites of the primers listed above. Further analysis of the RK308 staphylococcal cassette chromosome *mec* (SCC*mec*) showed that it contained a class A *mec* gene complex, harboring the *mecA*, *mecR1*, and *mecI* genes and a type 2 (A2B2) *ccr* gene complex, resulting in a SCCmec type II according to the nomenclature.[23] Further-

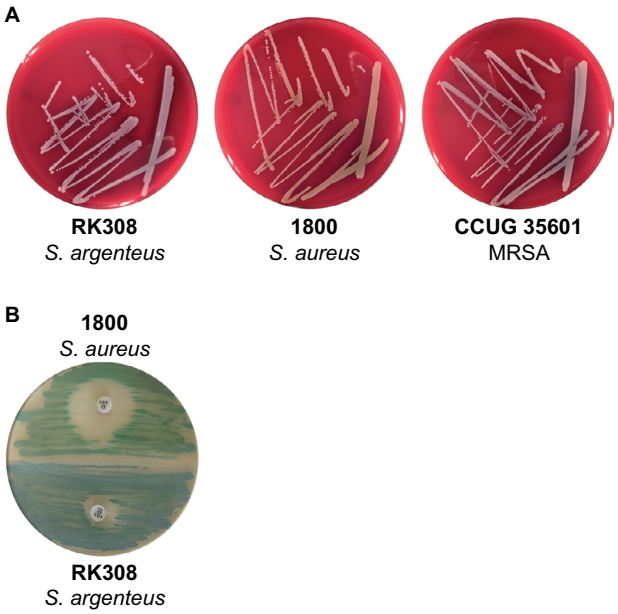

Figure 1 (**A**) *S. argenteus* RK308, *S. aureus* 1800 and MRSA CCUG 35601 on blood agar plates. (**B**) *S. aureus* 1800 (green) and *S. argenteus* RK308 (blue) on SAID agar plate with cefoxitin disc (30 µg).
Abbreviations: MRSA, methicillin-resistant Staphylococcus aureus; SAID, *S. aureus* ID.

Table 1 Summary of phenotypical characterization of *S. argenteus* RK308, *S. aureus* 1800 and MRSA CCUG 35601

	***S. argenteus* RK308**	***S. aureus* 1800**	**MRSA CCUG 35601**
Colony color on blood agar	White	Yellow	White
Hemolysis on blood agar	Beta	-*	–
Colony color on SAID agar	Blue	Green	Blue/green
Clear zone on DNase agar	+	+	+
StaphAurex	+	ND	ND
ID32 Staph urease (URE)	+	–	+
Arginine dehydrogenase (ADH)	+	–	+
Sucrose (SAC)	+	+	–
Susceptibility to cefoxitin	R	S	R
Vancomycin	S	ND	ND
Daptomycin	S	ND	ND
MALDI-TOF typing	*S. aureus*	*S. aureus*	*S. aureus*
nuc PCR	–	nd	+
mecA PCR	+	nd	+

Notes: +, positive result; –, negative result; R, resistant; S, sensitive; ND, not determined. *, unusual since many *S. aureus* strains are hemolysin positive
Abbreviations: MALDI-TOF, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MRSA, methicillin-resistant *Staphylococcus aureus*; SAID, *S. aureus* ID.

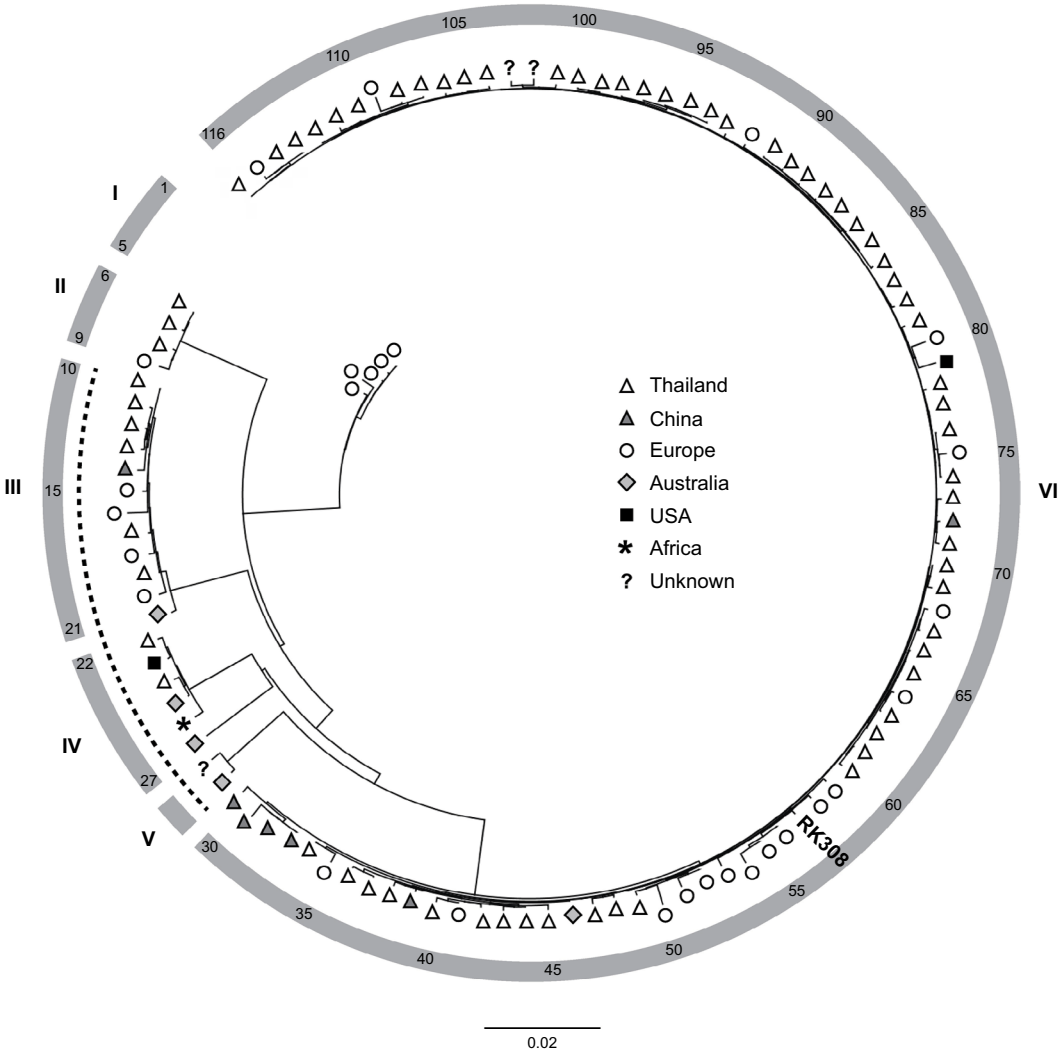

Figure 2 Phylogenetic ANI-based analysis of *S. argenteus* whole-genome sequences.
Notes: Symbols indicate geographical location of isolation. The position of isolate RK308 is indicated (#57). Numbers in outer circle refer to isolate numbers from Table S1. Roman numerals correspond to identified clusters I-VI. The dotted line indicates PVL-negative isolates.
Abbreviation: PVL, Panton-Valentine leucocidins.

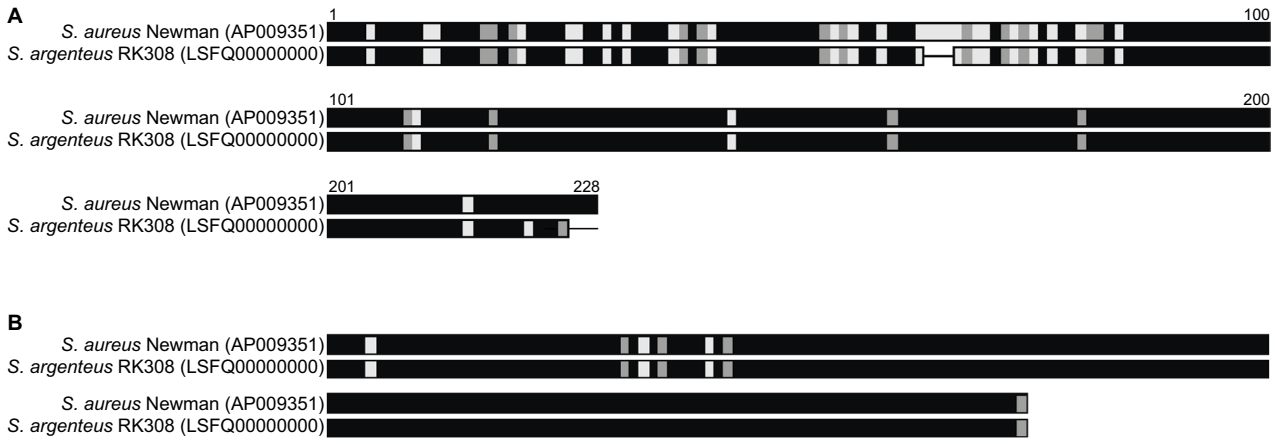

Figure 3 Amino acid alignment of two heat-stable nucleases of *S. aureus* Newman and *S. argenteus* RK308 (**A** and **B**).
Notes: Black: identical amino acids; Gray: similar amino acids; White: different amino acid; line: gaps.

more, the RK308 SCC*mec* had almost identical nucleotide sequence to that of MRSA USA300.

The *mprF* gene, involved in daptomycin resistance, was also detected in the whole genome but did not have any of the previously reported amino acid substitutions involved in resistance[24] and RK308 did not show resistance to daptomycin in in vitro susceptibility testing (data not shown). Neither did RK308 show vancomycin resistance in vitro, and nor were vancomycin resistance genes found in silico.

RK308 was predicted as a human pathogen with a probability of 95.3% and with 100 matched pathogenic families according to the database of the Center for Genomic Epidemiology Denmark.[19] Further in silico analyses revealed the presence of the Panton-Valentine leucocidins (PVL, *lukF*-PV, and *lukS*-PV) in RK308 and in 83% (96/116) of all the isolates included here. Interestingly, all PVL-negative isolates (isolates #10–29, Table S1), including the *S. argenteus* type strain MSHR1132, were found in clusters III, IV and V (Figure 2), while all isolates in clusters I, II and VI contained PVL. Other toxin genes, such as gamma-hemolysin (*hlg*) and exfoliative toxin A (*eta*) were also found in RK308, while the toxic shock syndrome toxin gene (*tsst*) and enterotoxins *sea-see* were not found in RK308.

Discussion

Despite the fact that *S. aureus* is known to have a clonal population structure,[25] a genetic divergence of 26% of the whole genome has been determined. Regarding the mutation frequency that was estimated to an average of 9.2 mutations per year,[26] *S. argenteus* and *S. aureus* have divided about 41, 000 years ago from the common ancestors.

The species determination of *S. argenteus* is challenging with classical methods,[9] and here RK308 was incorrectly identified as *S. aureus*. The cultivation on SAID agar plates on which the isolate grew with a deviant gray color raised doubts about the correct species identification. The isolate was therefore further analysed with an MRSA-specific multiplex PCR. The *mecA* gene was detected with PCR while the *nuc* gene (thermostable nuclease), which is a common marker for *S. aureus,* gave a negative result. However, two thermostable nuclease genes were found in the genome where the one for which diagnostic primers are used showed many variations between *S. aureus* strain Newman and *S. argenteus* RK308, possibly explaining the negative PCR. The positive reaction on DNase plates might, therefore, be due to redundant functions of the two thermostable nuclease genes, as previously reported.[27]

The variations in the binding site of the forward primer in the *spa* gene may also lead to difficulties in conventional PCR for *spa* typing.

With the results of WGS, the isolate RK308 could be assigned to the species *S. argenteus*. While *S. aureus* is a common species and an important cause of both nosocomial and community-acquired infections, *S. argenteus* occurs rarely but worldwide (Table S1). However, the real distribution and prevalence of *S. argenteus* are still unclear. Due to the difficulties in diagnostics, a high number of incorrectly diagnosed *S. aureus* / MRSA can be suspected if the methods are not appropriate to distinguish between *S. aureus* and *S. argenteus*. Thaipadungpanit et al[6] reported 4.1% of 246 molecularly typed *S. aureus* isolates to be *S. argenteus*. However, the patients might have received the appropriate treatment even

if the species identification has been wrong. The results from the in vitro susceptibility testing here showed that RK308 was susceptible to both vancomycin and daptomycin, which would offer good options for treatment. A worse scenario is the application of diagnostic methods specific for *S. aureus,* like the multiplex PCR described above, which might lead to the unrecognition and further unnoticed spread of *S. argenteus*. The PCR method described above[15] is used by national and reference laboratories worldwide and is considered to be robust and reliable, and a recommended method for detecting MRSA. However, the presence of the complete SCCmec cassette in RK308 might open up new options for screening and detection of *S. argenteus* in the future.

On the basis of the present results, it seems obvious that if both species need to be detected simultaneously, alternative methods have to be applied. There is no consensus, neither national nor international, whether *S. argenteus* should be included in or excluded from diagnostics of *mecA*-positive bacteria.[28] *S. argenteus* was previously denoted *S. aureus* CC75 and all detected MRSA CC75-cases were conclusively clinically treated as MRSA. Conclusively, the updated method for detection of MRSA at the laboratory of the Uppsala University Hospital now also includes *S. argenteus* and will do so until there is a clear definition in the Communicable Disease Act. Correct identification and separation of the two species will be important for epidemiological studies but might have an even larger impact on clinical practice and treatments as new studies point toward significant mortality rates for *S. argenteus* combined with sustained susceptibility to several antibiotics.[7,8]

Although the presence of several resistance genes and the high prevalence of PVL would suggest that *S. argenteus* is able to cause infections similar to those of *S. aureus*, the effect of RK308 on human cells remains to be tested.

Less is also known about the *S. argenteus* transmission ways, but several hosts and sources of isolation have been described. According to the metadata belonging to the deposited sequences in the NCBI database,[22] the majority of *S. argenteus* isolates have been isolated from humans but also from various animals, such as gorilla and bats,[29,30] and from environmental samples of unknown origin. Although globally distributed, most of the *S. argenteus* isolates originate from Asia. RK308 clustered among other European and Asian isolates of clonal complex 2250[31] in the phylogenetic tree.

Conclusion

The differentiation between methicillin-resistant *S. argenteus* and MRSA is challenging. In this study, all clinical diagnostic methods failed to identify *S. argenteus* correctly while identification to the species level was obtained by WGS. The *S. argenteus* isolates studied here, RK308, possessed several virulences and resistance genes usually detected in MRSA, which suggests that *S. argenteus* could be as virulent as MRSA and should not be left unnoticed. To prevent future spread, appropriate diagnostic methods should be used.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 *S. argenteus* genomes used in in silico analyses

#	Cluster	Isolate	Accession #	Origin of the isolate		Ref.
				Location	Year	
1	I	M5219	FXVN00000000	Denmark	2016	1
2		M5224	FXWC00000000	Denmark	2016	1
3		M5200	FXVY00000000	Denmark	2016	1
4		H1955	FXWA00000000	Denmark	2013	1
5		H115100079	CCEP00000000	UK	-	2
6	II	3688STDY6125129	FQRW00000000	Thailand	-	3
7		3688STDY6125130	FQMG00000000	Thailand	-	3
8		3688STDY6125134	FQMS00000000	Thailand	-	3
9		O-9	FXVM00000000	Denmark	2016	1
10	III	3688STDY6125128	FQME00000000	Thailand	-	3
11		3688STDY6125132	FQMJ00000000	Thailand	-	3
12		3688STDY6125135	FQMT00000000	Thailand	-	3
13		3688STDY6125143	FQNB00000000	Thailand	-	3
14		SJTU F20124	LWAN00000000	China	2005	4
15		D7903	FXVL00000000	Denmark	2014	1
16		M4185	FXVI00000000	Denmark	2013	1
17		3688STDY6125139	FQMX00000000	Thailand	-	3
18		M4528	FXWD00000000	Denmark	2013	1
19		3688STDY6125133	FQMI00000000	Thailand	-	3
20		CCUG69385	NSBX00000000	Sweden	2016	5
21		M051_MSHR	CCEN00000000	Australia	-	-
22	IV	3688STDY6125127	FQMH00000000	Thailand	-	3
23		ABFQM	LYLU00000000	USA	2015	-
24		3688STDY6125137	FQMV00000000	Thailand	-	3
25		M260_MSHR	CCEF00000000	Australia	-	-
26		BN75[a]	CP015758	Gabon	2012	6
27		JABA32044V6S1	CCEE00000000	Fiji	-	-
28	V	DSM_28299	PPPZ00000000	-	-	-
29		MSHR1132[b]	FR821777	Australia	2006	7
30	VI	SJTU F20419	LWAO00000000	China	2012	4
31		SJTU F21285[c]	LWAR00000000	China	2012	4
32		XNO62	CP023076	China	2014	8
33		XNO106	CP025023	China	2015	8
34		3688STDY6125106	FQKZ00000000	Thailand	-	3
35		CCUG69384	NSBY00000000	Sweden	2016	5
36		3688STDY6125063	FQKD00000000	Thailand	-	3
37		3688STDY6125069	FQRB00000000	Thailand	-	3
38		3688STDY6125109	FQKX00000000	Thailand	-	3
39		SJTU F21224	LWAQ00000000	China	2012	4
40		3688STDY6125118	FQLY00000000	Thailand	-	3
41		O-5	FXVO00000000	Denmark	2016	1
42		3688STDY6125123	FQRU00000000	Thailand	-	3
43		3688STDY6125120	FQMC00000000	Thailand	-	3
44		3688STDY6125138	FQMW00000000	Thailand	-	3
45		3688STDY6125131	FQMF00000000	Thailand	-	3
46		LBSA043	CCEM00000000	Australia	-	-
47		3688STDY6125111	FQLA00000000	Thailand	-	3
48		3688STDY6125125	FQLZ00000000	Thailand	-	3
49		3688STDY6125116	FQLH00000000	Thailand	-	3
50		H1864	FXVU00000000	Denmark	2013	1
51		H1826	FXWB00000000	Denmark	2014	1
52		M4146	FXVR00000000	Denmark	2013	1

(*Continued*)

Table S1 (*Continued*)

#	Cluster	Isolate	Accession #	Origin of the isolate		Ref.
53		H1540	FXVT00000000	Denmark	2014	1
54		M3040	FXVK00000000	Denmark	2013	1
55		O-2	FXWE00000000	Denmark	2016	1
56		M4611	FXWF00000000	Denmark	2013	1
57		RK308	LSFQ010000000	Sweden	2015	This paper
58		M4143	FXVV00000000	Denmark	2013	1
59		H2179	FXVS00000000	Denmark	2013	1
60		3688STDY6125074	FQKI00000000	Thailand	-	3
61		3688STDY6125081	FQKM00000000	Thailand	-	3
62		3688STDY6125080	FQKL00000000	Thailand	-	3
63		3688STDY6125105	FQKT00000000	Thailand	-	3
64		O-6	FXVZ00000000	Denmark	2016	1
65		3688STDY6125114	FQRS00000000	Thailand	-	3
66		3688STDY6125140	FQMZ00000000	Thailand	-	3
67		3688STDY6125126	FQMD00000000	Thailand	-	3
68		O-1	FXVH00000000	Denmark	2016	1
69		3688STDY6125100	FQKN00000000	Thailand	-	3
70		3688STDY6125112	FQLB00000000	Thailand	-	3
71		3688STDY6125062	FQRA00000000	Thailand	-	3
72		SJTU F21164	LWAP00000000	China	2011	4
73		3688STDY6125082	FQRG00000000	Thailand	-	3
74		3688STDY6125084	FQKQ00000000	Thailand	-	3
75		O-4	FXVQ00000000	Denmark	2016	1
76		3688STDY6125119	FQLX00000000	Thailand	-	3
77		3688STDY6125136	FQMU00000000	Thailand	-	3
78		3688STDY6125117	FQLO00000000	Thailand	-	3
79		1299_SAUR	JVUX00000000	USA	2012	9
80		H1604	FXVP00000000	Denmark	2014	1
81		3688STDY6125115	FQLC00000000	Thailand	-	3
82		3688STDY6125086	FQRJ00000000	Thailand	-	3
83		3688STDY6125087	FQKP00000000	Thailand	-	3
84		3688STDY6125064	FQMN00000000	Thailand	-	3
85		3688STDY6125068	FQRD00000000	Thailand	-	3
86		3688STDY6125092	FQRN00000000	Thailand	-	3
87		3688STDY6125110	FQKY00000000	Thailand	-	3
88		3688STDY6125122	FQRT00000000	Thailand	-	3
89		3688STDY6125093	FQKW00000000	Thailand	-	3
90		3688STDY6125113	FQRR00000000	Thailand	-	3
91		3688STDY6125124	FQRV00000000	Thailand	-	3
92		M4148	FXVX00000000	Denmark	2013	1
93		3688STDY6125091	FQRM00000000	Thailand	-	3
94		3688STDY6125072	FQMR00000000	Thailand	-	3
95		3688STDY6125075	FQRF00000000	Thailand	-	3
96		3688STDY6125077	FQRH00000000	Thailand	-	3
97		3688STDY6125079	FQKG00000000	Thailand	-	3
98		3688STDY6125121	FQMA00000000	Thailand	-	3
99		3688STDY6125088	FQRK00000000	Thailand	-	3
100		3688STDY6125090	FQKO00000000	Thailand	-	3
101		3688STDY6125089	FQRL00000000	Thailand	-	3
102		F87619	JGHK00000000	-	2013	-
103		M21126	JGMK00000000	-	2013	-
104		3688STDY6125065	FQML00000000	Thailand	-	3
105		3688STDY6125083	FQKR00000000	Thailand	-	3
106		3688STDY6125067	FQRC00000000	Thailand	-	3
107		3688STDY6125066	FQMM00000000	Thailand	-	3
108		3688STDY6125076	FQKJ00000000	Thailand	-	3

(*Continued*)

Table S1 (*Continued*)

#	Cluster	Isolate	Accession #	Origin of the isolate		Ref.
110		3688STDY6125070	FQMP00000000	Thailand	-	3
109		O-10	FXVJ00000000	Denmark	2016	1
111		3688STDY6125071	FQMQ00000000	Thailand	-	3
112		3688STDY6125085	FQRI00000000	Thailand	-	3
113		3688STDY6125073	FQRE00000000	Thailand	-	3
114		3688STDY6125108	FQLE00000000	Thailand	-	3
115		O-3	FXVW00000000	Denmark	2016	1
116		3688STDY6125078	FQKK00000000	Thailand	-	3

Note: [a]Origin: Gorilla, [b]Type strain of *S. argenteus*, [c]Origin: Pig, -; unknown

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