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Phenotypic and Genomic Profiling of *Staphylococcus argenteus* in Canada and the United States and Recommendations for Clinical Result Reporting

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ABSTRACT *Staphylococcus argenteus* is a newly described species, formerly known as *S. aureus* clonal complex 75 (CC75). Here, we describe the largest collection of *S. argenteus* isolates in North America, highlighting identification challenges. We present phenotypic and genomic characteristics and provide recommendations for clinical reporting. Between 2017 and 2019, 22 isolates of *S. argenteus* were received at 2 large reference laboratories for identification. Identification with routine methods (biochemical, matrix-assisted laser desorption ionization–time of flight mass spectrometry [MALDI-TOF MS], 16S rRNA gene analysis) proved challenging to confidently distinguish these isolates from *S. aureus*. Whole-genome sequencing analysis was employed to confirm identifications. Using several different sequence-based analyses, all clinical isolates under investigation were confirmed to be *S. argenteus* with clear differentiation from *S. aureus*. Seven of 22 isolates were recovered from sterile sites, 11 from nonsterile sites, and 4 from surveillance screens. While sequence types ST1223/*coa* type XV, ST2198/*coa* type XIV, and ST2793/*coa* type XId were identified among the Canadian isolates, the majority of isolates (73%) belonged to multilocus sequence types (MLST) ST2250/*coa* type XId and exhibited a high degree of homology at the genomic level. Despite this similarity, 5 *spa* types were identified among ST2250 isolates, demonstrating some diversity between strains. Several isolates carried *mecA*, as well as other resistance and virulence determinants (e.g., PVL, TSST-1) commonly associated with *S. aureus*. Based on our findings, the growing body of literature on *S. argenteus*, the potential severity of infections, and possible confusion associated with reporting, including use of incorrect breakpoints for susceptibility results, we make recommendations for clinical laboratories regarding this organism.

KEYWORDS *Staphylococcus aureus* clonal complex, *Staphylococcus argenteus*, whole-genome sequencing, sequence types, MALDI-TOF MS, bacterial identification, *mecA*

Staphylococcus aureus, including methicillin-resistant *S. aureus* (MRSA), is one of the best-studied and -described bacterial pathogens. It is well known to colonize humans and animals and to cause a range of skin and soft tissue infections, severe invasive diseases, and toxin-mediated illnesses (1). The rise and dissemination of MRSA is a major global antimicrobial resistance threat with significant clinical and infection prevention and control (IPAC) implications (2). To properly support clinical decision making and IPAC interventions, timely and accurate identification of *S. aureus*/MRSA in the clinical laboratory is imperative.

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There are many clonal complexes and sequence types of *S. aureus* which are often used to describe the molecular epidemiology of this pathogen. In 2015, results from investigations into two staphylococcal species which were almost identical to *S. aureus* phenotypically and by 16S rRNA gene sequences gave rise to the formal descriptions of *Staphylococcus argenteus* sp. nov., formerly known as *S. aureus* clonal complex 75 (CC75), and *Staphylococcus schweitzeri* sp. nov. (3, 4). *S. argenteus* and *S. schweitzeri* each have distinct multilocus sequence types (MLST), and genomic sequence analysis clearly demonstrates that they are phylogenetically divergent from *S. aureus*, displaying less than 95% average nucleotide identity (ANI) with one another and a predicted DNA-DNA hybridization of less than 70% between the species (3, 5–8). *S. argenteus* and *S. schweitzeri* have distinct spectral profiles on some research applications and/or databases of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), as well as unique cellular fatty acid signatures compared to those of *S. aureus*. While *S. aureus*, *S. argenteus*, and *S. schweitzeri* have nearly identical 16S rRNA gene sequences to one another, there are considerable differences in *nuc*, which is important given that some of these differences occur in areas of the gene often used as primer binding sites in PCR assays designed to detect *S. aureus* (3, 8–10).

S. schweitzeri is considered a zoonotic agent primarily associated with fruit bats (*Eidolon helvum*) and monkeys (*Cercopithecus ascanius*) in Africa (4, 11); that no human clinical cases have been reported to date suggests that this organism may inhabit a separate ecological niche from that of *S. aureus*.

In contrast, *S. argenteus* has now been established as an effective colonizer and pathogen of humans (6, 12–20). Initial reports of *S. argenteus*, both methicillin-susceptible and methicillin-resistant, were from Australia, the Pacific Islands, and Thailand and were thought to be geographically restricted (3, 5, 6, 13, 21–23). Based on early clinical observations, *S. argenteus* was considered less likely to cause nosocomial infections than *S. aureus*, appearing to be predominantly associated with community-onset superficial skin lesions (21). Based on this observation and murine studies, and because *S. argenteus* lacked some putative *S. aureus* virulence genes such as staphyloxanthin, the initial assessment was that *S. argenteus* was less virulent than *S. aureus* (7, 21, 24).

Awareness of *S. argenteus* was heightened after the formal description of the species as well as with the addition of the *S. argenteus* and *S. schweitzeri* spectra to the database of one commonly used commercial MALDI-TOF MS system (RUO database 2018, V8.0, 7854 Bruker; Bruker Daltonics). In the last few years, increased numbers of clinical reports and descriptions of *S. argenteus* resulted in increased attention on this organism and a recognition of a possible global distribution (12, 14–16, 25–30). It is now also evident that *S. argenteus* can cause serious invasive disease, including bacteremia, bone and joint infection, and purulent lymphadenitis, and has been directly linked to patient deaths (13, 16, 18, 19, 30). Like its relative *S. aureus*, *S. argenteus* has been shown to cause toxin-mediated foodborne illnesses (15, 20). In addition to community-associated spread, reports have documented nosocomial spread of *S. argenteus* (13, 24). Recent studies have also described strains of *S. argenteus* harboring traditional *S. aureus* virulence factors such as Panton-Valentine leukocidin (PVL), enterotoxins, and toxic shock syndrome toxin-1 (TSST-1), among others (12, 14, 20, 25, 28, 31, 32).

Considering the clinical and IPAC significance of *S. argenteus*, this organism should be correctly identified so that cases are not missed. For greater understanding of clinical, microbiological, and epidemiologic similarities and differences between *S. argenteus* and *S. aureus*, the two species should be distinguished. Despite the addition of *S. argenteus* spectra to at least one commercial MALDI-TOF MS database, confirmation of identification of and distinction from *S. aureus* and *S. schweitzeri* in the clinical laboratory is not straightforward. Laboratories may be identifying *S. argenteus* as *S. aureus*, as these species share equivalent reactions to most key biochemical tests traditionally used for characterization, such as catalase and tube coagulase positivity and beta-hemolysis on blood agar (3). One exception is that while *S. aureus* is often (but not exclusively) golden in color, *S. argenteus* colonies have been found to be nonpigmented,

appearing white/silver due to the absence of the staphyloxanthin gene cluster (*crtOPQMN*) which encodes carotenoid pigment (7). As mentioned previously, 16S rRNA gene sequence analysis is not able to discriminate between *S. argenteus*, *S. aureus*, and *S. schweitzeri*.

Here, our objective was to verify the identifications of *S. argenteus* identified by MALDI-TOF MS and characterize the isolates. Using several identification methods, including whole-genome sequencing (WGS), we describe initial isolates of *S. argenteus* received at two large North American microbiology reference laboratories, one in Canada and one in the United States. The description of the microbiological characteristics, including antimicrobial susceptibility profiles, of these North American isolates as well as basic clinical details of the patients contributes to a growing understanding of the epidemiology and the clinical presentations of this organism. Based on our laboratory experience as well as the growing body of literature on the clinical and IPAC significance of *S. argenteus*, we provide recommendations as to how to report this organism in the clinical laboratory, addressing breakpoint challenges with *S. argenteus*.

MATERIALS AND METHODS

Specimen collections. This study involved a collection of 22 clinical isolates of *S. argenteus* received between 2017 and 2019 by two large North American reference laboratories (Public Health Ontario [PHO], Toronto, ON, Canada [$n = 16$] and Mayo Clinic, Rochester, Minnesota, USA [$n = 6$]), along with strains *S. argenteus* DSM 28299^T/MSHR1132^T and *S. schweitzeri* DSM 28300^T/FSA084^T. Clinical isolates were received at the reference laboratories for identification and antibiotic susceptibility testing. Routine identification methods for *Staphylococcus* and related species were conducted, including tube coagulase, pyrrolidonyl aminopeptidase test (PYR), bacitracin disk, and MALDI-TOF MS (Bruker, BioTyper, databases V7.0 [7311] used 2017, V8.0 [7854] used 2018, and V9.0 [8468] used 2019) (Table 1). Of note, BioTyper RUO database V7.0 (7311) did not include *S. argenteus* or *S. schweitzeri* for which spectra were added in the V8.0 (7854) update. For closely related organisms, MALDI-TOF MS (Bruker, BioTyper) can occasionally result in multiple species scoring at or above the cutoff of >2.0 (confident to the species level). Different approaches were employed by each laboratory to avoid potential misidentifications that can occur as a result of reporting only the top-scoring identification by MALDI-TOF MS. At PHO, 16S rRNA gene PCR and sequence analysis were performed using universal primer pair 8FPL and 806R (33). 16S rRNA gene sequences were subjected to a BLAST search against the NCBI GenBank type strain and open nucleotide databases (34) with interpretation criteria described in the Clinical and Laboratory Standards Institute (CLSI) document MM18-A used to identify the isolates (35). At Mayo Clinic, if multiple species had scores of ≥2.0, a score separation of at least 10% was required between the top match and additional species in order to report the top-scoring organism to the species level. If a ≥10% score separation was not observed, organisms were only reported to the genus level. This 10% differential rule, or separation rule, has been used by other groups to aid in differentiating closely related organisms (36, 37).

The *S. argenteus* strain DSM 28299^T (MSHR1132^T) and the *S. schweitzeri* strain DSM 28300^T (FSA084^T), inoculated at Mayo Clinic, were used as controls. All isolates were frozen and maintained at −80°C.

Basic characteristics of the patients, including sex, age range, month and year of collection, geographic region, and specimen type, were recorded (Table 1). This work was approved by the PHO Research Office's Ethics Review Board, and a Privacy Impact Assessment was completed. This work was also approved by Mayo Clinic's Institutional Review Board.

Antimicrobial susceptibility testing and determination of MICs. Antimicrobial susceptibility testing was performed by agar dilution according to CLSI guidelines on all isolates to determine MICs (Table 2) (38). D-test for inducible clindamycin resistance and penicillin zone edge test (inducible beta-lactamase) were also performed in accordance with CLSI M100 recommendations (39). Cefoxitin disk (30 μg) diffusion and a PCR test to detect *mecA* (with *nuc* as a positive control for *S. aureus*) were also performed (10, 39).

Whole-genome sequencing. Genomic DNA from colonies were extracted and purified using a QiaAmp DNA minikit (Qiagen, Valencia, CA) or Zymo Research Quick-DNA Fungal/Bacterial MiniPrep kit (Zymo Research Corp., CA) from an overnight culture grown aerobically on sheep blood agar according to the manufacturer's protocol. The quality of isolated DNA was analyzed using gel electrophoresis and quantified using Qubit 2.0 (Invitrogen, Waltham, MA) fluorometer.

DNA libraries were prepared and multiplexed with a unique combination of two indexes of the Nextera XT index kit (Illumina, San Diego, CA). The sequencing library was quantified using Qubit 2.0 (Invitrogen, Waltham, MA) and qualified by Bioanalyzer (Agilent Technologies, Richardson, TX). The library was normalized and pulled together aiming for an average coverage of 100× and sequenced on the Illumina MiSeq platform, using Illumina MiSeq reagent kit v2 (2 × 150 bp), according to the manufacturer's instructions.

Genome assembly. FastQ files were imported into CLC Genomics Workbench version 8.0.1 (CLC bio, Germantown, MD, USA). Raw reads were trimmed to remove Nextera transposase adapter sequences and assembled using *de novo* assembler. Following *de novo* assembly, the largest contig was subjected

TABLE 1 Initial characteristics

Study no.	Date of isolation (mo-yr)	Region	Submitter	Site of isolation	Patient sex	Age range	Tube coagulase	PYR	Bacitracin disk	Cefoxitin 30 μg disk (mm zone)[a]	*mecA* PCR[b]	*nuc* PCR[b]	MALDI-TOF MS ID ≥2.0[c,d]	16S rRNA ≥99%[e]
PHL3431	10-2017	Ontario, Canada	Site 1	Rectal	M	≥75	+	−	+	13	+	−	*S. aureus*	ND[f]
PHL3432	10-2017	Ontario, Canada	Site 1	Nose	F	≥75	+	−	+	10	+	−	*S. aureus*	ND
PHL3433	10-2017	Ontario, Canada	Site 1	Nose	M	≥75	+	−	+	11	+	−	*S. aureus*	ND
PHL5740	10-2018	Ontario, Canada	Site 2	Eye wound	M	55–64	+	−	+	30	−	−	*S. aureus/S. argenteus/ S. schweitzeri*	*S. aureus/S. argenteus/S. haemolyticus/S. simiae*
PHL6344	11-2018	Ontario, Canada	Site 2	Cheek	F	≥75	+	−	+	28	−	−	*S. argenteus/S. schweitzeri/ S. aureus*	*S. aureus/S. argenteus/S. haemolyticus/S. simiae*
PHL8605	12-2018	Ontario, Canada	Site 2	Wound	M	≥75	+	−	+	29	−	−	*S. argenteus*	*S. aureus/S. argenteus/S. haemolyticus*
PHL3446	01-2019	Ontario, Canada	Site 3	Toe tissue	M	45–54	+	−	+	29	−	−	*S. argenteus/S. schweitzeri/ S. aureus*	*S. aureus/S. argenteus/S. haemolyticus*
PHL2420	01-2019	Ontario, Canada	Site 2	Stump wound	M	65–74	+	−	+	29	−	−	*S. argenteus/S. schweitzeri/ S. aureus*	*S. aureus/S. argenteus/S. haemolyticus*
PHL6318	01-2019	Ontario, Canada	Site 3	Sternal wound	M	55–64	+	−	+	26	−	−	*S. argenteus/S. schweitzeri/ S. aureus*	*S. aureus/S. argenteus/S. haemolyticus/S. simiae*
PHL1144	02-2019	Ontario, Canada	Site 1	Rectal & wound swab	F	0–1	+	−	+	28	−	−	*S. argenteus*	*S. aureus/S. argenteus/S. haemolyticus/S. simiae*
PHL2411	03-2019	Ontario, Canada	Site 2	Tendon tissue	M	45–54	+	−	+	30	−	−	*S. argenteus/S. schweitzeri*	*S. aureus/S. argenteus/S. haemolyticus/S. simiae*
PHL8642	03-2019	Ontario, Canada	Site 4	Eye	M	NA	+	−	+	31	−	−	*S. argenteus/S. aureus*	*S. aureus/S. argenteus/S. haemolyticus/S. simiae*
PHL4815	03-2019	Ontario, Canada	Site 5	Head wound	M	35–44	+	−	+	26	−	−	*S. argenteus/S. schweitzeri*	*S. aureus/S. argenteus/S. haemolyticus*
PHL4226	11-2019	Ontario, Canada	Site 6	Blood	M	45–54	+	−	+	30	−	−	*S. argenteus*	*S. aureus/S. schweitzeri/S. argenteus/S. haemolyticus*
PHL4313	11-2019	Ontario, Canada	Site 6	Synovial fluid	M	55–64	+	−	+	31	−	−	*S. argenteus/S. schweitzeri/ S. aureus*	*S. argenteus/S. aureus/S. schweitzeri/S. haemolyticus*
PHL4553	11-2019	Ontario, Canada	Site 2	Blood	F	45–54	+	−	+	32	−	−	*S. argenteus/S. schweitzeri/ S. aureus*	*S. argenteus/S. aureus/S. schweitzeri/S. haemolyticus*
WU1	08-2019	Missouri, USA	Site 7	Foot tissue	F	55–64	+	−	NA[g]	25	−	−	*S. aureus/S. schweitzeri/ S. argenteus*	ND
WU2	03-2019	Missouri, USA	Site 7	Buttocks wound	M	15–24	+	−	NA	25	−	−	*S. schweitzeri/S. argenteus/ S. aureus*	ND
WU3	12-2018	Missouri, USA	Site 7	Deep throat swab (cystic fibrosis)	M	15–24	+	−	NA	25	−	−	*S. argenteus*	*S. argenteus/S. aureus*
MC2	02-2019	Minnesota, USA	Site 8	Urine	M	2–14	+	−	NA	25	−	−	*S. argenteus/S. aureus*	ND
MC3	03-2019	Missouri, USA	Site 7	Buttocks wound	M	45–54	+	−	NA	25	−	−	*S. aureus*	ND
MC4	08-2019	Colorado, USA	Site 9	Blood	M	2–14	+	−	NA	24	−	−	*S. argenteus*	ND
DSM 28299[T] *S. argenteus*	2006	Northern Territory, Australia	Type strain	Blood	F	55	+	−	NA	12	+	−	*S. argenteus/S. schweitzeri/ S. aureus*[h]	ND
DSM 28300[T] *S. schweitzeri*	2010	Moyen-Ogooué, Gabon	Type strain	Nasal swab (monkey)	NA	NA	+	−	NA	26	−	−	*S. schweitzeri*[h]	ND

[a]For *S. aureus*, cefoxitin disk (30 μg disk content) zone diameter breakpoints ≥22 mm susceptible and ≤21 mm resistant; for other *Staphylococcus* spp. (excluding *S. aureus*, *S. lugdunensis*, *S. pseudintermedius*, *S. schleiferi*, *S. epidermidis*), zone diameter breakpoints ≥25 mm susceptible and ≤24 mm resistant (CLSI M100 2020).
[b]qPCR assay by Pichon et al. (10).
[c]Bruker MALDI BioTyper, databases: 2017, V7.0 (7,311 entries); 2018, V8.0 (7,854 entries), and 2019, V9.0 (8,468 entries). The identifications presented are those at the initial time of testing with the database that was in use at the time.
[d]Matches presented by descending score (top hit listed first).
[e]Partial 16S rRNA gene sequence (~700 bp) BLAST NCBI type strain database, presented by descending max score.
[f]Not done.
[g]Not available.
[h]Results with Bruker MALDI BioTyper database V9.0 (8,468 entries).

to NCBI BLAST to select the closest matching complete *S. argenteus* genome in GenBank to serve as the reference for reference-based assembly using CLC Genomic Workbench 8.5.3 (QIAGEN Digital Insights).

Genome analysis. The assembly was annotated using the RAST server (http://rast.nmpdr.org) (40) for gene prediction and annotation. Genome sequencing data of each isolate was assessed for *in silico* identification of MLST, SCC*mec*, and plasmid replicon and resistance genes using online tools such as CARD (41), MLST-1.8 server (42), ResFinder 2.1 (43), and SCC*mec*Finder 1.2 server (44) available by Center for Genomic Epidemiology (http://genomicepidemiology.org). Virulence factors (VFs) were identified by using VFanalyzer of the virulence factor database (VFDB) (http://mgc.ac.cn/VFs/) (45). To identify plasmids, the genome assemblies were screened using PlasmidFinder database for plasmid replicon (*rep*) genes (46).

Single nucleotide variant (SNV) analysis was conducted using a custom pipeline. Briefly, reads for all isolates were mapped against the chromosome of the strain MSHR1132^T (DSM 28299^T) available in GenBank (accession number NC_016941.1) for reference using SMALT software v 0.7.6 (https://www.sanger.ac.uk/tool/smalt-0/). Single nucleotide polymorphism (SNP) calling was performed using Freebayes with min-base-quality 30, min-mapping-quality 30, min-alternate-fraction 0.75, read-snp-limit 10, and min-coverage 15 (47). Additional variant confirmation was done using the SAMtools mpileup tool (48). Repetitive regions were removed by using MUMmer (49). The meta-alignment of core informative positions (SNVs) was used to create a maximum likelihood (ML) tree using MEGA 6 (50).

A whole-genome phylogenomic approach was performed by uploading the assembled sequences to Type Strain Genome Server (TYGS; https://tygs.dsmz.de) for a whole-genome-based phylogenetic analysis of isolates to the most closely related genome database (51).

ANI was calculated using EZBioCloud ANI calculator (52) between assembled genomes obtained in this study and publicly available genomes of *S. argenteus* MSHR1132^T (accession number NC_016941.1), *S. argenteus* strain XNO62 (accession number CP023076.1), *S. schweitzeri* NCTC 13712^T (accession number NZ_LR134304.1), and *S. aureus* NCTC 8325 (accession number NC_007795.1). An ANI threshold of ≥96% was considered to be the cutoff for species identification, which correlates to DNA-DNA hybridization studies (53). Whole-genome comparison was performed and visualized using the Gview tools (54).

***spa* typing, Sanger protocol.** All *S. argenteus* in this study were subjected for *spa* sequence-based typing using the previously published PCR primers (55). *spa* amplicons were sequenced with the same primers and analyzed using the *spa* type finder/identifier (http://spatyper.fortinbras.us) and the Ridom SpaServer (https://spaserver.ridom.de).

Data availability. WGS sequence data are available in the following databases. NCBI (https://www.ncbi.nlm.nih.gov/): accession numbers QQOV00000000 (PHL3431), PUXC00000000 (PHL3432), and QQOW00000000 (PHL3433). BioProject accession number PRJNA666697: WGS numbers JADANH000000000 (PHL6344), JADANG000000000 (PHL5740), JADANF000000000 (PHL3446), JADANE000000000 (PHL2420), JADAND000000000 (PHL6318), JADANC000000000 (PHL8605), JADANB000000000 (PHL2411), JADANA000000000 (PHL1144), JADAMZ000000000 (PHL4815), JADAMY000000000 (PHL8642), JADAMX000000000 (PHL4226), JADAMW000000000 (PHL4313), JADAMV000000000 (PHL4553), JADAMU000000000 (DSM 28299^T), JADAMT000000000 (DSM 28300^T), JADAMS000000000 (MC2), JADAMQ000000000 (MC3), JADAMP000000000 (MC4), JADAMO000000000 (WU1), JADAMN000000000 (WU2), and JADAMR000000000 (WU3).

RESULTS

Initial description of isolates. In total, 22 isolates were received at the PHO laboratory (n = 16) and Mayo Clinic (n = 6) for confirmation of identification of *S. aureus*, MRSA, or *S. argenteus* from 2017 to 2019 (Table 1). All isolates were tube coagulase positive and PYR negative.

The initial 3 *S. argenteus* isolates arrived at the PHO laboratory in 2017, as MRSA isolates (PHL3431, PHL3432, and PHL3433) for confirmation of identification. At the time, MALDI-TOF MS provided confident identifications of *S. aureus* (≥2.0), with positive tube coagulase and PYR reactions; of note, the BioTyper RUO database V7.0 (7311) used at the time did not include *S. argenteus* or *S. schweitzeri*. To determine the presence of *mecA* and confirm the identification of MRSA, these isolates were run on an in-house *mecA* PCR assay (10). All three isolates were positive for *mecA*; however, the positive control for *S. aureus*, *nuc*, was negative (Table 1). These results prompted further investigation of the identification of the organisms, and they were referred for WGS analysis, which determined that they were *S. argenteus* (details below).

In 2018, the Bruker BioTyper RUO database was updated to include spectra for *S. argenteus* and *S. schweitzeri* for the first time (RUO databases V8.0 [7854 MSP] and V9.0 [8468]), after which both reference laboratories were technically able to use MALDI-TOF MS to identify *S. argenteus* and *S. schweitzeri*. When the single *S. schweitzeri* strain available in this study (DSM 28300^T) was tested by MALDI-TOF MS using the new databases, it resulted in a single identification of ≥2.0 of *S. schweitzeri*; of note, this same strain was used to produce the spectrum in the library (Table 1). However, use of the

TABLE 2 Antibiotic susceptibility test results (phenotypic and genomic)

Isolate no.	MIC (μg/ml)[a]																							
	Ampicillin	Cefazolin	Ciprofloxacin	Clindamycin	Erythromycin	Gentamicin	Linezolid	Penicillin	Tetracycline	Trimethoprim-sulfamethoxazole	Vancomycin	Ceftaroline	Levofloxacin	Mupirocin	Minocycline	Nitrofurantoin	Rifampin	Doxycycline	Delafloxacin	Oxacillin	D-test	Penicillin zone edge test (inducible beta-lactamase)	CARD resistance database (genes)
PHL3431	≥**16**[b]	≥32	≥4	≤0.5	≤0.5	≤4	≤2	**8**	≤4	≤2/38	1	1	≤1	≤256	≤4	≤32	≤0.5	≤4	0.12	>2	−	NA[c]	*mgrA, arlR, mepR, lmrS, fosB, mecA*
PHL3432	≥**16**	≥32	≥4	≤0.5	≤0.5	≤4	≤2	**8**	≤4	≤2/38	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	*mgrA, arlR, mepR, lmrS, fosB, mecA*
PHL3433	≥**16**	≥32	≥4	≤0.5	≤0.5	≤4	≤2	**8**	≤4	≤2/38	1	0.5	1	≤256	≤4	≤32	≤0.5	≤4	0.12	>2	−	NA	*mgrA, arlR, mepR, lmrS, fosB, mecA*
PHL5740	≤8	≤8	≤1	≤0.5	≤0.5	≤4	≤2	**4**	≤4	≤2/38	1	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	1	−	NA	*mgrA, arlR, mepR, lmrS, fosB, blaZ*
PHL6344	≤8	≤8	≤1	≤0.5	≤0.5	≤4	≤2	**4**	≤4	≤2/38	1	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	1	−	NA	*mgrA, arlR, mepR, lmrS, fosB, blaZ*
PHL8605	≤8	≤8	≤1	≤0.5	≤0.5	≤4	≤2	≤0.12	≤4	≤2/38	1	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	−	*mgrA, arlR, mepR, lmrS, fosB*
PHL3446	≤8	≤8	≤1	≤0.5	≤0.5	≤4	≤2	≤0.12	≤4	≤2/38	1	≤0.12	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	−	*mgrA, arlR, mepR, lmrS, fosB*
PHL2420	≤8	≤8	≤1	≤0.5	≤0.5	≤4	≤2	≤0.12	≤4	≤2/38	1	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	−	*mgrA, arlR, mepR, lmrS, fosB*
PHL6318	≤8	≤8	≤1	≤0.5	≤0.5	≤4	≤2	≤0.12	≤4	≤2/38	1	≤0.12	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	NA	*mgrA, arlR, mepR, lmrS, fosB*
PHL1144	≤8	≤8	≤1	≤0.5	≤0.5	≥**16**	≤2	**4**	≤4	≤2/38	1	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	1	−	NA	*mgrA, arlR, mepR, lmrS, fosB, blaZ, AAC(6′)-Ie-APH(2′′)-Ia*
PHL2411	≤8	≤8	≤1	≤0.5	≤0.5	≤4	≤2	≤0.12	≤4	≤2/38	1	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	−	*mgrA, arlR, mepR, lmrS*
PHL8642	≤8	≤8	≤1	≤0.5	≤0.5	≤4	≤2	0.5	≤4	≤2/38	1	≤0.12	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	NA	*mgrA, arlR, mepR, lmrS, blaZ*
PHL4815	≤8	≤8	≤1	≤0.5	≤0.5	≤4	≤2	≤0.12	≤4	≤2/38	1	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	NA	*mgrA, arlR, mepR, lmrS, fosB*
PHL4226	≤8	≤8	≤1	≤0.5	≤0.5	≤4	≤2	≤0.12	≤4	≤2/38	1	0.25	≤0.5	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25			*mgrA, arlR, mepR, lmrS, fosB*
PHL4313	≤8	≤8	≤1	≤0.5	≤0.5	≤4	≤2	**4**	≤4	≤2/38	1	0.25	≤0.5	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25		NA	*mgrA, arlR, mepR, fosB, lmrS, blaZ*
PHL4553	≤8	≤8	≤1	≤0.5	≤0.5	≤4	≤2	**0.5**	≤4	≤2/38	1	0.25	≤0.5	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25		NA	*mgrA, arlR, lmrS, blaZ*
WU1	NA	NA	NA	≤0.5	≤0.5	≤1	≤2	≤0.12	≤4	≤2/38	2	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	−	*mgrA, arlR, mepR, lmrS, fosB*
WU2	NA	NA	NA	≤0.5	≤0.5	≤1	≤2	≤0.12	≤4	≤2/38	2	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	−	*mgrA, arlR, mepR, lmrS, fosB*
WU3	NA	NA	NA	≤0.5	≤0.5	≤1	≤2	≤0.12	≤4	≤2/38	2	≤0.12	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	−	*mgrA, arlR, mepR, lmrS, fosB*
MC2	NA	NA	NA	≤0.5	≤0.5	≤1	≤2	**4**	≤4	≤2/38	2	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	1	−	NA	*mgrA, arlR, mepR, lmrS, fosB, blaZ, fusB*
MC3	NA	NA	NA	≤0.5	≤0.5	≤1	≤2	≤0.12	≤4	≤2/38	2	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	−	*mgrA, arlR, mepR, lmrS, fosB*
MC4	NA	NA	NA	≤0.5	≤0.5	≤1	≤2	≤0.12	≤4	≤2/38	1	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	−	*mgrA, arlR, mepR, mexT, lmrS, fosB*
S. argenteus DSM 28299^T	NA	NA	NA	≤0.5	≤0.5	≤1	≤2	**8**	≤4	≤2/38	1	0.5	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	>2	−	+	*mgrA, arlR, mepR, lmrS, fosB, mecA, blaZ*
S. schweitzeri DSM 28300^T	NA	NA	NA	≤0.5	≤0.5	≤1	≤2	≤0.12	≤4	≤2/38	1	0.25	≤1	≤256	≤4	≤32	≤0.5	≤4	≤0.008	≤0.25	−	−	*mgrA, arlR, mepR, lmrS, murA* with mutation

[a]MICs were obtained by CLSI agar dilution method, with the exception of delafloxacin for which agar dilution was performed on all isolates at Mayo Clinic and D-test was performed on all isolates at Mayo Clinic by the disk diffusion method.

[b]Bold, underlined MICs indicate resistance according to CLSI M100 (39) using *S. aureus* breakpoints.

[c]NA, not available.

updated databases challenged with *S. argenteus* typically resulted in multiple species (*S. aureus*, *S. argenteus*, and *S. schweitzeri*) with high (≥2.0) scores (Table 1), resulting in an inability to confidently identify the organism to species level. This prompted additional testing to confirm identification, including attempts at using 16S rRNA gene sequence analysis.

Partial 16S rRNA gene PCR and sequence analysis of these isolates were not able to reliably differentiate between *S. aureus*, *S. argenteus*, and *S. schweitzeri* as well as a strain of *Staphylococcus haemolyticus* (accession number Z26896.1); all had ≥99.0% homology to reference strain deposits within NCBI GenBank for these species (Table 1). Of note, *S. haemolyticus* could be ruled out as it is coagulase negative, and Z26896.1 was likely deposited into NCBI incorrectly.

Isolates were also tested with the in-house *nuc* quantitative PCR (qPCR) in which the primers are specific to *S. aureus* (10) (the *nuc* target is used as a control for *S. aureus* in a *mecA* PCR assay). All isolates of *S. argenteus* for which the described *nuc* qPCR assay was run were negative (Table 1), while control isolates of *S. aureus* were positive (data not shown). While this result is evidence that the isolates under investigation were not *S. aureus*, the negative results of this assay do not specifically identify *S. argenteus* and cannot rule out *S. schweitzeri*. The lack of definitive identification prompted the use of WGS.

Patient demographics and specimen descriptions. *S. argenteus* isolates were cultured from patients in Ontario, Canada ($n = 16$), Missouri ($n = 4$), Minnesota ($n = 1$), or Colorado ($n = 1$) and identified between October 2017 and November 2019. Detailed patient information for these isolates was not available. Of the 22 isolates described, 7 were from sterile sites, 11 from nonsterile sites, and 4 from surveillance screens. Seventy-seven percent ($n = 17$) were recovered from males and 23% ($n = 5$) from females. Ages of patients ranged from <1 to ≥75 years (Table 1).

Phenotypic antimicrobial susceptibility. MICs for all isolates and antibiotics tested are presented in Table 2. All *S. argenteus* identified in this collection demonstrated low MICs to clindamycin, erythromycin, linezolid, trimethoprim-sulfamethoxazole, and vancomycin and would be considered susceptible using *S. aureus* breakpoints (39). Ten of 22 (45.4%) isolates were resistant to penicillin (MIC range 0.5 to 8 μg/ml); 3 of these isolates (PHL3431, PHL3432, PHL3433) were also *mecA* PCR positive, demonstrating oxacillin MICs of >2 μg/ml (PHL3431, PHL3433) and resistance to ciprofloxacin. *S. argenteus* isolates with penicillin MICs of ≤0.12 μg/ml were negative by the penicillin zone edge test. One isolate (PHL1144) was resistant to gentamicin with an MIC of 16 μg/ml.

As there are different oxacillin breakpoints for different *Staphylococcus* species (39), we evaluated multiple species-specific breakpoints for oxacillin susceptibility testing. Using *S. aureus* breakpoints, 3 of 21 isolates (14.3%) would be considered oxacillin resistant, while when using the "other *Staphylococcus* species" breakpoints, 7 of 21 (33.3%) would be considered resistant. All *S. argenteus* isolates identified as oxacillin resistant using *S. aureus* breakpoints harboring *mecA* (as determined by *mecA*-specific qPCR as well as WGS analysis). PHL3432 was identified as being *mecA* positive but was not subjected to oxacillin susceptibility testing due to strain loss.

Genome features and comparative genomics. *De novo* assembly of sequence reads using CLC Genomics Workbench resulted in 1,610,728 to 7,425,408 bp. Between 12 and 315 contigs were obtained, with the largest contig size of 1,088,191 bp length and average coverage of 153×. The N_{50} values of assemblies were 23,043 to 693,825 bp.

The sequences of all 23 *S. argenteus* (22 clinical isolates and 1 type strain, DSM 28299^T) and 1 *S. schweitzeri* genome (DSM 28300^T) were assembled by mapping short reads to the reference sequence XNO62 (accession number CP023076.1). Assembled genomes ranged from 2,599,989 to 2,724,271 bp in size with a GC content of 32.42%. Genome annotation by RAST showed an average of 2,665 (median 2,603) coding sequences and average of 63 RNAs (tRNAs and rRNAs) among all *S. argenteus*. *De novo* assembly of 4,014,380 reads obtained for type strain *S. schweitzeri* (DSM 28300^T) resulted in 37 contigs between 200 and 343,130 bp; the assembled genome was

2,784,939 bp long with 305× coverage and N_{50} of 200,036. Using RAST annotation, 2,885 coding DNA sequences (CDSs) and 65 RNAs (tRNAs and rRNAs) were identified.

The clinical isolates' pairwise ANI values between isolates studied and *S. argenteus* XNO62 (accession number CP023076.1) were 98.70 to 99.96% and were 92.61 to 92.23% and 87.58 to 89.0% compared against *S. schweitzeri* and *S. aureus*, respectively (see Table S1 in the supplemental material).

A circular alignment of genomes from this study compared with reference genomes *S. argenteus* XNO62 and MSHR1132^T is presented in Fig. S1. This alignment, as well as other features such as sequence similarity and distribution of GC content, demonstrated a high level of homology among members of these groups.

The WGS data for all 22 clinical isolates were interrogated for MLST and other features commonly used for typing *S. aureus*. Four MLSTs were identified in the collection: ST2250 (16/22) predominated, followed by ST1223 (3/22), ST2198 (2/22), and ST2793 (1/22) (Fig. 1A). MLSTs for type strains of *S. argenteus* (DSM 28299^T) and *S. schweitzeri* (DSM 28300^T) were determined to be ST1850 and ST2022, respectively, as previous described (3). Using Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi) on extracted staphylocoagulase (*coa*) sequences from assemblies, three *coa* types were identified: XId, XV, and XIV, with *coa*-type XId being the predominant type (17/22) (Fig. 1A). We were unable to assign *spa* type from *in silico* whole-genome data due to the repetitive region span being greater than the read length of 150 bp. Using traditional endpoint PCR and Sanger sequencing, we were able to confirm presence of at least 8 *spa* types among clinical isolates of *S. argenteus* in this study. All *mecA*-positive *S. argenteus* identified in this study (3/22) were assigned the *spa* type t7960, while the majority of isolates were *spa* type t5078 (9/22). Other *spa* types identified include t10900 (1/22), t9385 (2/22), and 3 novel *spa* types, t19456 (1/22), t19457 (1/22), and t19497 (2/22). We were unable to assign a *spa* type to 3 isolates, all of which belonged to ST1223 (https://spa.ridom.de/spatypes.shtml) (Table S2). Strains DSM 28299^T and DSM 28300^T were t17252 and t6705, respectively.

Phylogenetic relationships of *S. argenteus* genomes were assessed using core genome SNV as well as whole-genome sequence analysis, with both methods yielding phylogenetic trees with similar topologies. Examination of clinical isolates as well as the strain DSM 28299^T resulted in 5 clusters of *S. argenteus* which coincided with the 5 MLSTs (WGS phylogenetic tree shown in Fig. 1, SNV tree not shown). The major cluster was comprised of 16 isolates (10 from Canada and 6 from the United States), all MLST 2250, with little variation in the core genome (0.2 to 0.3%).

The full lengths of *S. argenteus*, *S. aureus*, and *S. schweitzeri* 16S rRNA and *nuc* genes were extracted from the assemblies. As shown before by others, our results demonstrate that there is insufficient demarcation between the 16S rRNA gene of these three species to be used for species identification (Fig. S1). However, the *nuc* gene allows for clear separation of the three species (Fig. 2) (3, 8, 9).

Antibiotic resistance determinants identified through WGS analysis. A total of 9 antibiotic resistance genes were identified among the 22 *S. argenteus* clinical isolates; each isolate harbored multiple genes associated with resistance (Table 2). Genes identified include *mecA* (3/22), *mgrA* (*norR*) and *arlR* (both 22/22) (encode regulatory components for a major facilitator superfamily [MFS] antibiotic efflux pump associated with fluoroquinolone, tetracycline, penicillin, cephalosporin, and peptide antibiotic resistance) (56, 57), *mepR* (21/22) (encodes a repressor that helps to regulate expression of a multidrug and toxic compound extrusion [MATE] efflux pump [58, 59]), *blaZ* (7/22) (encodes class A β-lactamase) (60), *fosB* (18/22) (encodes a fosfomycin thiol transferase which inactivates fosfomycin) (61), *lmrS* (22/22) (encodes a component of an MFS efflux pump associated with resistance to several antibiotics, including macrolides, aminoglycosides, diaminopyrimidines, and oxazolidinones) (62), and *aac(6′)-Ie-aph(2′′)-Ia* (1/22) (encodes an aminoglycoside-modifying enzyme which inactivates aminoglycoside, including gentamicin) (63). As demonstrated in Table 2, many of these molecular determinants of resistance correlated with phenotypic resistance. Further analysis of isolates

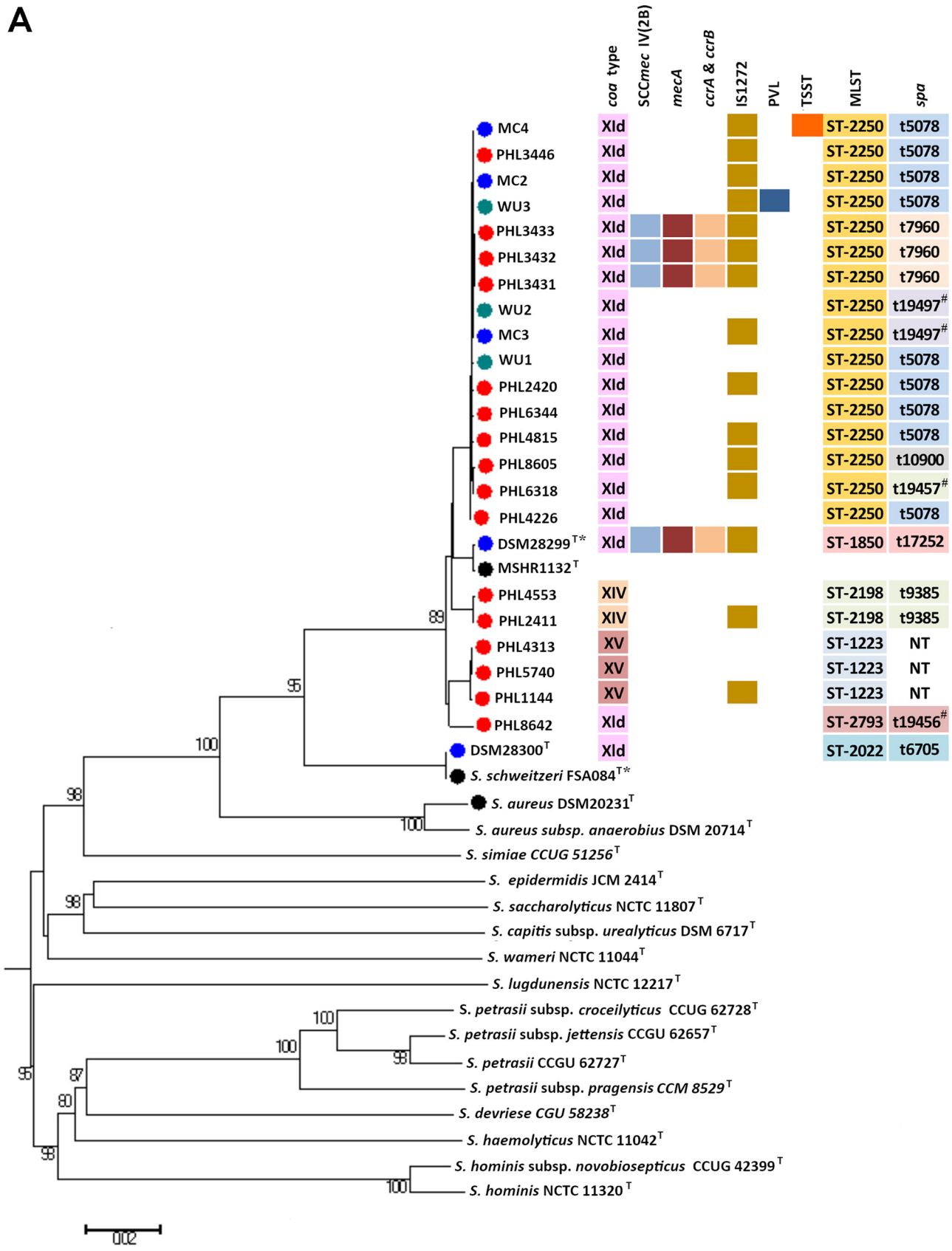

FIG 1 Whole-genome sequence phylogenetic tree of *S. argenteus*. (A) Whole-genome sequence phylogenetic tree of *S. argenteus* from this study and related type strains of the genus *Staphylococcus* obtained from TYGS. Tree inferred with FastME 2.1.6.1 from Genome BLAST Distance Phylogeny

(Continued on next page)

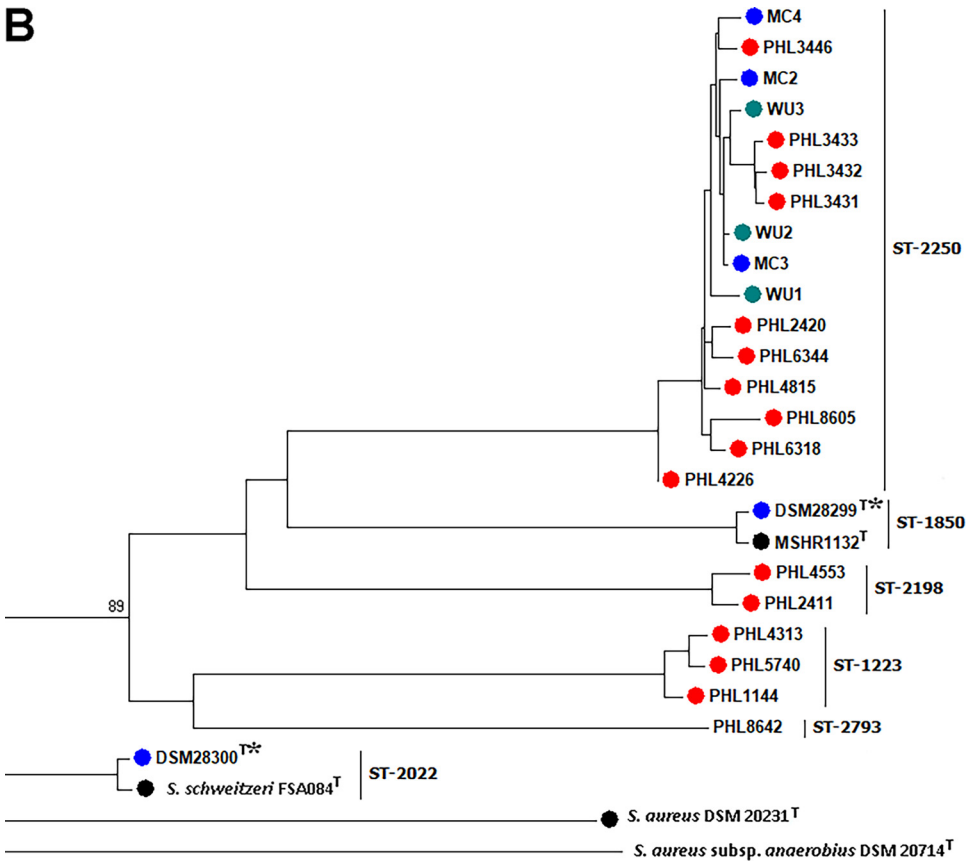

FIG 1 (Continued)

with *mecA* showed that their SCC*mec* type IV possesses the class B *mec* gene complex, harboring the *mecA*, *mecR1*, IS*1272*, and *ccrA*/*ccrB* gene complex (Fig. 1A).

Given a recent report of daptomycin resistance in *S. argenteus* associated with a point mutation in *mprF*, we analyzed the predicted protein of this gene in all isolates of this study and found all to be wild type (S337, data not shown) (30).

As plasmids often carry antibiotic resistance determinants, we investigated the presence of plasmids using genomic analysis. Using the PlasmidFinder, a total of 7 plasmid replicon types were detected in our isolates, characterized as rep16, rep5, repUS5, rep5a, repUS9, rep20, and rep21 with accession numbers BX571858, NC005011, NC003265, AP003139, AF203376, FN433597, and NC007790, respectively, with an alignment similarity ranging from 99.24 to 100%. The *S. argenteus* isolates in this study were found to harbor multireplicons in various combinations, including rep16/rep5, rep16/rep5a, rep16/repUS5, rep16/rep5/repUS9, and rep21/rep20 (Table S2).

Putative virulence determinants. Analysis of putative virulence gene composition based on the VF database revealed a number of virulence determinants in all *S. argenteus* isolates from this study, including autolysin (*atlE*), elastin binding protein (*ebp*), fibrinogen binding protein (*efb*), intercellular adhesion (*icaA*, *icab*, *icaC*, and

FIG 1 Legend (Continued)
approach (GBDP) distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d5. The numbers above branches are GBDP pseudo-bootstrap support values >60% from 100 replications. The reference sequences available in GenBank and the type strains sequenced in this study are indicated by solid black and blue dots, respectively. The tree was rooted at the midpoint. Staphylocoagulase (*coa*), SCC *mec* IV (2B), *mecA*, *ccrA* and *ccrB*, IS*1272*, PVL, TSST, MLST, and *spa* type are indicated for each strain. Presence of a given gene/element is identified by colored boxes. NT, nontypable. (B) Magnified main branch of phylogenetic tree containing *S. argenteus* representing clustering of strains possessing same MLST. *, sequences for these type strains (DSM 28229^T, DSM 28300^T) were generated in this study; #, novel *spa* types identified in this study.

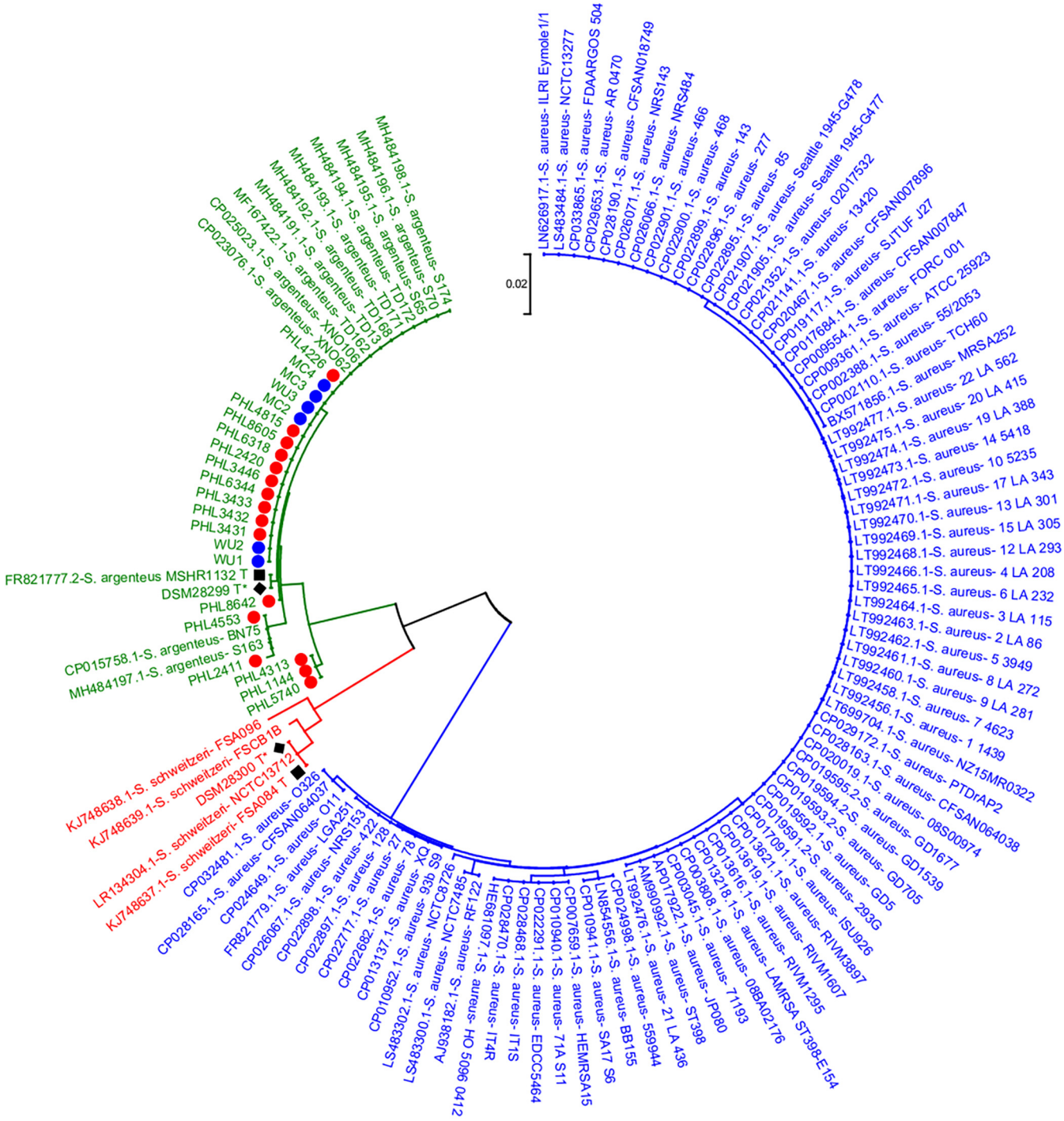

FIG 2 *nuc* phylogenetic tree. Phylogenetic dendrogram of *nuc* sequences of *S. argenteus* from this study and *S. argenteus*, *S. schweitzeri*, and *S. aureus* strains from GenBank constructed by neighbor-joining method. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. The analysis involved 125 isolates. There were a total of 426 positions in the final data set. Evolutionary analyses were conducted in MEGA6. Green text, *S. argenteus*; red text, *S. schweitzeri*; blue text, *S. aureus*; black diamond, genome sequence of type strains generated in this study; black square, genome sequence of type strains obtained from GenBank; red circles, sequences from PHO isolates; blue circles, sequences from Mayo Clinic isolates.

icaR), staphylokinase (*sak*), staphylococcal complement inhibitor (*scn*), staphylococcal protein A (*spa*), cysteine protease (*sspB*), hyaluronate lyase (*hysA*), lipase (*geh* and *lip*), staphylocoagulase (*coa*), thermonuclease (*nuc*), capsular (*cap5* and *cap8*), type VII secretion system (*esaG*, *essA*, *essB*, *essC*, and *esxA*), alpha hemolysin (*hly*/*hla*), delta hemolysin (*hld*), exfoliative toxin type A (*eta*), and gamma hemolysin (*hlgA*, *hlgB*, and

hlgC) genes (Table S3). Additionally, one clinical *S. argenteus* isolate (WU3, a throat swab isolate) harbored the gene that encodes Panton-Valentine leukocidin (PVL), and another single isolate (MC4, a blood isolate) harbored the gene for toxic shock syndrome toxin (TSST-1).

DISCUSSION

Through many reports over the past several years, including this one, it is clear that *S. argenteus* is a distinct species from *S. aureus* and that it is clinically relevant as a cause of skin and soft tissue infections as well as severe invasive disease (3, 7, 12–14, 16–19, 23, 24, 64). Several reports have indicated international detection of *S. argenteus* (12, 14–16, 25–29, 65–68); here, we report the first large collection of clinical isolates of *S. argenteus* in North America, from both Canada and the United States. As large reference laboratories, we each receive isolates to confirm bacterial identification. From 2017 to 2019, collectively, we received and identified 22 isolates of *S. argenteus*.

We encountered some of the same initial challenges as other investigators in the differentiation of *S. argenteus* from *S. aureus* using both traditional biochemical assays and a commercial MALDI-TOF MS (Bruker Biotyper) system, despite having spectra for both *S. argenteus* and *S. schweitzeri* in the databases. Although not investigated here, another commercial MALDI-TOF MS system (Vitek MS, bioMérieux) does not currently have spectra for *S. argenteus* or *S. schweitzeri*, and users of this system may erroneously call these organisms *S. aureus*. Further investigation must be undertaken concerning the ability of commercial MALDI-TOF MS platforms and database/software versions to differentiate among the *S. aureus* complex members. At this time, current routine methods used in clinical microbiology laboratories will not conclusively differentiate *S. argenteus* from *S. aureus*.

All *S. argenteus* encountered during this investigation period were definitively identified using a WGS approach. Our WGS data are consistent with previous reports that *S. argenteus* is distinct from *S. aureus* and *S. schweitzeri* (3, 7, 14, 25, 69). All isolates of *S. argenteus* for which the described *nuc* PCR assay was run were negative, while isolates of *S. aureus* were positive (data not shown) (10); however, depending on where the primers are, this may not always be the case. The *nuc* sequences examined demonstrate considerable sequence diversity between *S. aureus*, *S. argenteus*, and *S. schweitzeri*, consistent with what other investigators have found (3, 8, 9, 14, 70). This sequence diversity can be exploited as with the appropriate primers the *nuc* gene can be a target for discriminating *S. aureus* from other members of the complex, thus avoiding the need for WGS to distinguish *S. aureus* from *S. argenteus/S. schweitzeri*. An example of such an assay is the one developed by the Mayo Clinic (71). As many laboratories already include a *nuc* target in their *mecA* PCR as a control, this may not require significant workflow changes. Other groups have identified *crtM* and *sodA* as useful targets for differentiating these species (29, 72).

S. argenteus from this collection were isolated from a range of sites, including normally sterile and nonsterile specimen sources, and from surveillance swabs. Patients ranged in age from <1 to ≥ 75 years, with most being older than 45 years of age. One limitation of this study is that we do not have detailed information on the patients, including if they were inpatients or outpatients and whether the *S. argenteus* was likely acquired in the community or via a nosocomial route. It is possible that 3 isolates in this study were part of a nosocomial transmission event, as these isolates were collected from patients being investigated as part of a MRSA cluster in a health care facility (PHL3431, PHL3432, and PHL3433). These three isolates were identified through surveillance screens and are all temporally related from the same health care facility; all were *mecA*-positive ST2250, *spa* type t7960, SCC*mec* type IV (2B) with similar antimicrobial susceptibility patterns and were $\geq 99.94\%$ similar to one another by ANI comparison. Another limitation of this work is that we have no treatment or clinical outcome data on these patients. This type of information needs to be collected to better understand the transmission dynamics,

reservoirs, and best treatment options for patients; however, in order for this to be done, widespread, accurate identification of *S. argenteus* must be available.

This study is unique in that it includes a large number of *S. argenteus* isolates from North America, from both Canada and the United States. Although it has been postulated to have spread globally, this is the first report to demonstrate that *S. argenteus* is present in North America beyond a single case report and is likely circulating (30). Like many global studies, the majority of isolates (73%) in this study belong to MLST ST2250 (14, 16, 18, 19, 23, 32, 65, 73). These ST2250 isolates were also part of *coa* XId, described as the predominant type by Aung et al. in their study (69). There was a high degree of homology among these ST2250/*coa* XId isolates; however, 5 different *spa* types were identified, showing some diversity between strains.

Three additional previously reported but less frequently described MLSTs (ST1223, ST2198, and ST2793) were identified from the Canadian specimens, with corresponding *coa* types XV, XIV, and XId, respectively, thus demonstrating some diversity in the North American isolates (14, 16, 23, 25, 31, 32, 69, 72, 74–77). Most isolates in this study belonged to the *coa* types XIV and XV. *spa* typing is based on the highly variable and repetitive X-region of a single gene (*spa*) that encodes protein A. Use of reads generated by WGS in order to infer *spa* type has been done for *S. aureus*; however, factors such as length of the reads and repetitive sequence regions affect the *de novo* assembly and may result in incorrect assignments of *spa* type. To overcome these challenges, here we relied on traditional Sanger sequencing to confirm *spa* types and identified 7 different *spa* types, including 3 previously undescribed types (all in ST1223 isolates), adding evidence of the diversity of this collection of *S. argenteus*. A wide array of virulence determinants commonly associated with *S. aureus* were also detected, with PVL and TSST-1 of note. Further detailed investigation of isolates is needed to truly understand the diversity and epidemiology of the organism in North America.

It is interesting to consider why this organism appears to be emerging at this time. It is possible that *S. argenteus*, as part of the *S. aureus* clonal complex, has always been a human pathogen and had gone undetected until recently due to implementation of newer, more specific identification methods (e.g., WGS). However, others have provided evidence that supports a fairly recent host adaption which has allowed it to colonize and infect humans (the same does not appear to have happened with the closely related *S. schweitzeri*, for which there are no reports of human infection) (25, 75). Regardless of whether it is a newly emergent pathogen or an organism that we can only now differentiate from *S. aureus*, it is clear that *S. argenteus* is pathogenic and possesses many of the same virulence determinants and resistance mechanisms (including *mecA*) as *S. aureus*. Based on our experience as well as the emerging findings reported in the literature, we support the proposal that *S. argenteus* should be treated in a similar manner as *S. aureus* in terms of IPAC measures, as well as clinically, for the following reasons: (i) evidence points to similar disease presentations (including invasive disease), (ii) there is evidence of both community and nosocomial spread, and (iii) *S. argenteus*, like *S. aureus*, can carry *mecA*, PVL, TSST-1, and other virulence genes, suggesting it has similar pathogenic potential (78).

Clinical laboratories and other members of the health care teams should recognize that *S. argenteus* and *S. schweitzeri* are members of the *S. aureus* complex; this is significant, as classification guides appropriate clinical interpretation of culture as well as oxacillin susceptibility. If the oxacillin MIC breakpoints of the non-*S. aureus* group ($S \leq 0.25\,\mu$g/ml; $R \geq 0.5\,\mu$g/ml) are assigned to *S. argenteus* instead of *S. aureus* oxacillin MIC breakpoints ($S \leq 2\,\mu$g/ml; $R \geq 4\,\mu$g/ml), isolates may be inappropriately reported as oxacillin resistant (39). Likewise, cefoxitin disk diffusion breakpoints differ between these two groups. Furthermore, allowable methods for detection of methicillin resistance differ according to *Staphylococcus* species; for example, cefoxitin MIC is an appropriate testing method for *S. aureus*, while it is not appropriate for the "other *Staphylococcus* spp." group. Given that *S. argenteus* appears to be more like *S. aureus* than other staphylococci applying the genomic, phenotypic, and clinical data to date,

we propose that *S. aureus* antimicrobial breakpoints be applied for *S. argenteus* and *S. schweitzeri* as members of the *S. aureus* complex. At the January 2020 meeting of the CLSI Antimicrobial Susceptibility Testing Subcommittee, CLSI members voted to approve a motion to accept the recommendation, when a definitive identification cannot be made, to report *S. argenteus* as "*S. aureus* complex" or, when identified to species level, to report it as "*S. aureus* complex (*S. argenteus*)" so that it is not overlooked as a less pathogenic/nonpathogenic species (79). Additionally, it was approved to use *S. aureus* breakpoints and interpretive categories for reporting. This change will ensure accurate reporting of susceptibility results in all laboratories, including those currently unable to differentiate species within the complex, as well as provide guidance for laboratories that can identify these bacteria to the species level.

The availability of enhanced technology has dramatically improved our ability to accurately describe and identify microorganisms, but access to this technology as well as changing taxonomy can be challenging for the clinical laboratory as well as health care providers. If both organisms result in similar clinical presentations, some may ask if clinical laboratories should distinguish between *S. aureus* and *S. argenteus.* A recent position paper by the ESCMID Study Group for Staphylococci and Staphylococcal Diseases (ESGS) has proposed that due to known pathogenic and clinical similarities between *S. aureus* and *S. argenteus*, there is currently no need to distinguish within the *S. aureus* complex (78). However, in order to better understand the population epidemiology and pathogenicity of *S. argenteus* (and *S. schweitzeri*), it is important to distinguish the members of the *S. aureus* complex from a research and surveillance perspective. Based on the data to date, we recommend that clinical laboratories, where possible, (i) clearly report the organism as "*Staphylococcus argenteus*, member of *Staphylococcus aureus* clonal complex" or something similar in order to capture both the unique nature of the organism as well as its close link to the well-known pathogen (see Table S4 in the supplemental material) and (ii) apply "*S. aureus/S. aureus* complex" breakpoints for antibiotics, including oxacillin. This method of reporting will hopefully allow for education of clinical staff and better data collection as well as larger studies on the clinical and treatment outcomes of patients with these infections.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

SUPPLEMENTAL FILE 2, PDF file, 0.5 MB.

SUPPLEMENTAL FILE 3, PDF file, 0.4 MB.

SUPPLEMENTAL FILE 4, PDF file, 0.6 MB.

SUPPLEMENTAL FILE 5, PDF file, 0.5 MB.

SUPPLEMENTAL FILE 6, PDF file, 0.4 MB.

SUPPLEMENTAL FILE 7, PDF file, 0.5 MB.

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REFERENCES

1. Lowy FD. 1998. *Staphylococcus aureus* infections. N Engl J Med 339:520–532. https://doi.org/10.1056/NEJM199808203390806.
2. Hassoun A, Linden PK, Friedman B. 2017. Incidence, prevalence, and management of MRSA bacteremia across patient populations-a review of recent developments in MRSA management and treatment. Crit Care 21:211. https://doi.org/10.1186/s13054-017-1801-3.
3. Tong SYC, Schaumburg F, Ellington MJ, Corander J, Pichon B, Leendertz F, Bentley SD, Parkhill J, Holt DC, Peters G, Giffard PM. 2015. Novel staphylococcal species that form part of a *Staphylococcus aureus*-related complex: the non-pigmented *Staphylococcus argenteus* sp. nov. and the non-human primate-associated *Staphylococcus schweitzeri* sp. nov. Int J Syst Evol Microbiol 65:15–22. https://doi.org/10.1099/ijs.0.062752-0.
4. Schaumburg F, Pauly M, Anoh E, Mossoun A, Wiersma L, Schubert G, Flammen A, Alabi AS, Muyembe-Tamfum J-J, Grobusch MP, Karhemere S, Akoua-Koffi C, Couacy-Hymann E, Kremsner PG, Mellmann A, Becker K, Leendertz FH, Peters G. 2015. *Staphylococcus aureus* complex from animals and humans in three remote African regions. Clin Microbiol Infect 21:345.e1–345.e8. https://doi.org/10.1016/j.cmi.2014.12.001.
5. Ng JWS, Holt DC, Lilliebridge RA, Stephens AJ, Huygens F, Tong SYC, Currie BJ, Giffard PM. 2007. Phylogenetically distinct *Staphylococcus*

aureus lineage prevalent among indigenous communities in northern Australia. J Clin Microbiol 47:6. https://doi.org/10.1128/JCM.00122-09.
6. Ruimy R, Armand-Lefevre L, Barbier F, Ruppé E, Cocojaru R, Mesli Y, Maiga A, Benkalfat M, Benchouk S, Hassaine H, Dufourcq J-B, Nareth C, Sarthou J-L, Andremont A, Feil EJ. 2009. Comparisons between geographically diverse samples of carried *Staphylococcus aureus*. J Bacteriol 191:5577–5583. https://doi.org/10.1128/JB.00493-09.
7. Holt DC, Holden MTG, Tong SYC, Castillo-Ramirez S, Clarke L, Quail MA, Currie BJ, Parkhill J, Bentley SD, Feil EJ, Giffard PM. 2011. A very early-branching *Staphylococcus aureus* lineage lacking the carotenoid pigment staphyloxanthin. Genome Biol Evol 3:881–895. https://doi.org/10.1093/gbe/evr078.
8. Kaden R, Engstrand L, Rautelin H, Johansson C. 2018. Which methods are appropriate for the detection of Staphylococcus argenteus and is it worthwhile to distinguish S. argenteus from S. aureus? Infect Drug Resist 11:2335–2344. https://doi.org/10.2147/IDR.S179390.
9. Bogestam K, Vondracek M, Karlsson M, Fang H, Giske CG. 2018. Introduction of a hydrolysis probe PCR assay for high-throughput screening of methicillin-resistant *Staphylococcus aureus* with the ability to include or exclude detection of *Staphylococcus argenteus*. PLoS One 13:e0192782. https://doi.org/10.1371/journal.pone.0192782.
10. Pichon B, Hill R, Laurent F, Larsen AR, Skov RL, Holmes M, Edwards GF, Teale C, Kearns AM. 2012. Development of a real-time quadruplex PCR assay for simultaneous detection of *nuc*, Panton-Valentine leucocidin (PVL), *mecA* and homologue mecALGA251. J Antimicrob Chemother 67:2338–2341. https://doi.org/10.1093/jac/dks221.
11. Akobi B, Aboderin O, Sasaki T, Shittu A. 2012. Characterization of *Staphylococcus aureus* isolates from faecal samples of the Straw-Coloured Fruit Bat (*Eidolon helvum*) in Obafemi Awolowo University (OAU), Nigeria. BMC Microbiol 12:279. https://doi.org/10.1186/1471-2180-12-279.
12. Dupieux C, Blondé R, Bouchiat C, Meugnier H, Bes M, Laurent S, Vandenesch F, Laurent F, Tristan A. 2015. Community-acquired infections due to *Staphylococcus argenteus* lineage isolates harbouring the Panton-Valentine leucocidin, France, 2014. Eurosurveillance 20:8–10. https://doi.org/10.2807/1560-7917.ES2015.20.23.21154.
13. Thaipadungpanit J, Amornchai P, Nickerson EK, Wongsuvan G, Wuthiekanun V, Limmathurotsakul D, Peacock SJ. 2015. Clinical and molecular epidemiology of *Staphylococcus argenteus* infections in Thailand. J Clin Microbiol 53:1005–1008. https://doi.org/10.1128/JCM.03049-14.
14. Hansen TA, Bartels MD, Høgh SV, Dons LE, Pedersen M, Jensen TG, Kemp M, Skov MN, Gumpert H, Worning P, Westh H. 2017. Whole genome sequencing of Danish *Staphylococcus argenteus* reveals a genetically diverse collection with clear separation from *Staphylococcus aureus*. Front Microbiol 8:1–7. https://doi.org/10.3389/fmicb.2017.01512.
15. Suzuki Y, Kubota H, Ono HK, Kobayashi M, Murauchi K, Kato R, Hirai A, Sadamasu K. 2017. Food poisoning outbreak in Tokyo, Japan caused by *Staphylococcus argenteus*. Int J Food Microbiol 262:31–37. https://doi.org/10.1016/j.ijfoodmicro.2017.09.005.
16. Chen SY, Lee H, Wang XM, Lee TF, Liao C-H, Teng LJ, Hsueh PR. 2018. High mortality impact of *Staphylococcus argenteus* on patients with community-onset staphylococcal bacteraemia. Int J Antimicrob Agents 52:747–753. https://doi.org/10.1016/j.ijantimicag.2018.08.017.
17. Jiang B, You B, Tan L, Yu S, Li H, Bai G, Li S, Rao X, Xie Z, Shi X, Peng Y, Hu X. 2018. Clinical *Staphylococcus argenteus* develops to small colony variants to promote persistent infection. Front Microbiol 9:1–10. https://doi.org/10.3389/fmicb.2018.01347.
18. Rigaill J, Grattard F, Grange S, Forest F, Haddad E, Carricajo A, Tristan A, Laurent F, Botelho-Nevers EVP. 2018. Community-acquired *Staphylococcus argenteus* sequence type 2250 bone and joint infection, France, 2017. Emerg Infect Dis 24:1958–1961. https://doi.org/10.3201/eid2410.180727.
19. Ohnishi T, Shinjoh M, Ohara H, Kawai T, Kamimaki I, Mizushima R, Kamada K, Itakura Y, Iguchi S, Uzawa Y, Yoshida A, Kikuchi K. 2018. Purulent lymphadenitis caused by *Staphylococcus argenteus*, representing the first Japanese case of *Staphylococcus argenteus* (multilocus sequence type 2250) infection in a 12-year-old boy. J Infect Chemother 24:925–927. https://doi.org/10.1016/j.jiac.2018.03.018.
20. Wakabayashi Y, Umeda K, Yonogi S, Nakamura H, Yamamoto K, Kumeda Y, Kawatsu K. 2018. Staphylococcal food poisoning caused by *Staphylococcus argenteus* harboring staphylococcal enterotoxin genes. Int J Food Microbiol 265:23–29. https://doi.org/10.1016/j.ijfoodmicro.2017.10.022.
21. Tong SYC, Sharma-Kuinkel BK, Thaden JT, Whitney AR, Yang SJ, Mishra NN, Rude T, Lilliebridge RA, Selim MA, Ahn SH, Holt DC, Giffard PM, Bayer AS, Deleo FR, Fowler VG. 2013. Virulence of endemic nonpigmented northern Australian *Staphylococcus aureus* clone (clonal complex 75, S. argenteus) is not augmented by staphyloxanthin. J Infect Dis 208:520–527. https://doi.org/10.1093/infdis/jit173.
22. Williamson DA, Coombs GW, Nimmo GR. 2014. *Staphylococcus aureus* "Down Under": contemporary epidemiology of *S. aureus* in Australia, New Zealand, and the South West Pacific. Clin Microbiol Infect 20:597–604. https://doi.org/10.1111/1469-0691.12702.
23. Chantratita N, Wikraiphat C, Tandhavanant S, Wongsuvan G, Ariyaprasert P, Suntornsut P, Thaipadungpanit J, Teerawattanasook N, Jutrakul Y, Srisurat N, Chaimanee P, Anukunananchai J, Phiphitaporn S, Srisamang P, Chetchotisakd P, West TE, Peacock SJ. 2016. Comparison of community-onset *Staphylococcus argenteus* and *Staphylococcus aureus* sepsis in Thailand: a prospective multicentre observational study. Clin Microbiol Infect 22:458.e11–458.e19. https://doi.org/10.1016/j.cmi.2016.01.008.
24. Tong SYC, Lilliebridge RA, Bishop EJ, Cheng AC, Holt DC, McDonald MI, Giffard PM, Currie BJ, Boutlis CS. 2010. Clinical correlates of Panton-Valentine leukocidin (PVL), PVL isoforms, and clonal complex in the *Staphylococcus aureus* population of Northern Australia. J Infect Dis 202:760–769. https://doi.org/10.1086/655396.
25. Zhang DF, Zhi XY, Zhang J, Paoli GC, Cui Y, Shi C, Shi X. 2017. Preliminary comparative genomics revealed pathogenic potential and international spread of *Staphylococcus argenteus*. BMC Genomics 18:808. https://doi.org/10.1186/s12864-017-4149-9.
26. Monecke S, Stieber B, Roberts R, Akpaka PE, Slickers P, Ehricht R. 2014. Population structure of *Staphylococcus aureus* from Trinidad & Tobago. PloS One 9:e89120. https://doi.org/10.1371/journal.pone.0089120.
27. Argudín MA, Dodémont M, Vandendriessche S, Rottiers S, Tribes C, Roisin S, de Mendonça R, Nonhoff C, Deplano A, Denis O. 2016. Low occurrence of the new species *Staphylococcus argenteus* in a *Staphylococcus aureus* collection of human isolates from Belgium. Eur J Clin Microbiol Infect Dis 35:1017–1022. https://doi.org/10.1007/s10096-016-2632-x.
28. Aung MS, San T, Aye MM, Mya S, Maw WW, Zan KN, Htut WHW, Kawaguchiya M, Urushibara N, Kobayashi N. 2017. Prevalence and genetic characteristics of *Staphylococcus aureus* and *Staphylococcus argenteus* isolates harboring Panton-Valentine leukocidin, enterotoxins, and TSST-1 genes from food handlers in Myanmar. Toxins (Basel) 9:241. https://doi.org/10.3390/toxins9080241.
29. Hallbäck ET, Karami N, Adlerberth I, Cardew S, Ohlén M, Jakobsson HE, Stadler LS. 2018. Methicillin-resistant *Staphylococcus argenteus* misidentified as methicillin-resistant *Staphylococcus aureus* emerging in western Sweden. J Med Microbiol 67:968–971. https://doi.org/10.1099/jmm.0.000760.
30. Hao S, Abdelghany M, Lyden A, Sit R, Tan M, Tato C, DeRisi J, Miller S, Doernberg S, Langelier C. 2020. Genomic profiling of evolving daptomycin resistance in a patient with recurrent *Staphylococcus argenteus* sepsis. Antimicrob Agents Chemother 64. https://doi.org/10.1128/AAC.00961-20.
31. Johansson C, Rautelin H, Kaden R. 2019. *Staphylococcus argenteus* and *Staphylococcus schweitzeri* are cytotoxic to human cells in vitro due to high expression of alpha-hemolysin Hla. Virulence 10:502–510. https://doi.org/10.1080/21505594.2019.1620062.
32. Aung MS, San T, San N, Oo WM, Ko PM, Thet KT, Urushibara N, Kawaguchiya M, Sumi A, Kobayashi N. 2019. Molecular characterization of *Staphylococcus argenteus* in myanmar: identification of novel genotypes/clusters in staphylocoagulase, protein a, alpha-haemolysin and other virulence factors. J Med Microbiol 68:95–104. https://doi.org/10.1099/jmm.0.000869.
33. McCabe KM, Zhang YH, Huang BL, Wagar EA, McCabe ER. 1999. Bacterial species identification after DNA amplification with a universal primer pair. Mol Genet Metab 66:205–211. https://doi.org/10.1006/mgme.1998.2795.
34. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.
35. CLSI. 2008. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing; approved guideline. CLSI document MM18-A. Clinical and Laboratory Standards Institute. Wayne, PA.
36. Saffert RT, Cunningham SA, Ihde SM, Jobe KEM, Mandrekar J, Patel R. 2011. Comparison of Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometer to BD Phoenix automated microbiology system for identification of gram-negative bacilli. J Clin Microbiol 49:887–892. https://doi.org/10.1128/JCM.01890-10.
37. Degand N, Carbonnelle E, Dauphin B, Beretti JL, Le Bourgeois M, Sermet-Gaudelus I, Segonds C, Berche P, Nassif X, Ferroni A. 2008. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of nonfermenting gram-negative bacilli isolated from cystic

fibrosis patients. J Clin Microbiol 46:3361–3367. https://doi.org/10.1128/JCM.00569-08.
38. CLSI. 2018. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 11th ed CLSI standard M07. Clinical and Laboratory Standards Institute. Wayne, PA.
39. CLSI. 2020. Performance standards for antimicrobial susceptibility testing. 30th ed CLSI supplement M100. Clinical and Laboratory Standards Institute. Wayne, PA.
40. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
41. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, Huynh W, Nguyen A-LV, Cheng AA, Liu S, Min SY, Miroshnichenko A, Tran H-K, Werfalli RE, Nasir JA, Oloni M, Speicher DJ, Florescu A, Singh B, Faltyn M, Hernandez-Koutoucheva A, Sharma AN, Bordeleau E, Pawlowski AC, Zubyk HL, Dooley D, Griffiths E, Maguire F, Winsor GL, Beiko RG, Brinkman FSL, Hsiao WWL, Domselaar GV, McArthur AG. 2020. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 48:D517–D525. https://doi.org/10.1093/nar/gkz935.
42. Jolley KA, Maiden MCJ. 2010. BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics 11:595. https://doi.org/10.1186/1471-2105-11-595.
43. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261.
44. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, Hiramatsu K. 2007. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. Antimicrob Agents Chemother 51:264–274. https://doi.org/10.1128/AAC.00165-06.
45. Liu B, Zheng D, Jin Q, Chen L, Yang J. 2019. VFDB 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic Acids Res 47:D687–D692. https://doi.org/10.1093/nar/gky1080.
46. Carattoli A, Hasman H. 2020. PlasmidFinder and in silico pMLST: identification and typing of plasmid replicons in whole-genome sequencing (WGS). Methods Mol Biol 2075:285–294. https://doi.org/10.1007/978-1-4939-9877-7_20.
47. Garrison E, Marth G. 2012. Haplotype-based variant detection from short-read sequencing.
48. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078–2079. https://doi.org/10.1093/bioinformatics/btp352.
49. Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. 2004. Versatile and open software for comparing large genomes. Genome Biol 5:R12. https://doi.org/10.1186/gb-2004-5-2-r12.
50. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. https://doi.org/10.1093/molbev/mst197.
51. Meier-Kolthoff JP, Göker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182. https://doi.org/10.1038/s41467-019-10210-3.
52. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, Seo H, Chun J. 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613–1617. https://doi.org/10.1099/ijsem.0.001755.
53. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91. https://doi.org/10.1099/ijs.0.64483-0.
54. Petkau A, Stuart-Edwards M, Stothard P, Van Domselaar G. 2010. Interactive microbial genome visualization with Gview. Bioinformatics 26:3125–3126. https://doi.org/10.1093/bioinformatics/btq588.
55. Schuster D, Rickmeyer J, Gajdiss M, Thye T, Lorenzen S, Reif M, Josten M, Szekat C, Melo LDR, Schmithausen RM, Liégeois F, Sahl H, Gonzalez JJ, Nagel M, Bierbaum G. 2017. Differentiation of Staphylococcus argenteus (formerly: Staphylococcus aureus clonal complex 75) by mass spectrometry from S. aureus using the first strain isolated from a wild African great ape. Int J Med Microbiol 307:57–63. https://doi.org/10.1016/j.ijmm.2016.11.003.
56. Truong-Bolduc QC, Zhang X, Hooper DC. 2003. Characterization of NorR protein, a multifunctional regulator of norA expression in *Staphylococcus aureus*. J Bacteriol 185:3127–3138. https://doi.org/10.1128/jb.185.10.3127-3138.2003.
57. Fournier B, Aras R, Hooper DC. 2000. Expression of the multidrug resistance transporter NorA from *Staphylococcus aureus* is modified by a two-component regulatory system. J Bacteriol 182:664–671. https://doi.org/10.1128/jb.182.3.664-671.2000.
58. Kaatz GW, McAleese F, Seo SM. 2005. Multidrug resistance in *Staphylococcus aureus* due to overexpression of a novel multidrug and toxin extrusion (MATE) transport protein. Antimicrob Agents Chemother 49:1857–1864. https://doi.org/10.1128/AAC.49.5.1857-1864.2005.
59. McAleese F, Petersen P, Ruzin A, Dunman PM, Murphy E, Projan SJ, Bradford PA. 2005. A novel MATE family efflux pump contributes to the reduced susceptibility of laboratory-derived *Staphylococcus aureus* mutants to tigecycline. Antimicrob Agents Chemother 49:1865–1871. https://doi.org/10.1128/AAC.49.5.1865-1871.2005.
60. McLaughlin JR, Murray CL, Rabinowitz JC. 1981. Unique features in the ribosome binding site sequence of the gram-positive *Staphylococcus aureus* beta-lactamase gene. J Biol Chem 256:11283–11291. https://doi.org/10.1016/S0021-9258(19)68589-3.
61. Cao M, Bernat BA, Wang Z, Armstrong RN, Helmann JD. 2001. FosB, a cysteine-dependent fosfomycin resistance protein under the control of sigma(W), an extracytoplasmic-function sigma factor in *Bacillus subtilis*. J Bacteriol 183:2380–2383. https://doi.org/10.1128/JB.183.7.2380-2383.2001.
62. Floyd JL, Smith KP, Kumar SH, Floyd JT, Varela MF. 2010. LmrS is a multidrug efflux pump of the major facilitator superfamily from *Staphylococcus aureus*. Antimicrob Agents Chemother 54:5406–5412. https://doi.org/10.1128/AAC.00580-10.
63. Daiglel DM, Hughes W, Wright D, Daigle DM, Hughes DW, Wright GD. 1999. Prodigious substrate specificity of AAC(6′)-APH(′′), an aminoglycoside antibiotic resistance determinant in enterococci and staphylococci. Chem Biol 6:99–110. https://doi.org/10.1016/S1074-5521(99)80006-4.
64. Söderquist B, Wildeman P, Stenmark B, Stegger M. 2020. *Staphylococcus argenteus* as an etiological agent of prosthetic hip joint infection: a case presentation. J Bone Jt Infect 5:172–175. https://doi.org/10.7150/jbji.44848.
65. Alhussein F, Fürstenberg J, Gaupp R, Eisenbeis J, Last K, Becker SL, Papan C. 2020. Human infections caused by *Staphylococcus argenteus* in Germany: genetic characterisation and clinical implications of novel species designation. Eur J Clin Microbiol Infect Dis 39:2461–2468. https://doi.org/10.1007/s10096-020-03950-4.
66. Kukla R, Neradová K, Petráš P, Kekláková J, Ryšková L, Žemličková H. 2020. The first confirmed detection of *Staphylococcus argenteus* in the Czech Republic. Epidemiol Mikrobiol Imunol 69:48–52.
67. Pumipuntu N. 2019. *Staphylococcus argenteus*: an emerging subclinical bovine mastitis pathogen in Thailand. Vet World 12:1940–1944. https://doi.org/10.14202/vetworld.2019.1940-1944.
68. Rossi BF, Bonsaglia ÉCR, Castilho IG, Dantas STA, Langoni H, Pantoja JCF, Júnior AF, Gonçalves JL, Santos MV, Mota RA, Rall VLM. 2020. First investigation of *Staphylococcus argenteus* in a Brazilian collections of *S. aureus* isolated from bovine mastitis. BMC Vet Res 16:7–11. https://doi.org/10.1186/s12917-020-02472-7.
69. Aung MS, Urushibara N, Kawaguchiya M, Sumi A, Takahashi S, Ike M, Ito M, Habadera S, Kobayashi N. 2019. Molecular epidemiological characterization of *Staphylococcus argenteus* clinical isolates in Japan: identification of three clones (ST1223, ST2198, and ST2550) and a novel staphylocoagulase genotype XV. Microorganisms 7:27–29. https://doi.org/10.3390/microorganisms7100389.
70. Tunsjø HS, Kalyanasundaram S, Charnock C, Leegaard TM, Moen AEF. 2018. Challenges in the identification of methicillin-resistant *Staphylococcus argenteus* by routine diagnostics. APMIS Denmark 126:533–537. https://doi.org/10.1111/apm.12843.
71. Bommersbach CE, Cole NC, Heitman AK, Dylla BL, Mainella JM, Jeraldo Maldonado PR, Kus JV, Patel S, Burnham C-A, Dien Bard J, Patel RSA. 2020. Identification of Staphylococcus aureus complex members *Staphylococcus argenteus* and *Staphylococcus schweitzeri* by real-time PCR. ASM Microbe, Chicago, IL.
72. Chen S-Y, Lee H, Teng S-H, Wang X-M, Lee T-F, Huang Y-C, Liao C-H, Teng L-J, Hsueh P-R. 2018. Accurate differentiation of novel

Staphylococcus argenteus from *Staphylococcus aureus* using MALDI-TOF MS. Future Microbiol 13:997–1006. https://doi.org/10.2217/fmb-2018-0015.
73. Kitagawa H, Ohge H, Hisatsune J, Masuda K, Aziz F, Hara T, Kuroo Y, Sugai M. 2020. Low incidence of *Staphylococcus argenteus* bacteremia in Hiroshima, Japan. J Infect Chemother 26:140–143. https://doi.org/10.1016/j.jiac.2019.07.011.
74. Wu S, Huang J, Wu Q, Zhang J, Zhang F, Yang X, Wu H, Zeng H, Chen M, Ding Y, Wang J, Lei T, Zhang S, Xue L. 2020. *Staphylococcus aureus* isolated from retail meat and meat products in China: incidence, antibiotic resistance and genetic diversity. Food Microbiol 9:2767. https://doi.org/10.3389/fmicb.2018.02767.
75. Moradigaravand D, Jamrozy D, Mostowy R, Anderson A, Nickerson EK, Thaipadungpanit J, Wuthiekanun V, Limmathurotsakul D, Tandhavanant S, Wikraiphat C, Wongsuvan G, Teerawattanasook N, Jutrakul Y, Srisurat N, Chaimanee P, Eoin West T, Blane B, Parkhill J, Chantratita N, Peacock SJ, Staphylococcal L. 2017. Evolution of the *Staphylococcus argenteus* ST2250 clone in Northeastern Thailand is linked with the acquisition of livestock-associated staphylococcal genes. mBio 8:1–13. https://doi.org/10.1128/mBio.00802-17.
76. Giske CG, Dyrkell F, Arnellos D, Vestberg N, Hermansson Panna S, Fröding I, Ullberg M, Fang H. 2019. Transmission events and antimicrobial susceptibilities of methicillin-resistant *Staphylococcus argenteus* in Stockholm. Clin Microbiol Infect 25:1289.e5–1289.e8. https://doi.org/10.1016/j.cmi.2019.06.003.
77. Pumipuntu N, Tunyong W, Chantratita N, Diraphat P, Pumirat P, Sookrung N, Chaicumpa W, Indrawattana N. 2019. *Staphylococcus* spp. associated with subclinical bovine mastitis in central and northeast provinces of Thailand. PeerJ 7:e6587-21. https://doi.org/10.7717/peerj.6587.
78. Becker K, Schaumburg F, Kearns A, Larsen AR, Lindsay JA, Skov RL, Westh H. 2019. Implications of identifying the recently defined members of the *Staphylococcus aureus* complex *S. argenteus* and *S. schweitzeri*: a position paper of members of the ESCMID study group for Staphylococci and Staphylococcal diseases (ESGS). Clin Microbiol Infect 25:1064–1070. https://doi.org/10.1016/j.cmi.2019.02.028.
79. CLSI. 2021. Performance standards for antimicrobial susceptibility testing, 31st ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.