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Identification and characterization of *Staphylococcus argenteus* from Indonesia

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ABSTRACT

Background: In 2015, *Staphylococcus argenteus* was reported for the first time as a novel species of the *Staphylococcus aureus* complex. While *S. argenteus* has been found in many countries, its presence in Indonesia has not been reported yet. Our aim is to confirm *S. argenteus* presence in Indonesia, describe its characteristics and analyze its genomic diversity.

Methods: The *S. aureus* isolates used in this study were collected from patients with skin and soft tissue infections in Indonesia, between July 2009 to February 2010. Randomly selected isolates were recultured from −80 C° stocks and analyzed using matrix-assisted laser desorption/ionization – time of flight (MALDI-TOF). Isolates identified as *S. argenteus*, *S. roterodami*, or *S. schweitzeri* and *S. aureus* with a low score in the MALDI-TOF analysis were analyzed by a real-time PCR targeting the *nucA* gene able to identify true *S. argenteus*. Isolates identified as *S. argenteus* were further characterized by whole genome sequencing. Vitek®2 (bioMérieux) was used for antimicrobial susceptibility testing.

Results: Fifteen isolates were identified as *S. argenteus*, with the majority belonging to ST2250. Two pairs of isolates proved to be identical by core genome multilocus sequence typing analysis. Most isolates were susceptible to all antibiotics tested, except for seven isolates (46.7 %) that were resistant to benzylpenicillin, and one isolate was resistant to tetracycline (6.7 %). The presence of resistance genes *blaZ* and *tet(45)* correlated with these findings. Notably, the *sey* enterotoxin gene was prevalent in 80 % of the isolates. Other virulence factor genes were less prevalent. Plasmid replicon types in *S. argenteus* were also known to *S. aureus*.

Conclusion: Our study reveals the occurrence of *S. argenteus* in Indonesia. The diversity within Indonesian *S. argenteus* matches the global diversity of *S. argenteus*. Identical isolates between patients indicate potential transmission events. A lower prevalence of a broad panel of virulence factors suggests that *S. argenteus* is less virulent than *S. aureus*.

1. Background

Staphylococcus argenteus was first isolated from the blood culture of a female patient from Australia in 2006 and described as a novel species within the *Staphylococcus aureus* complex in 2015 (Tong et al. 2015). However, several reports thereafter showed that the case report from Australia was not a rare finding and that *S. argenteus* does cause a range of infections such as bloodstream infections (Thaipadungpanit et al.

Abbreviations: MALDI-TOF MS;, Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry; *ESCMID;*, European Society of Clinical Microbiology and Infectious Disease; *ESGS;*, European Society of Clinical Microbiology and Infectious Disease Study Group for *Staphylococci* and *Staphylococcal* Diseases; *SSTI;*, Skin and soft tissue infections; *PHC;*, Primary healthcare centres; *MSSA;*, Methicillin-susceptible *S. aureus*; *MH;*, Mueller-Hinton; *RT-PCR;*, Real-time polymerase chain reaction; *GP;*, Gram positive; *AST;*, Antimicrobial susceptibility test; *cgMLST;*, Core genome multilocus sequence typing.

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2015; Chantratita et al. 2016) and sepsis (Hao et al. 2020), bone and joint infections (Rigaill et al. 2018; Diot et al. 2020; Söderquist et al. 2020; Jiang et al. 2018), soft tissue and skin infections, impetigo, necrotizing fasciitis, and toxin-mediated syndromes, like staphylococcal food poisoning (Tong et al. 2013; McDonald et al. 2006; Holt et al. 2011), resembling *S. aureus*. Recent reports even showed that the rates of healthcare-associated infections, morbidity and death caused by *S. argenteus* was comparable to *S. aureus* (Thaipadungpanit et al. 2015; Chen et al. 2018). Based on these findings, it is suggested that the pathogenicity of *S. argenteus* is comparable to *S. aureus* (Becker et al. 2019). However, the place of *S. argenteus* within the pathogenicity spectrum of the *S. aureus* complex has not yet been completely elucidated. A major reason for this is that standard diagnostic tests are unable to reliably identify *S. argenteus*. The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Staphylococci and Staphylococcal Diseases (ESGS) stated in their 2019 position paper that routine differentiation within the *S. aureus* complex is unnecessary unless there is substantial evidence of different pathogenicity or clinical outcome among the species. Additional research is therefore needed to understand the epidemiology, clinical consequences and implications for infection prevention and control (IPC) related to the different species (Becker et al. 2019).

S. argenteus has been reported in several countries (Zhang et al. 2017), including countries in Southeast Asia (Thailand, Myanmar) (Thaipadungpanit et al. 2015; Aung et al. 2019). However, from Indonesia, the most populated country in Southeast Asia, no information is available yet. Therefore, this study aimed to investigate whether *S. argenteus* is also present in Indonesia using isolates from a former study collection of (presumably) *S. aureus* and if present, describe their characteristics and analyze their genomic diversity.

2. Methods

2.1. Bacterial isolates

The *S. aureus* isolates were taken from a previous study conducted in Indonesia, where *S. aureus* was cultured from anterior nares, throats, and wounds from patients presenting with skin and soft tissue infections (SSTI) (Santosaningsih et al. 2018). Isolates were collected between July 2009 and February 2010 in several primary healthcare centers (PHC) and dermatology clinics on the islands of Java and Bali, Indonesia. Identification at the time was performed by Slidex Staph Plus (bioMérieux, Marcy l'Etoile, France) and the Vitek®2 system (bioMérieux). A subset of these *S. aureus* isolates (from Denpasar, island of Bali) was additionally confirmed by matrix-assisted laser desorption/ionization – time of flight (Maldi Biotyper, Bruker Microflex LT, Bruker, London, UK).

For the present study, a total of 357 isolates, stored at −80°C in tryptic soy broth (TSB) medium + 10 % glycerol, were randomly selected from the collection. Reculturing of isolates was done by subculturing the stored isolates on blood agar (Becton Dickinson, New Jersey, USA), followed by incubation at 35°C for 24–48 hours. Subsequently, plates were checked for growth and purity. Identification was performed by MALDI-TOF MS. Isolates that were identified as *S. argenteus*, *S. schweitzeri*, S. *roterodami* or *S. aureus* (but with low score) were further subjected to a real-time PCR targeting the *nucA* gene to identify true *S. argenteus*.

2.2. nucA PCR

Primers and probes were based on conserved regions of the *nucA/nuc* gene and amplified both the *S. aureus* and *S. argenteus* target, discrimination between the two was based on species specific probes (Supplementary Table 1). First, DNA was isolated from freshly grown cultures using the MagNA Pure96 platform in combination with the DNA and Viral Nucleic Acid Small Volume Kit (Roche Diagnostics, Almere, the Netherlands). Amplification reactions (20 μL) consisted of 5 μL of isolated DNA, 0.5 μM of both amplification primers and 0.2 μM of each probe in 1x LightCycler 480 Probes Master (Roche Diagnostics). Amplification was performed on a LightCycler 480 platform (Roche Diagnostics). Cycling parameters involved an initial denaturation for 5 min at 95°C followed by 50 amplification cycles of 95° for 5 s and 60°C for 30 s with maximum heating and cooling settings, after which the samples were cooled down. No cross-reactivity between *S. aureus* and *S. argenteus* was observed.

2.3. Antimicrobial susceptibility testing

Susceptibility testing was performed by Vitek®2 AST-P657 cards (bioMérieux). Furthermore, susceptibility to teicoplanin and vancomycin was assessed by performing a direct E-test on Mueller-Hinton (MH) agar based on the study by Walsh et al. (Walsh et al. 2001). Breakpoints were used according to EUCAST guideline version 11 (vancomycin <=2 mg/L and teicoplanin <2=mg/L).

2.4. Biochemical profiling

Biochemical profiling for staphylococcal species was performed using Vitek®2 Gram positive (GP) Card.

2.5. Whole genome sequencing

DNA was isolated from freshly grown cultures using the Quick-DNA Fungal/Bacterial Miniprep kit (Zymo Research) isolation kit. Libraries were constructed using the Illumina DNA prep kit (Illumina, San Diego, CA, USA) and sequenced on an iSEQ-100 platform (Illumina) generating 150 nt paired-end reads and a minimal coverage of >30x. De-novo assemblies were created using CLC Genomics Workbench v21 using default parameters (Qiagen, Hilden, Germany). Presence of antimicrobial resistance genes was investigated using the online comprehensive antimicrobial resistance database (CARD) interface (https://card.mcmaster.ca/analyze/rgi) restricted to perfect and strict hits. Plasmid replicons were detected using a stand-alone version of Plasmidfinder V2.1. Virulence factors were identified using the sequence extraction module of BioNumerics v7.6 (Applied Maths, St-Martens-Latem, Belgium) using the *S. aureus* sequences as references and a panel of 70 virulence factors as described elsewhere (Slingerland et al. 2020). Hits were considered with a minimum of 80 % sequence identity and 95 % coverage.

A core-genome multilocus sequence typing (cgMLST) scheme was constructed in SeqSphere+ software v5 (Ridom, Munster, Germany) using the cgMLST target definer module. The seed genome consisted of the genomic sequence of the *S. argenteus* type strain (MSHR1132; Genbank Accession nr. NC_016941.1). As penetration genomes, available *S. argenteus* genome sequences from NCBI (N=173) were used (Supplementary file 2). The resulting scheme consisted of 2028 core genes and 463 accessory genes. Conventional multilocus sequence types (MLST) were determined in SeqSphere+ by applying the *S. aureus* scheme. The flowchart of *S. argenteus* detection can be found in Supplementary Figure 1.

2.6. Data availability

Sequence data from the isolates in this study have been deposited in the SRA archive and are available under BioProject nr PRJNA1101927 and BioSample accession nrs SAMN41006555 through SAMR41006569.

3. Results

In this collection of *S. aureus* isolates, a total of 15 isolates were identified as *S. argenteus*. The majority of these isolates (8 isolates) originated from Denpasar. Almost half of the isolates (7 isolates) were cultured from wound, five isolates from throat and three isolates from

nose. When considering the age group, five isolates were from children below 18 years of age and six isolates were from adults. The details of the isolates are provided in Table 1.

The Vitek®2 GP card was employed to conduct biochemical profiling for staphylococcal species as detailed in Table 2. Interestingly, urease was positive for 60.0 % of the isolates, alpha-glucosidase for 53.5 % and d-mannose for 86.7 %.

All *S. argenteus* isolates were susceptible to cefoxitin, oxacillin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, clindamycin, linezolid, teicoplanin, vancomycin, fosfomycin, fusidic acid, mupirocin, rifampicin, and trimethoprim/sulfamethoxazole. Seven isolates (46.7 %) were resistant to benzylpenicillin, and one isolate was resistant to tetracycline (6.7 %).

The results of AST for all *S. argenteus* isolates can be found in Table 3.

All isolates were tested for the presence of a panel of known virulence factors of *S. aureus*. Remarkably, the *sey* enterotoxin gene was detected in 80 % of the isolates, while *seg*, *sei*, *sem*, *sen*, *seo,* and *seo* were found in two isolates Fig. 1.

Antibiotic resistance genes were identified using CARD, the presence of *arlR*, *mepR*, *mgrA, and lmrS* in all isolates were reported. The *blaZ* gene was present in seven isolates corresponding with benzylpenicillin resistant isolates. The *cat* gene was detected in one isolate and *tet(45)* was found in another, corresponding with the AST result. None of the isolates harboured *mecA* or *mecC*. Furthermore, with one exception the *fosB* gene was present in all isolates. However, despite of the presence of the gene, all isolates were reported to be susceptible to the antibiotic fosfomycin based on the Vitek®2 results.

All identified *S. argenteus* were subjected to whole genome sequencing. Assembly statistics of all isolates can be found in Supplementary Table 3. Genotyping of the isolates using an in-house developed cgMLST scheme showed that two pairs of identical isolates were found, namely N/14/VIII/9 and T112, and W/447/XII/9/1 and W/6/VII/9. Among our isolates, 80 % were of sequence type (ST) 2250 (12 isolates), followed by ST1223 (2 isolates) and ST2793 (1 isolate).

The genotypic diversity of Indonesian isolates compared to available *S. argenteus* genomes from around the globe (N=173) is shown in Fig. 2. *S. argenteus* isolates from Indonesia show the same sequence types as the global *S. argenteus* collection with ST2250 being the most prevalent ST. The developed cgMLST scheme is specific for *S. argenteus* since on average an allele number will be assigned to >99 % of core genes. Instead, when genomes of other members from the *S. aureus* complex are analyzed lower percentages are obtained (e.g. *S. schweitzeri* 78 %, S. *roterodami* 85 %, *S. aureus* 36 %, results not shown).

Table 1
Details of the *S. argenteus* isolates.

Isolate name	Patient's gender	Patient's age (years)	Specimen	City
72	Male	3	Wound	Surabaya
86	Male	1	Wound	Surabaya
88	Female	10	Wound	Surabaya
M1203	Male	16	Nose	Malang
M2206	Male	62	Throat	Malang
M2224	Male	14	Throat	Malang
N14	Female	37	Nose	Denpasar
N/14/VIII-9	Female	37	Nose	Denpasar
P3T	Male	25	Throat	Surabaya
T112	Male	27	Throat	Denpasar
T/146/VIII-9 1	Unknown	Unknown	Throat	Denpasar
W/6/VII-9	Male	38	Wound	Denpasar
W16	Unknown	Unknown	Wound	Denpasar
W/14/VIII-9	Unknown	Unknown	Wound	Denpasar
W/447/XII-9	Unknown	Unknown	Wound	Denpasar

Table 2
Biochemical characterization of *Staphylococcus argenteus* isolates from Indonesia.

Isolates	Biochemicals																							
	dRIB	OPTO	ILATk	NC6.5	O129R	dMAN	ADH1	URE	NAG	dMNE	SAC	BGAL	PyrA	POLYB	dMAL	MBdG	dTRE	AGLU	PHOS	dGAL	BACI
72	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	-	+	+	+
86	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	-
88	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M1203	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	-	+	+	
M2206	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	+	-	+	+	+
M2224	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+
N14	+	+	+	+	-	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+
N14/VIII-9	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+
P3T	-	+	+	+	-	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+
T112	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
T146/VIII/9	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+		+
W6/VII/9	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+		+	+	+
W16	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+
W14/VIII/9	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+
W/447/XII/9	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
	93.3	100	100	100	80.0	100	100	60.0	53.3	86.7	100	6.7	100	86.7	100	100	100	53.3	100	93.3	86.7

Abbreviations: dRIB, d-ribose; OPTO, optochin resistance; ILATk, L-lactate alkalinization; NC6.5, growth in 6.5 % NaCl; 0129 R, 0/129 resistance (comp.vibrio); dMAN,;d-mannitol; ADH1, arginine dihydrolase 1, URE, urease; NAG, n-acetyl-d-glucosamine; dMNE, d-mannose; SAC, saccharose/sucrose; BGAL, beta-galactosidase; PyRA, L-Pyrrolidonyl-arylamidase; POLYB, polymyxin B resistance; dMAL, d-maltose; MBdG, methyl-B-D-glucopyranoside; dTRE, D-trehalose; AGLU, alpha-glucosidase; PHOS, alkaline phosphatase; dGAL, d-galactose; BACI, bacitracin resistance. The bottom line shows percentage of positive test results for each biochemical test.

Table 3
Susceptibility test results of *S. argenteus* isolates by Vitek® 2.

Isolates	ANTIBIOTICS																		CARD	
	CXM	PEN	OXA	GEN	TOB	CIP	LVX	ICR	ERY	CLI	LNZ	TEC*	VAN*	TET	FOS	FUS	MUP	RIF	SXT	tet(45)	blaZ
72	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	+	+
86	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	+
88	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	+
M1203	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	-
M2206	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	-
M2224	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	+
N14	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	+
N14/VIII-9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	-
P3T	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	+
T112	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	-
T146/VIII/9	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	+
W6/VII/9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	-
W16	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	-
W14/VIII/9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	-
W/447/XII/9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	-

S, susceptible; R, resistant; CXM, cefixime; PEN, benzylpenicillin; OXA, oxacillin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; LVX, levofloxacin; ICR, inducible clindamycin resistance; ERY, erythromycin; CLI, clindamycin; LNZ, linezolid; TEC, teicoplanin; VAN, vancomycin; TET, tetracyclin; FOS, fosfomycin;FUS, fusidic acid; MUP, mupirocin; RIF, rifampicin; SXT, trimethoprim-sulfamethoxazole.
*Measured by direct E-test on MH agar

4. Discussion

This study provides the initial documentation of *S. argenteus* in Indonesia, thereby enhancing our understanding of its global distribution. From a study among patients with SSTI performed in 2009–2010, 15 isolates were identified.

Routine diagnostic microbiological testing is unable to differentiate between *S. argenteus* and *S. aureus*, leading to potential misidentification, as seen in a study from Thailand where 4.1 % of isolates initially identified as *S. aureus*, were actually *S. argenteus* (Thaipadungpanit et al. 2015). Additionally, researchers from the USA and Canada emphasized the importance of distinguishing between different members of the *S. aureus* complex for research and surveillance. However, the ESCMID study group for Staphylococci and Staphylococcal Diseases (ESGS) recommends this distinction only for research, not diagnostics, as the clinical implication of infections caused by *S. argenteus* compared to *S. aureus* remains unclear (Becker et al. 2019).

The *S. argenteus* isolates from Indonesia belonged to three STs, predominantly ST2250, a globally prevalent clone (Hansen et al. 2017; Eshaghi et al. 2021; Wu et al. 2021; Goswami et al. 2021; Aung et al. 2019). This suggests that the sequence type is not specific to any region, indicating a widespread distribution of this clone across different geographical locations. Nevertheless, eight of the isolates in this study originated from Denpasar, suggesting tourism might contribute to *S. argenteus* transmission. We also found one isolate that belonged to ST2793, previously only found in Europe and USA, but not in Asia except Northern Taiwan (Hao et al. 2020; Hansen et al. 2017; Giske et al. 2019; Hsu et al. 2020).

The discovery of two identical pairs of isolates indicates potential transmission between persons consistent with earlier findings (Jauneikaite et al. 2021). However, the small sample size and lack of metadata limited further transmission analysis. Further studies with larger sample and comprehensive data are needed to better understand transmission dynamics of *S. argenteus*.

The *S. argenteus* isolates from our study were identified by Vitek®2 as *S. aureus* but with a positive urease test as discordant result in 60 % of the isolates, consistent with findings from Northern Taiwan (65 %) (Hsu et al. 2020). However, all isolates contained the *ure* gene cluster with fully conserved sequences (results not shown) indicating that the observed phenotype results from differences at the gene regulation level. In *S. aureus* urease plays a significant role in the acid response network in the presence of urea (Zhou et al. 2019). Additionally, a study conducted in England and Wales reported urease presence in over 90 % of *S. aureus* (Murchan et al. 2004). Our results indicate that regulation of urease activity is different in *S. argenteus* and (although not with 100 % sensitivity) that urease activity could serve as a marker to identify *S. argenteus* within *S. aureus* complex. Furthermore, discrepancies were also reported for alpha-glucosidase and d-mannose (in 53.5 and 86.7 % of the isolates, respectively) suggesting that these substrates could be indicators for identifying *S. argenteus*.

The AST results indicated that isolates resistant to benzylpenicillin and tetracycline were associated with the presence of resistance genes *blaZ* and *tet(45)*, respectively, and confirmed the discrepancy between the presence of the *fosB* gene in *S. argenteus* and the observed phenotypic susceptibility to fosfomycin (Chantratita et al. 2016; Goswami et al. 2021). Further investigations are necessary to elucidate the correlation between these findings.

The *sey* gene was detected in 80 % of the isolates, consistent with studies from France and China, identifying it as most prevalent enterotoxin gene in *S. argenteus* (Cavaiuolo et al. 2023) (Zhang et al. 2017) and along with its significant lower presence in *S. aureus* (5.8 %) (Slingerland et al. 2020), suggests that the *sey* gene likely originated from *S. argenteus*. Additionally, a combination of *seg, sei, sem, sen* and *seo* genes was detected in our isolates, which have also been reported in *S. aureus* from several studies (Nashev et al. 2007; Becker et al. 2004; Omoe et al., 2002).

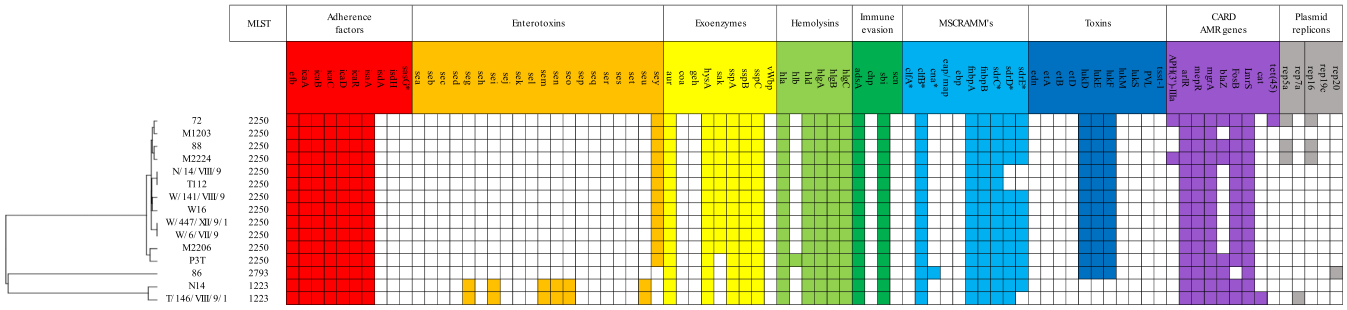

Fig. 1. Strains, virulence factors, resistance genes and plasmid replicons of S. argenteus isolates. The dendrogram is based on cgMLST analysis. Colored boxes indicate presence, white boxes indicate absence. MLST: multilocus sequence typing; MSCRAMM's: microbial surface component recognizing adhesive matrix molecules; AMR, antimicrobial resistance.

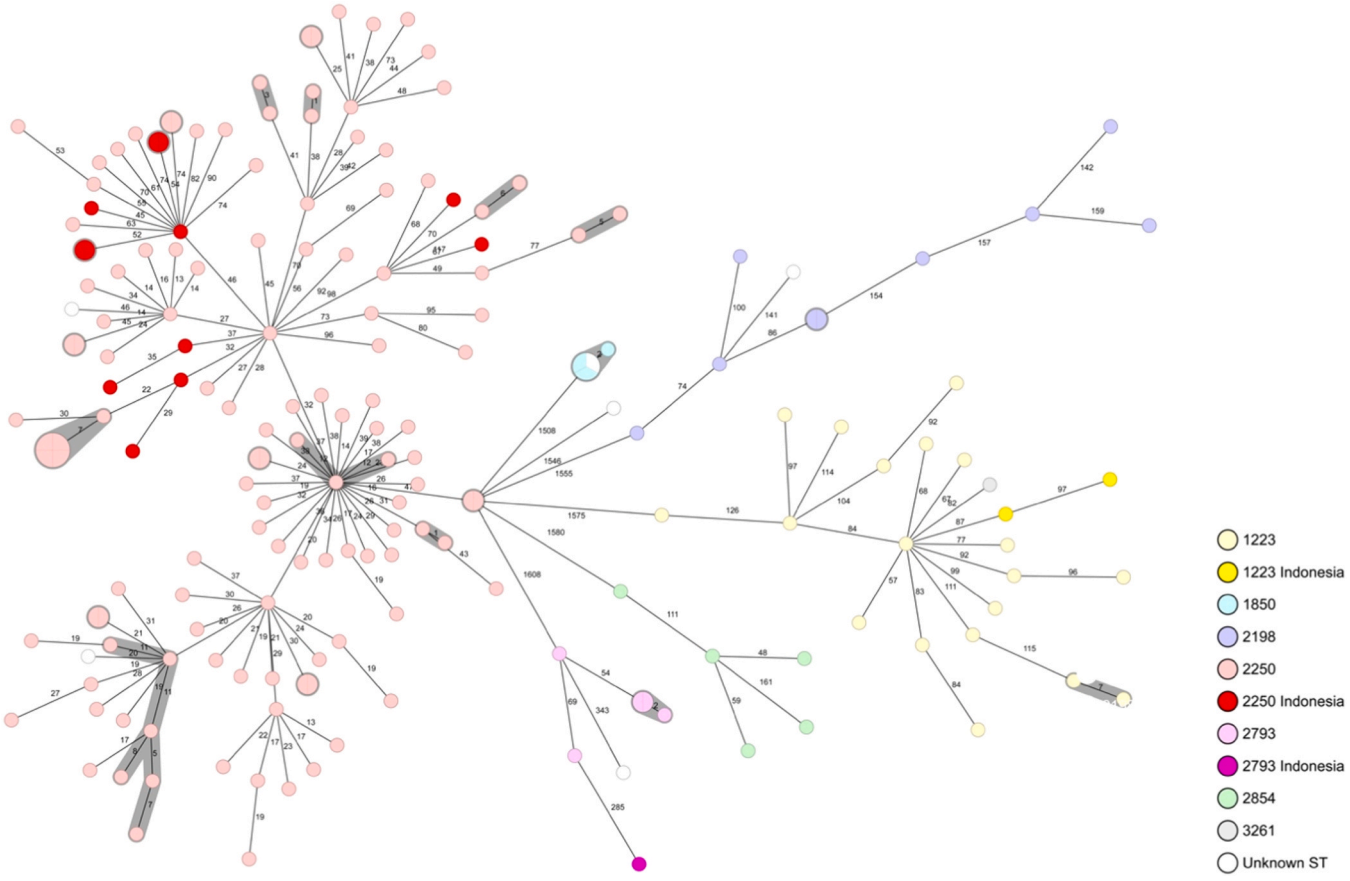

Fig. 2. Minimum spanning tree of *S. argenteus* based on cgMLST analysis. Darker colors indicate *S. argenteus* isolates from Indonesia. The size of the circle corresponds to the number of isolates of the same genotype. Numbers on connecting lines indicate the number of different alleles between the genotypes (pairwise ignoring missing values). Isolates connected by a grey background are considered to be related genotypes based on a provisional cut-off 12 differences.

The majority of the isolates carried both *lukD* and *lukE* genes which are also present in *S. aureus* (Morinaga, Kaihou, and Noda, 2003). The plasmid replicon types identified in our isolates are also known to *S. aureus* (McCarthy and Lindsay, 2012). These findings showed that both *S. argenteus* and *S. aureus* share similar toxins and plasmid replicons.

Currently, confirmation of *S. argenteus* identification typically relies on sequencing. However, where sequencing is not available, phenotypic testing followed by screening biochemicals test for Gram positive bacteria using Vitek®2 can be utilized. In Indonesia, the identification of *S. argenteus* is feasible without sequencing, as Vitek®2 is commonly used in routine diagnostic, and (real-time) PCR testing has become widely available in most laboratories following the COVID-19 pandemic.

4.1. Strengths and limitations of the study

This study marks the first identification of *S. argenteus* in Indonesia using a robust identification method combining MALDI-TOF MS and PCR, further confirmed by sequencing. These methodologies allowed for accurate identification of *S. argenteus*. However, our study encountered certain limitations. One limitation was the small sample size, which was followed by the unavailability of denominator data, resulting in a lack of information on the prevalence of *S. argenteus*. Another limitation was the absence of metadata, which prevented the tracing of the clinical impact and transmission pattern related to *S. argenteus*.

5. Conclusions

Our study reveals the occurrence of *S. argenteus* in Indonesia among *S. aureus* isolates. The genotypic diversity observed within Indonesian *S. argenteus* corresponds to the global diversity of *S. argenteus*. Identical isolates among patients indicate potential transmission. The lower prevalence of virulence factors suggests that *S. argenteus* is less virulent than *S. aureus*. Plasmid replicon types were known to *S. aureus*. For Indonesia, the identification of *S. argenteus* can be performed using Vitek®2, followed by PCR in the absence of MALDI-TOF. The clinical impact of the identification of *S. argenteus* is still unclear, however, our results contribute to better understanding of *S. argenteus*.

Ethics approval and consent to participate

Ethical approval was obtained from medical ethics committee of Faculty of Medicine, Brawijaya University, Malang, Indonesia (the ethical approval was not assigned by a number).

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CRediT authorship contribution statement

Corne H.W Klaassen: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Juliëtte Severin:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Indri Rooslamiati Supriadi:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dewi Santosaningsih:** Writing – review & editing, Investigation. **Nyoman S Budayanti:** Writing – review & editing, Investigation. **Willemien H. A Zandijk:** Investigation, Formal analysis. **Amber Amber Rijfkogel:** Investigation, Formal analysis.

Data availability

Data will be made available on request. The datasets generated and/or analyzed during the current study are publicly available upon request to the corresponding author. Sequence data from the isolates in this study have been deposited in the SRA archive and are available under BioProject nr PRJNA1101927 and BioSample accession nrs SAMN41006555 through SAMR41006569.

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Consent for publication

Not applicable

Competing interest

All authors declare that they have no competing interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ijmm.2024.151629.

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