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Genetic characterization of methicillin-resistant / susceptible *Staphylococcus aureus* (MRSA/MSSA) and *Staphylococcus argenteus* clinical isolates in Bangladesh: Dominance of ST6-MRSA-IV/t304 and detection of *cfr/fexA* in ST8-MSSA/t008

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ABSTRACT

Objectives: Coagulase-positive staphylococcus (CoPS), represented by *Staphylococcus aureus*, is a major cause of infections in humans. This study aimed to investigate molecular epidemiological characteristics, antimicrobial resistance, and their trends of CoPS in Bangladesh.

Methods: Clinical isolates of CoPS were collected from two medical institutions in Bangladesh for a 2-year period and analyzed for their species, genotypes, virulence factors, antimicrobial susceptibility, and resistance determinants.

Results: 172 CoPS isolates collected were identified as *S. aureus* or *S. argenteus* (170 and two, respectively). Methicillin-resistant *S. aureus* (MRSA) accounted for 36% (n = 61), having Staphylococcal cassette chromosome *mec* (SCC*mec*)-IV (82%) or V (18%). Panton-Valentine leukocidin (PVL) genes were detected at higher rate in methicillin-susceptible *S. aureus* (MSSA) (62%) than MRSA (26%). MRSA comprised 11 STs, including a dominant type ST6 (46%) associated with mostly SCC*mec*-IVa/*spa*-t304, and one isolate had genetic features of the USA300 clone (ST8/SCC*mec*-IVa/*coa*-IIIa/*spa*-t008/ACME-I/ΦSa2USA). STs of CC1, CC88, and CC398 were common in MSSA, with CC88 showing the highest PVL-positive rate. One MSSA isolate (ST8/*spa*-t008) harbored *fexA* and *cfr* showing susceptibility to linezolid. *S. argenteus* was methicillin-susceptible and belonged to ST2250/*coa*-XId.

Conclusions: Genetic characteristics of current MRSA/MSSA in Bangladesh were revealed, with first identification of *S. argenteus* at low prevalence.

Introduction

Coagulase-positive staphylococci (CoPS) represented by *Staphylococcus aureus*, is a common infectious pathogen in humans, causing wide spectrum of diseases ranging from superficial skin infection to severe invasive diseases [1]. Similarly, *Staphylococcus argenteus*, one of the minor species among CoPS, has been also recognized as a common bacterium of infectious diseases worldwide [2]. *S. aureus* and *S. argenteus* contain bacterial populations that are genetically diverse and classified into numerous types based on different genotyping schemes. Global spread of various CoPS clones having multiple antimicrobial resistance (AMR) determinants and virulence factor/toxin genes poses public health concern. Staphylococcal cassette chromosome *mec* (SCC*mec*) is a chromosomal determinant that defines methicillin-resistant (MR) *S. aureus* (MRSA) and other MR staphylococcal species [1,3]. MRSA is one of the most well-known drug-resistant bacteria that causes infections in health-

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care settings as well as in communities worldwide. Most of all, or a subset of *S. aureus* produces variable virulence factors including exotoxins such as hemolysin, enterotoxin, TSST-1, exfoliative toxin, leukocidin that are related to symptoms/types of disease. Particularly, Panton-Valentine leukocidin (PVL), a two component pore-forming toxin, has been noted because it is related to severe skin and soft tissue infections, as well as invasive diseases, including necrotizing pneumonia [1,4]. Recently, the rising prevalence of infections with community-acquired MRSA has been related to increase in severe infections due to strains with virulence factors, such as PVL. Monitoring of CoPS, with regard to their AMR and its determinants, virulence factors in relation to genotypes is significant as basic information directed to control MRSA and virulent CoPS clones.

In Bangladesh, although only limited information is available for the molecular epidemiology of *S aureus*, notable findings have been observed previously. In early study of hospital isolates in 2004, ST772 was first identified in PVL-positive methicillin-susceptible (MS) *S. aureus* (MSSA) [5]. This type was later revealed to be a major sequence type (ST) of MRSA prevailing in South Asia, which is colloquially referred to as Bengal Bay clone [6]. A small scale study of *S. aureus* from diabetic skin infections revealed the presence of USA300 clone (ST8-MRSA-IVa having PVL genes and arginine catabolic mobile element (ACME), which is prevalent in the US, and exfoliative toxin D gene (*etd*)-positive ST1931 (CC80) MRSA-IVa that was genetically close to European clone [7]. Another molecular epidemiological study of *S. aureus* from skin and soft tissue infections (2015-2016) described higher detection rate of PVL genes in MSSA than in MRSA, and main genotypes/CCs of PVL-positive isolates (CC1, CC6, CC88) and PVL-negative isolates (CC5, etc.) [8]. However, thereafter, no information is available for the prevalence of recent MRSA/MSSA clones.

S. argenteus constitutes *S. aureus* complex (SAC) with three more species (*S. schweitzeri, S. singaporensis*, and *S. roterodami*) [9], being a representative species of non-*S. aureus* CoPS. *S. argenteus* is distributed globally with considerably lower prevalence than *S. aureus*. Although this species is prevalent in Northern Australia and Southeast Asia including Thailand and Myanmar [10,11], there is no information of its prevalence and genotypes in South Asia including Bangladesh. The present study was aimed to reveal the clonal structure of recent clinical isolates of CoPS (MRSA, MSSA, and *S. argenteus*) in Bangladesh and observe the recent trend of clones with regard to AMR and virulence factors.

Materials and methods

Bacterial isolates

CoPS isolates were collected from various clinical specimens of inpatients and outpatients attending Mymensingh Medical College hospital (MMCH), or Bangladesh Institute of Health Sciences hospital (BIHSH), for two-year period between January 2021 and December 2022. MMCH is a 1000-bed hospital that is only a tertiary referral medical center in Mymensingh division, located north-central part of the country. BIHSH is a 250-bed hospital for both inpatient and outpatient care, situated in the capital city, Dhaka. All the clinical specimens were collected through aseptic procedures and transported to microbiology department from the hospital within 1 hour after their collection. Wound swabs were collected by sterile swab sticks applying gentle rubbing and pus was also collected by immersing the swab stick into free flow pus. The clinical specimens were inoculated onto blood agar and mannitol salt agar plates, followed by incubation at 37°C for 48 hours aerobically. Isolates were provisionally identified as *S. aureus* by colony morphology, positive results in catalase test and coagulase test, and mannitol fermentation. Although only one colony on agar plate was picked up for further analysis, when presence of multiple clones from a single specimen was suspected by genetic analysis as described below (coagulase genotyping and nonribosomal peptide synthetase [NRPS] gene detection), pure clones with distinct genetic traits were obtained by cloning of bacterial isolates on blood agar plates at least three times. All of such clones recovered simultaneously from a single specimen were genetically analyzed. Only one isolate per patient was included in this study, except for three patients having isolates of two genotypes from a single specimen.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was measured by broth microdilution test using Dry Plate Eiken DP42 (Eiken Chemical, Tokyo, Japan) for antimicrobials including oxacillin (OXA), cefoxitin (FOX), penicillin (PEN), gentamicin (GEN), arbekacin (ABK), erythromycin (ERY), clindamycin (CLI), vancomycin (VAN), teicoplanin (TEC), daptomycin (DAP), linezolid (LZD), minocycline (MIN), fosfomycin (FOF), rifampicin (RIF), levofloxacin (LVX), and trimethoprim/sulfamethoxazole (SXT). Resistance was judged according to Clinical Laboratory Standards Institute (CLSI) breakpoints for broth microdilution [12].

Genotyping and genetic characterization

All the isolates were confirmed genetically for the presence of staphylococcal 16S rRNA gene, *nuc, mecA, lukS-PV-lukF-PV*, ACME-*arcA* by multiplex polymerase chain reaction (PCR) (initial M-PCR assay) as described previously [13,14]. In addition, PCR targeting the NRPS gene with the primers nrps-F and nrps-R [15] was performed for all the isolates to discriminate non-*S. aureus* SAC species (e.g., *S. argenteus*) from *S. aureus*. SCC*mec* type and subtype of SCC*mec* IV were determined by multiplex PCR [14], and PVL phage was typed by PCR assay as described previously [16,17]. For ACME-*arcA*-positive strain, ACME types I, II, II' were classified by long-range PCR (LR-PCR) as described previously [18]. Staphylocoagulase genotype (*coa* type), *spa* type, and *agr* type were determined according to methods shown previously [19]. ST was determined by the scheme of multilocus sequence typing (MLST) and assigned to clonal complex (CC), referring to the PubMLST website (https://pubmlst.org/organisms/staphylococcus-aureus).

For all the MRSA, *S. argenteus*, and selected MSSA isolates, the presence of genes encoding virulence factors and antimicrobial resistance determinants was analyzed by multiplex or uniplex PCR as described previously [17,20]. Nucleotide sequences of *cfr* and *fexA* with their surrounding regions, variant of elastin-binding protein gene (*ebpS-v*), and *sey* were determined by PCR and direct sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an automated DNA sequencer (ABI PRISM 3100). The primers used for sequencing of *cfr/fexA* regions are shown in Table S1. For The multiple alignment of nucleotide/amino acid sequences determined in the present study and those retrieved from the GenBank database was performed by the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/), which was also used for the calculation of sequence identity. Phylogenetic dendrogram of *ebpS/ebpS-v* based on maximum likelihood method was constructed by using the MEGA.6 software package (https://www.megasoftware.net/).

Statistical analysis

Antimicrobial resistance rates between MRSA and MSSA and the proportion of PVL prevalence in MRSA and MSSA were statistically analyzed by Fisher's exact test using the js-STAR XR ver.1.1.9 software (https://www.kisnet.or.jp/nappa/software/star/index.htm). A *P*-value <0.01 was considered statistically significant.

GenBank accession numbers

The nucleotide sequences of *cfr* and *fexA*, including their surrounding regions of an isolate (M98), were deposited in the GenBank database under accession number OR453537 and OR462193, respectively. For sequences of *ebp-S*-variant identified in ST573-MSSA, *sey* detected in *S. argenteus* and MSSA, GenBank accession numbers OR682200-OR682202 were assigned.

Table 1
Prevalence of PVL gene among MRSA, MSSA, and MS *Staphylococcus argenteus* isolates with different *coa* genotypes and SCC*mec* types.

coa genotype	MRSA					MSSA		*S. argenteus*
	No. of isolates	PVL(+)	SCC*mec* type			No. of isolates	PVL(+)	No. of isolates[b]
			IVa	IVg	V			
Ia	4	0	0	1	3	3	0	0
IIa	2	0	2	0	0	1	0	0
IIIa	10	8	10[a]	0	0	44	39	0
IVa	7	1	7	0	0	0	0	0
IVb	21	1	19	0	2	0	0	0
Va	0	0	0	0	0	15	14	0
VIa	8	5	4	0	4	15	10	0
VIIa	2	1	1	0	1	13	2	0
VIIb	0	0	0	0	0	7	0	0
Xa	0	0	0	0	0	5	2	0
XIc	7	0	7	0	0	0	0	0
XId	0	0	0	0	0	0	0	2
XIIIa	0	0	0	0	0	1	1	0
XVI	0	0	0	0	0	5	0	0
Total (n = 172)	61	16	50	1	10	109	68	2

Abbreviations: MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; PVL, Panton-Valentine leukocidin; SCC*mec*, Staphylococcal cassette chromosome *mec*.
[a] One isolate had both PVL genes and ACME-I (USA300 clone);
[b] *S. argenteus* isolates were negative for PVL genes.

Results

CoPS species, coa genotype, prevalence of mecA/*PVL*

A total of 172 presumptive *S. aureus* isolates were collected consecutively from clinical specimens derived from 169 patients. Majority of isolates were derived from pus (44%, n = 76) and wound swab (44%, n = 75), followed by urine (6%, n = 11), blood (3%, n = 5), and sputum (3%, n = 5). The age range of patients was 10 days to 90 years (median age = 45 years), and the ratio of males to females was 1.5 (101 males and 68 females). Among all the presumptive *S. aureus* isolates, two isolates were identified as *S. argenteus*, with the ratio of *S. argenteus* to *S. aureus* being 0.012 (2/170, 1.2% of presumptive *S. aureus*). These *S. argenteus* isolates were derived from urine and pus specimens from two inpatients of MMCH. Sixty-one isolates among *S. aureus* isolates (36%) were judged as MRSA (*mecA*-positive), having SCC*mec* IV (n = 51) or V (n = 10) (Table 1). Thirteen *coa* genotypes were identified for *S. aureus*, with *coa* IIIa and IVb being dominant in MSSA (40%, 44/109) and MRSA (34%, 21/61), respectively. PVL genes were detected in 84 isolates (49%) of all the isolates, and their prevalence was significantly higher in MSSA (62%, 68/109) than in MRSA (26%, 16/61) (P <0.01).

ST, spa type, agr type, PVL phage type, SCCmec type

Further molecular analysis was performed for all the 61 MRSA isolates, 56 MSSA selected from various specimens with different *coa* genotypes, and two *S. argenteus* isolates. A total of 11 STs (7CCs) and 19 STs (8CCs) were identified in MRSA and MSSA isolates, respectively (Tables 2 and 3). Two *S. argenteus* isolates were *mecA*/PVL-negative and genotyped as *coa*-XId, *agr* type I, ST2250, and *spa* t5078. Among MRSA (n = 61), the dominant lineage was ST6 (CC5)-SCC*mec* IVa/V (46%, n = 28; 26-IVa and 2-V), followed by ST88 (CC88)-SCC*mec* IVa (15%, n = 9), ST80-SCC*mec* IVa (11%, n = 7), ST772-SCC*mec* IVa/V (8%, n = 5), and ST361-SCC*mec* V/IV (7%, n = 4). Most of ST6-MRSA-IVa belonged to *spa*-t304 (39% in MRSA; 24/61). PVL genes were commonly identified in ST88-*agr*III (*spa*-t729/t1339/t1855/t2393) and ST772 (CC1)-*agr* II (*spa*-t345/t1839). One MRSA isolate genotyped as ST8/*spa*-t008/*agr*I/*coa*-IIIa harbored PVL genes on Φsa2USA and type I ACME, which are characteristics of the USA300 clone [21]. This isolate was recovered from pus of a 24-year-old female hospitalized in MMCH in October 2022. Among MSSA, PVL genes were detected in nine STs, among which ST8, ST88, ST2884 (CC88), ST121, ST772 (CC1), and ST15 included two or more PVL-positive isolates. PVL phage ΦSa119 was detected in only ST772 isolates, while ΦPVL was commonly identified in other MRSA and MSSA isolates. From specimens of three patients, two isolates with distinct genetic traits were recovered, as shown in Table 4. Either of these co-isolated strains was PVL-positive.

AMR and its determinants

Antimicrobial susceptibility was measured for all the MRSA/MSSA and *S. argenteus* isolates, and resistance rates were compared among them (Table 5). Resistance rates to ERY, CLI, and LVX were comparable between MRSA and MSSA, while a higher rate to ERY and a lower rate to SXT in MRSA were noted compared with MSSA. Among MRSA, ST6-SCC*mec* IVa/V and ST80-SCC*mec* IVa showed resistance to five antimicrobials including ERY and LVX (Table 2). ERY-resistant isolates mostly harbored *erm(C)*, while ST8/*spa* t008 isolate (USA300 clone) and ST1570 (CC398) MSSA harbored *msrA* and *erm(*T), respectively (Table 2 and 3). LVX resistance was more common in CC1, CC5, CC8, CC88, ST121, ST361, and ST1156.

An isolate of ST8-t008-PVL/ACME-negative MSSA (strain M98) was resistant to chloramphenicol (minimal inhibitory concentration [MIC], 64 μg/ml) and florfenicol (MIC, 256 μg/ml), while showing susceptibility to LZD (MIC, 4 μg/ml) and tedizolid (MIC, <0.5 μg/ml). In this isolate, *cfr* and *fexA* were detected, despite being negative for *optrA* and *poxtA*. Genetic context surrounding *fexA* of this isolate showed high identity (98%) to those in chromosome of many *S. aureus* strains, and its most part (87%) was similar to a Tn*558* region of *S. aureus* plasmid (Figure S1[a]). *cfr* region was close to that in plasmid of staphylococci, with highest identity to *S. arlettae* strain SA-01 plasmid (99.7%), having also similarity in sequence/genetic organization to plasmids of *S. cohnii, M. sciuri*, and *S. aureus* (Figure S1[b]).

Virulence factors

Majority of the isolates harbored alpha-, beta-, delta-, and gamma-hemolysin genes (*hla, hlb, hld*, and *hlg*), virulence factor genes attributed to biofilm formation (*fib, icaA, icaD*, etc.), and immune evasion cluster genes (*sak, scn*, and *chp*). Nevertheless, it was noted that *cna* encoding collagen binding protein was found in several limited clones, including CC1, CC5, ST80, CC88, ST121, ST1156, ST1930, and also in

Table 2
Genotypes and AMR/virulence factor profiles of MRSA isolates (n = 61) in this study.

coa genotype	SCC*mec* type	ST	CC	*spa* type	*agr* type	No. of isolates	PVL-positive isolates	PVL-phage	ACME type	AMR profile[b] (%)	AMR genes[c] (%)	Virulence factors genes[d] (%)
Ia	V (3), IVg (1)	ST361	CC361	t315	I	4	0			ERY, LVX (25), FOF (25)	*aph(3′)-IIIa, erm(C)*	*lukDE* (25), *hld* (25), *hlg, fib, ebpS, eno, seg, sei, sem, sen, seo, sak, scn* (50), *chp*
IIa	IVa	ST5	CC5	t6100	II	1	0			ERY, CLI, GEN, ABK, LVX	*aac(6′)-Ie-aph(2′′)-Ia, erm(C)*	*lukDE, hlg, fib, ebpS, eno, seg, sei, sem, sen, seo, seu, sak, scn*
		ST8324[a]		t6100	II	1	0			ERY, LVX	*erm(C)*	*hlg, fib, ebpS, eno, seg, sei, sem, sen, seo, seu, sak, scn, chp*
IIIa	IVa	ST8	CC8	t008	I	1	1	ΦSa2usa	ACME-I	ERY, LVX	*msrA*	*lukDE, hlg, fib, ebpS, eno, sek, seq, sak, scn, chp*
	IVa	ST88	CC88	t690 (1), t729 (1), t1339 (1), t1855 (4), t2393 (2)	III	9	7	ΦPVL		ERY, LVX	*erm(C)*	*lukDE* (88.9), *hlg, fib, ebpS, eno, sak* (55.6), *scn* (88.9), *chp*
IVa/IVb	IVa (26), V (2)	ST6	CC5	t304 (26), t15595 (1), t18457 (1)	I	28	2	ΦPVL		ERY (82.1), CLI (14.3), LVX (60.7), GEN (17.9), ABK (14.3)	*aph(3′)-IIIa* (25), *aac(6′)-Ie-aph(2′′)-Ia* (10.7), *erm(C)* (82.1)	*lukDE, hld, hlg, fib, ebpS, eno, cna, sea, seb* (7.1), *sec* (3.6), *sak, scn*
VIa	V (4), IVa (1)	ST772	CC1	t345 (2), t1839 (3)	II	5	5	ΦSa119		ERY (60), CLI-i (60)	*erm(C)* (60)	*hld* (20), *hlg, ebpS, eno, cna, sea, scn* (80), *chp* (20)
	IVa	ST1930	CC96	t934 (1), t2849 (1), t11023 (1)	III	3	0			ERY, LVX (66.7)	*erm(C)*	*hlg, fib, ebpS, eno, cna, sea, sec, scn* (33.3), *chp* (33.3)
VIIa	V	ST1156		t213	III	1	1	ΦPVL		LVX, SXT		*fib, ebpS, eno, sea, scn, chp*
	IVa	ST2885	CC1	t13849	I	1	0			ERY	*erm(C)*	*ebpS, eno, cna*
XIc	IVa	ST80		t416 (1), t934 (1), t8154 (3), t20819 (2)	III	7	0			ERY (85.7), CLI (28.6), GEN (28.6), ABK (28.6), LVX (71.4)	*aph(3′)-IIIa* (28.6), *aac(6′)-Ie-aph(2′′)-Ia* (28.6), *erm(C)* (85.7)	*lukDE* (42.9), *fib, ebpS, eno, cna* (14.3), *seb, sek, seq, etd, ednB, sak* (85.7), *scn* (85.7), *chp*
Total						61	16					

Abbreviations: ABK, Arbekacin; AMR, antimicrobial resistance; CLI, Clindamycin; DAP, Daptomycin; ERY, Erythromycin; FOF, Fosfomycin; FOX, Cefoxitin; GEN, Gentamycin; LVX, Levofloxacin; LZD, Linezolid; MIN, Minocycline; MRSA, methicillin-resistant *S. aureus*; OXA, Oxacillin; PEN, Penicillin; RIF, Rifampicin; ST, sequence type; SXT, Sulfamethoxazole-Trimethoprim; TEC, Teicoplanin; VAN, Vancomycin.

[a] Novel ST detected in this study;

[b] All the isolates were resistant to PEN, OXA, and FOX, while susceptible to MIN, LZD, DAP, VAN, and TEC. None of isolate showed high-level gentamicin resistance;

[c] *blaZ* was detected in all the isolates. None of the isolate harbored *erm(A), erm(B), aac(6')-Im, ant(3'')- Ia, ant(9)-Ib, aph(2'')-Ic, aph(2'')-Id/Ie, optrA, fexA, cfr,* and *poxtA*;

[d] All isolates had *hla, hlb, eno, clfA, clfB, fnbA, fnbB, selx*, and *selw*.

Table 3
Genotypes and AMR/virulence factor profiles of selected MSSA (n = 56) and MS *S. argenteus* (n = 2) isolates.

MSSA / MS *S. argenteus*	*coa* genotype	ST	CC	*spa* type	*agr* type	No. of isolates	PVL-positive isolates	PVL phage	AMR profile[b] (%)	AMR genes[c] (%)	Virulence factors genes[d] (%)
MSSA	Ia	ST361	CC361	t7262 (1), t3841 (1)	I	2	0		PEN, ERY (50), CLI-i (50), LVX	*blaZ, aph(3′)-Ia, erm*(C) (50)	*lukDE, hlb, hlg, fib, fnbB, eno, ebpS, sec* (50), *seg, sei, sem, sen, seo, sak* (50), *scn* (50), *chp* (50)
	IIa	ST2867		t2016	II	1	0		PEN	*blaZ*	*lukDE, hlb, hld, hlg, fib, fnbB, eno, ebpS, sak, scn, ednB*
	IIIa	ST8	CC8	t008 (1), t121 (1), t2393 (1)	I	3	2	ΦPVL	PEN, ERY, CLI, LVX, SXT (33.3)	*blaZ, ant(4′)-Ia, erm(C)*(33.3), *fexA* (33.3), *cfr* (33.3)	*lukDE* (33.3), *hlb, hld* (33.3), *hlg, fib, fnbB, eno, ebpS, sak, scn*
		ST88	CC88	t448 (3), t2393 (1), t10740 (1)	III	5	4	ΦPVL	PEN (80), ERY (80), LVX (80), SXT (40)	*blaZ* (80), *erm(C)*(80)	*lukDE* (60), *hlb, hld* (60), *hlg* (80), *fib, fnbB, eno, ebpS, cna* (20), *sak, scn, chp*
		ST2884	CC88	t2393 (7), t7263 (1)	III	8	8	ΦPVL	PEN, ERY (87.5), LVX, SXT (12.5)	*blaZ, erm(C)*(87.5)	*lukDE* (75), *hlb, hld* (62.5), *hlg, fib, fnbB, eno* (75), *ebpS, cna* (20), *sak* (87.5), *scn* (87.5), *chp* (87.5)
	Va	ST121	CC121	t159 (1), t270 (1), t645 (1), NT (2)	IV	6	5	ΦPVL (4), NT (1)	PEN, ERY (83.3), LVX	*blaZ, erm(C)*(83.3)	*lukDE, hlb, hld, hlg, fib* (83.3), *fnbB, eno, ebpS-v, seg, sei, sem, sen, seo, seu, sey, cna* (83.3), *sak, scn, chp* (50)
	VIa	ST772	CC1	t345 (3), t1236 (1) t3992 (1)	II	5	3	ΦSa119	PEN, ERY	*blaZ, erm(C)*	*hlb, hld* (60), *hlg, fib, fnbB, eno, ebpS, sea, cna, scn* (60)
		ST573 (ST772 SLV)	CC1	t345	II	1	0		PEN, ERY, LVX	*blaZ, erm(C)*	*lukDE, hlb, hld, hlg, fnbB, eno, ebpS-v, sec, seg, sei, sem, sen, seo, cna*
		ST8617[a] (ST772 SLV)	CC1	t9498	II	1	0		PEN, ERY, LVX	*blaZ, erm(C)*	*lukDE, hlb, hld, hlg, fib, fnbB, eno, ebpS, sea, cna, sak, scn, chp*
	VIIa	ST1	CC1	t127	III	1	0		PEN, LVX	*blaZ*	*hlb, hlg, fib, fnbB, eno, ebpS, sea, seh, sek, seq, sak, scn*
		ST1156		t213	I	4	0		PEN, ERY (50), LVX (75), FOF (25)	*blaZ, erm(C)* (50)	*lukDE* (75), *hlb, hld* (75), *hlg* (25), *fib, fnbB, eno, ebpS, sea* (50), *cna* (20), *sak* (25), *scn* (50), *chp* (50)
		ST2233		t2663	III	1	1	NT	PEN, ERY	*blaZ, erm(C)*	*lukDE, hlb, hlg, eno*
	VIIb	ST291 (ST398 DLV)	CC398	t1149	I	4	0		PEN, ERY (50), LVX (25)	*blaZ, erm(C)* (50)	*lukDE* (75), *hlb, hld* (75), *hlg, fnbB, eno* (75), *ebpS, etd*(75), *ednB, sak, scn* (25), *chp* (75)
		ST1570 (ST398 SLV)	CC398	t6606	I	2	0		ERY	*erm(T)*	*lukDE* (50), *hlb* (50), *hlg, fnbB* (50), *eno* (50), *ebpS, cna* (50), *sak* (50), *scn* (50), *chp* (50)
		ST7565 (ST398 TLV)	CC398	t1250	I	1	0		PEN, CLI	*blaZ*	*hlb, hld, hlg, fib, fnbB, eno, ebpS, cna, sak, chp*
	Xa	ST15	CC15	t368 (1), t1509 (1), t4188 (1), t15578 (1)	II	4	2	ΦPVL	PEN (75), ERY (25), LVX (25)	*blaZ* (75), *aph(3′)-Ia, erm(C)* (25)	*lukDE* (25), *hlb, hlg, fib, fnbB, eno, ebpS, seb* (25), *cna* (25), *sak* (50), *scn* (25), *chp* (75)
	XIc	ST22		t223	I	1	1	ΦPVL	PEN	*blaZ*	*hlb, hlg, fnbB, eno, ebpS, cna, sec, seg, sei, sem, sen, seo, seu, tst-1, sak, scn, chp*
	XIIIa	ST1153	CC97	t903	II	1	1	NT	PEN, ERY	*blaZ, aph(3′)-Ia*	*hlb, hlg, fib, fnbB, eno, ebpS, sek, seq*
	XVI	ST1290	CC1	t131	I	5	0		PEN, ERY (60), LVX (25)	*blaZ*	*lukDE* (20), *hlb, hld* (20), *fib, fnbB, eno, ebpS, eta* (60) *sak* (60), *scn* (40), *chp* (60)
MS *S. argenteus*	XId	ST2250		t5078	I	2	0		All susceptible		*hlb, hld, hlg, fnbB, eno, ebpS, sey, cna, sak, scn*

Abbreviations: ABK, Arbekacin; AMR, antimicrobial resistance; CLI, Clindamycin; DAP, Daptomycin; DLV, Double-locus variant; ERY, Erythromycin; FOF, Fosfomycin; FOX, Cefoxitin; GEN, Gentamycin; LVX, Levofloxacin; LZD, Linezolid; MIN, Minocycline; MRSA, methicillin-resistant *S. aureus*; OXA, Oxacillin; PEN, Penicillin; RIF, Rifampicin; SLV, Single-locus variant; ST, sequence type; SXT, Sulfamethoxazole-Trimethoprim; TEC, Teicoplanin; VAN, Vancomycin.

[a] Novel ST detected in this study;

[b] All the isolates were susceptible to MIN, LZD, ABK, FOF, DAP, VAN, TEC, and RIF. None of isolate showed high-level gentamicin resistance;

[c] None of the isolate harbored *erm(A), erm(B), msrA, aac(6')-Im, ant(3'')- Ia, ant(9)-Ib, aph(2'')-Ic, aph(2'')-Id/Ie, optrA*, and *poxtA*;

[d] All isolates had *hla, icaA, icaD, clfA, clfB, fnbA, selx,* and *selw*. *ebpS-v* is a variant of *ebpS* encoding elastin binding protein.

Table 4
S. aureus / *S. argenteus* co-isolated from a single specimen of a patient in this study.

Subject no.	Age / Sex	Specimen	Isolate ID	Results of initial PCRs[a]	*coa* genotype	ST (CC)[b]	*spa* type[b]	*agr* type	AMR profile	AMR genes
1	11/F	pus	M115	MRSA, PVL(+)	VIa	ST772	t345	II	OXA, FOX, PEN, ERY	*blaZ, erm(C)*
			M115-1	MS *S. argenteus*	XId	ST2250	t5078	I	All susceptible	
2	52/M	wound	M170-1	MRSA, PVL(+)	IIIa	ST8	t121	I	OXA, FOX, PEN, ERY, LVX	*blaZ, erm(C)*
			M170-2	MRSA, PVL(+)	Va	ST121	NT	IV	OXA, FOX, PEN, ERY	*blaZ, erm(C)*
3	70/M	sputum	M215-1	MSSA, PVL(+)	IIIa	ST88 (CC88)	t2393	III	PEN, ERY, LVX	*blaZ, erm(C)*
			M215-2	MSSA	VIIb	ST1570 (ST398 SLV)	t6606	I	ERY	

MRSA, methicillin-resistant *S. aureus*; MRSA, methicillin-susceptible *S. aureus*; PCR, polymerase chain reaction.
[a] Initial PCRs include two PCR assays, one for detection of staphylococcal 16S rRNA, *nuc, mecA*, PVL genes, and ACME-*arcA*, and the other for discrimination of *S. aureus* and *S. argenteus* by NRPS gene (see Materials and Methods section);
[b] Abbreviations; ST, sequence type; CC, clonal complex; SLV, single-locus variant; NT, non-typable.

Table 5
Antimicrobial resistance rate among MRSA, MSSA, MS *S. argenteus* isolates.

Antimicrobials (MIC Breakpoint, μg/ml)	MRSA (n = 61) (%)	MSSA (n = 109) (%)	MS *S. argenteus* (n = 2) (%)
PEN (≥0.25)	61 (100)	100 (91.7)	0 (0)
OXA (≥4)	61 (100)	0 (0)	0 (0)
FOX (>8)	61 (100)	0 (0)	0 (0)
ERY (≥8)	52 (85.2[a])	75 (68.8)	0 (0)
CLI (≥4)	9 (14.8)	6 (5.5)	0 (0)
GEN (≥16)	8 (13.1)	0 (0)	0 (0)
ABK (≥4)	7 (11.4)	0 (0)	0 (0)
LVX (≥4)	40 (65.6)	61 (56)	0 (0)
SXT (≥4/76)	1 (1.6)	14 (12.8)	0 (0)
FOF (≥32)	1 (1.6)	2 (1.8)	0 (0)

Abbreviations: see footnote of Table 2. MRSA, methicillin-resistant *S. aureus*; MRSA, methicillin-susceptible *S. aureus*.
[a] Significantly higher rate than in MSSA (P <0.01).None of the isolates showed resistance to MIN (≥16 μg/ml), RIF (≥4 μg/ml), VAN (≥16 μg/ml), TEC (≥32 μg/ml), DAP (≥2 μg/ml), LZD (≥8 μg/ml), and high-level resistance to GEN (≥1000 μg/ml) (not shown in this Table).For antimicrobials whose breakpoints are not defined by CLSI guidelines, we employed the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint for FOF (32 μg/ml, *Staphylococcus* spp.), and a unique breakpoint for ABK (4 μg/ml, which is higher than the 2 μg/ml defined by the Japanese Society of Chemotherapy for respiratory infection.

S. argenteus (ST2250). Enterotoxin gene cluster *seg-sei-sem-sen-seo-*(*seu*) was detected in CC5 (ST5, ST8324), ST22, ST121, ST361, and ST573 (ST772 single-locus variant (SLV)). Although a rare enterotoxin gene *sey* was detected in ST121 and ST2250, its sequence was slightly divergent (98% identity) between these two STs (Table S2). Genetic cluster encoding exfoliative toxin D-epidermal cell differentiation inhibitor B (*etd-ednB*) was detected in only ST80-MRSA-IVa and ST291 (CC398) MSSA, while another exfoliative toxin gene *eta* was identified in 60% of ST1290 isolates. Although elastin-binding protein gene (*ebpS*) was detected in all the isolates, its shorter variant with internal deletion (*ebpS-v*) was identified in ST121 and ST573 (CC1, ST772-SLV) MSSA. Because *ebpS-v* was first identified in ST573 (strain M41), its sequence was determined and phylogenetically analyzed with those from other *S. aureus* lineages. ST573 (strain M41) *ebpS-v* is included in clade I, along with *ebpS* of CC1, CC5, and CC8 strains (Figure S2). Alignment of deduced Ebps amino acid sequence of ST573 with other Ebps(-v) indicated that strain M41 lacked 60-amino acid sequence including presumptive hydrophobic domain H1, at the same position of deletion as EbpS-v of ST22 and ST121 [22,23] (Figure S3).

Discussion

The present study first revealed the clonal structure of MRSA clinical isolates in Bangladesh, because previous studies reported profiles of only representative isolates from some infection types [7,8,24]. Proportion of MRSA among all the *S. aureus* isolates (36%) was comparable to that reported previously for isolates from skin/soft tissue infections in Bangladesh (31%, 2015-2016) [8]. Similarly, higher proportion of SCC*mec* IV than V and a higher prevalence of PVL among MSSA than MRSA were observed in the present study. However, it was notable that ST6-MRSA-IVa belonging to mostly *spa*-t304 was dominant, accounting for 46% of MRSA. These ST6-IVa isolates were obtained from both study sites (eight and 20 isolates from MMCH and BIHSH, respectively), while this genotype was described in Bangladesh previously (from 2010 until 2016) as a minor clone [8,24]. Therefore, the present dominance of ST6-IVa suggests its increasing trend within the country. ST6-MRSA-IV, which was originally known as WA MRSA-51 in Australia [25], has been distributed to Middle East countries [26,27], with ST6-IV/t304 being described as a dominant clone in Oman [27]. In northern European countries including Denmark, this clone is recognized as emerging clone presumably brought from the Middle East via flow of people [28] and also isolated from bovine mastitis and wild animal [29,30], indicating its successful spread. Therefore, movement of people between Bangladesh and the Middle East is suggested to be one of the reasons for the dominance of this clone in Bangladesh. *coa* XVI, which was recently reported as novel type in Myanmar [31], was detected in five MSSA isolates, associated with the same genetic traits (ST1290(CC1)/*spa*-t131). This may suggest the local prevalence of this rare clone in an area including Bangladesh and Myanmar.

Other common MRSA clones (ST88, ST772, ST80, and ST361) were also described in Bangladesh [8,24], indicating their constant prevalence. In the present study, one MRSA isolate was genotyped as ST8/*spa*-t008/*agr*-I/*coa*-IIIa, harboring Φsa2USA and type I ACME, which are characteristics of the USA300 clone [21]. This identification of the

USA300 clone is the second report in this country followed by first detection in Dhaka (2008-2009) [7], suggesting its persistence at low prevalence. In the previous study in Dhaka in 2008-2009, we identified *etd*-positive ST1931 (CC80) MRSA-IVa at high rate among MRSA from skin lesion of diabetic patients [7], which was genetically related to European MRSA clone [32]. Although ST1931 was not detected in the present study, ST80-IVa harboring *etd-edinB* was detected in seven isolates (11%, 7/61) that were all derived from pus or wound swab. Same clone was isolated from skin/soft tissue infections in MMCH, in 2015-2016 [8]. Thus, CC80 MRSA-IVa with *etd* has still been a common cause of skin infection, indicating the endemicity of this *etd*-carrying clone in Bangladesh.

Among AMR traits, it was remarkable to find an ST8/t008 MSSA (strain M98) harboring *cfr* and *fexA*, which were suggested to reside in different genetic loci by genetic analysis. Co-detection of *cfr* and *fexA* mediated by plasmid has been reported for mostly livestock-associated clones (e.g., CC398) from animals [33]. However, only a few studies in Europe (e.g., France) described detection in humans of *cfr* in ST8-MRSA [34]. BLAST search revealed that the Cfr amino acid sequence of M98 was identical to ST9 MRSA strain from bovine mastitis in China that showed susceptibility to LZD [35]. Because the association of *cfr/fexA* with animals is suggested, monitoring may be necessary for *cfr*-carrying *S. aureus* in humans and livestock to control the spread of oxazolidinone resistance.

EbpS is one of the adhesins on cell surface that binds to host cellular matrix factors involved in biofilm formation produced by most of all *S. aureus* clinical isolates. The shorter variant EbpS-v with internal deletion has been reported for ST121 and ST22 *S. aureus* strains [8,22,23]. Although the functional affect by the deletion has not been clarified, ST121 and ST22 have been recognized as one of the lineages of hypervirulent and pandemic clones [25]. In the present study, *ebpS-v* was newly detected in distinct lineage CC1 (ST573, SLV of ST772) and revealed to be presumably originated from intact *ebpS* of CC1. Attention may be noted for the emergence of *ebpS-v* among *S. aureus* and its association with virulence.

In the present study, *S. argenteus* was first identified in Bangladesh, and its genotype ST2250 was globally most common [2]. The prevalence of this species in the present study (1.2% of presumptive *S. aureus*) was considerably low as those seen in East Asia and Europe. One of the *S. argenteus* isolates was co-recovered from pus with PVL-positive MRSA. Co-isolation of *S. aureus* and *S. argenteus* was also reported in our recent study in Myanmar [31]. Though we detected only three cases in our present study, such mixed infection with different clones is considered to increase the risk for transmission of resistance/virulence traits among *S. aureus* isolates and may cause confusion in characterization of isolates.

In conclusion, we first comprehensively described the clonal structure and genetic characteristics of CoPS clinical isolates in Bangladesh and revealed the dominance of ST6-MRSA-IVa/t304. Further study is necessary for the trend of CoPS clones associated with the change in AMR and prevalence of virulence factors.

Declarations of competing interest

The authors have no competing interests to declare.

CRediT authorship contribution statement

Sangjukta Roy: Conceptualization, Methodology, Investigation. **Meiji Soe Aung:** Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing, Funding acquisition. **Shyamal Kumar Paul:** Resources. **Syeda Anjuman Nasreen:** Resources. **Nazia Haque:** Resources. **Rummana Mazid:** Resources. **Md. Shahed Khan:** Resources. **Tridip Kanti Barman:** Resources. **Parvez Arafa:** Resources. **Fardousi Akter Sathi:** Resources. **Sultana Shabnam Nila:** Resources. **Afsana Jahan:** Resources. **Noriko Urushibara:** Investigation, Funding acquisition. **Mitsuyo Kawaguchiya:** Investigation. **Nobuhide Ohashi:** Investigation. **Nobumichi Kobayashi:** Conceptualization, Methodology, Data curation, Writing – review & editing, Funding acquisition, Supervision, Project administration.

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Ethical approval

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Mymensingh Medical College (IRB approval no. MMC/IRB/2022/510).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2023.12.006.

References

[1] Lakhundi S, Zhang K. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. *Clin Microbiol Rev* 2018;**31** e00020-18. doi:10.1128/CMR.00020-18.

[2] Becker K, Schaumburg F, Kearns A, Larsen AR, Lindsay JA, Skov RL, et al. 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* 2019;**25**:1064–70. doi:10.1016/j.cmi.2019.02.028.

[3] David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev* 2010;**23**:616–87. doi:10.1128/CMR.00081-09.

[4] Boyle-Vavra S, Daum RS. Community-acquired methicillin-resistant Staphylococcus aureus: the role of Panton-Valentine leukocidin. *Lab Invest* 2007;**87**:3–9. doi:10.1038/labinvest.3700501.

[5] Afroz S, Kobayashi N, Nagashima S, Alam MM, Hossain AB, Rahman MA, et al. Genetic characterization of *Staphylococcus aureus* isolates carrying Panton-Valentine leukocidin genes in Bangladesh. *Jpn J Infect Dis* 2008;**61**:393–6 PMID: 18806351. doi:10.7883/yoken.JJID.2008.393.

[6] Steinig EJ, Duchene S, Robinson DA, Monecke S, Yokoyama M, Laabei M, et al. Evolution and global transmission of a multidrug-resistant, community-associated methicillin-resistant *Staphylococcus aureus* lineage from the Indian subcontinent. *mBio* 2019;**10**:e01105–19. doi:10.1128/mBio.01105-19.

[7] Paul SK, Ghosh S, Kawaguchiya M, Urushibara N, Hossain MA, Ahmed S, et al. Detection and genetic characterization of PVL-positive ST8-MRSA-IVa and exfoliative toxin D-positive European CA-MRSA-Like ST1931 (CC80) MRSA-IVa strains in Bangladesh. *Microb Drug Resist* 2014;**20**:325–36. doi:10.1089/mdr.2013.0153.

[8] Haque N, Aung MS, Paul SK, Bari MS, Ahmed S, Sarkar SR, et al. Molecular epidemiological characterization of methicillin-susceptible and -resistant *Staphylococcus aureus* isolated from skin and soft tissue infections in Bangladesh. *Microb Drug Resist* 2019;**25**:241–50. doi:10.1089/mdr.2018.0123.

[9] Tong SYC, Schaumburg F, Ellington MJ, Corander J, Pichon B, Leendertz F, et al. 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* 2015;**65**:15–22. doi:10.1099/ijs.0.062752-0.

[10] Chantratita N, Wikraiphat C, Tandhavanant S, Wongsuvan G, Ariyaprasert P, Suntornsut P, et al. Comparison of community-onset *Staphylococcus argenteus* and *Staphylococcus aureus* sepsis in Thailand: A prospective multicentre observational study. *Clin Microbiol Infect* 2016;**22** 458.e11–e458.e19. doi:10.1016/j.cmi.2016.01.008.

[11] Aung MS, San T, San N, Oo WM, Ko PM, Thet KT, et al. 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* 2019;**68**:95–104. doi:10.1099/jmm.0.000869.

[12] Clinical and Laboratory Standards Institute (CLSI) *Performance standards for antimicrobial susceptibility testing*, Wayne: Clinical and Laboratory Standards Institute; 2022. M100-Ed32.

[13] Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for simultaneous identification of community-associated methicillin-resistant *Staphylococcus aureus* strains USA300 and USA400 and detection of *mecA* and Panton-Valentine leukocidin genes, with discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. *J Clin Microbiol* 2008;**46**:1118–22. doi:10.1128/JCM.01309-07.

[14] Aung MS, Kawaguchiya M, Urushibara N, Sumi A, Ito M, Kudo K, et al. Molecular characterization of methicillin-resistant *Staphylococcus aureus* from outpatients in Northern Japan: increasing tendency of ST5/ST764 MRSA-IIa

with arginine catabolic mobile element. *Microb Drug Resist* 2017;**23**:616–25. doi:10.1089/mdr.2016.0176.

[15] Zhang DF, Xu X, Song Q, Bai Y, Zhang Y, Song M, et al. Identification of *Staphylococcus argenteus* in Eastern China based on a nonribosomal peptide synthetase (NRPS) gene. *Future Microbiol* 2016;**11**:1113–21. doi:10.2217/fmb-2016-0017.

[16] Boakes E, Kearns AM, Ganner M, Perry C, Hill RL, Ellington MJ. Distinct bacteriophages encoding Panton-Valentine leukocidin (PVL) among international methicillin-resistant Staphylococcus aureus clones harboring PVL. *J Clin Microbiol* 2011;**49**:684–92. doi:10.1128/JCM.01917-10.

[17] Aung MS, San T, Urushibara N, San N, Oo WM, Soe PE, et al. Molecular characterization of methicillin-susceptible and -resistant *Staphylococcus aureus* harboring panton-valentine leukocidin-encoding bacteriophages in a tertiary Care Hospital in myanmar. *Microb Drug Resist* 2020;**26**:360–7. doi:10.1089/mdr.2019.0208.

[18] Urushibara N, Kawaguchiya M, Kobayashi N. Two novel arginine catabolic mobile elements and staphylococcal chromosome cassette *mec* composite islands in community-acquired methicillin-resistant Staphylococcus aureus genotypes ST5-MRSA-V and ST5-MRSA-II. *J Antimicrob Chemother* 2012;**67**:1828–34. doi:10.1093/jac/dks157.

[19] Aung MS, Urushibara N, Kawaguchiya M, Sumi A, Shinagawa M, Takahashi S, et al. Clonal diversity and genetic characteristics of methicillin-resistant *Staphylococcus aureus* isolates from a Tertiary Care Hospital in Japan. *Microb Drug Resist* 2019;**25**:1164–75. doi:10.1089/mdr.2018.0468.

[20] Aung MS, Urushibara N, Kawaguchiya M, Hirose M, Ito M, Habadera S, et al. Clonal diversity of methicillin-resistant Staphylococcus aureus (MRSA) from bloodstream infections in northern Japan: identification of spermidine N-acetyltransferase gene (*speG*) in staphylococcal cassette chromosomes (SCCs) associated with type II and IV SCC*mec*. *J Glob Antimicrob Resist* 2021;**24**:207–14. doi:10.1016/j.jgar.2020.12.008.

[21] Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. *Lancet* 2006;**367**:731–9. doi:10.1016/S0140-6736(06)68231-7.

[22] Aung MS, Urushibara N, Kawaguchiya M, Aung TS, Mya S, San T, et al. Virulence factors and genetic characteristics of methicillin-resistant and -susceptible *Staphylococcus aureus* isolates in Myanmar. *Microb Drug Resist* 2011;**17**:525–35. doi:10.1089/mdr.2011.0061.

[23] Pokhrel RH, Aung MS, Thapa B, Chaudhary R, Mishra SK, Kawaguchiya M, et al. Detection of ST772 Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* (Bengal Bay clone) and ST22 *S. aureus* isolates with a genetic variant of elastin binding protein in Nepal. *New Microbes New Infect* 2016;**11**:20–7. doi:10.1016/j.nmni.2016.02.001.

[24] Ahmed S, Kawaguchiya M, Ghosh S, Paul SK, Urushibara N, Mahmud C, et al. Drug resistance and molecular epidemiology of aerobic bacteria isolated from puerperal infections in Bangladesh. *Microb Drug Resist* 2015;**21**:297–306. doi:10.1089/mdr.2014.0219.

[25] Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant Staphylococcus aureus. *PLoS One* 2011;**6**:e17936. doi:10.1371/journal.pone.0017936.

[26] Monecke S, Skakni L, Hasan R, Ruppelt A, Ghazal SS, Hakawi A, et al. Characterisation of MRSA strains isolated from patients in a hospital in Riyadh, Kingdom of Saudi Arabia. *BMC Microbiol* 2012;**12**:146. doi:10.1186/1471-2180-12-146.

[27] Udo EE, Al-Lawati BA, Al-Muharmi Z, Thukral SS. Genotyping of methicillin-resistant *Staphylococcus aureus* in the Sultan Qaboos University Hospital, Oman reveals the dominance of Panton-Valentine leucocidin-negative ST6-IV/t304 clone. *New Microbes New Infect* 2014;**2**:100–5. doi:10.1002/nmi2.47.

[28] Bartels MD, Worning P, Andersen LP, Bes M, Enger H, Ås CG, et al. Repeated introduction and spread of the MRSA clone t304/ST6 in northern Europe. *Clin Microbiol Infect* 2021;**27** 284.e1–5. doi:10.1016/j.cmi.2020.05.004.

[29] Kløve DC, Jensen VF, Astrup LB. First finding of a methicillin-Resistant *Staphylococcus aureus* (MRSA) t304/ST6 from bovine clinical mastitis. *Antibiotics (Basel)* 2022;**11**:1393. doi:10.3390/antibiotics11101393.

[30] Venla J, Ahmad AM, Viivi H, Laura L, Saara S, Sanna S, et al. Clonal dissemination of successful emerging clone *mecA*-MRSA t304/ST6 among humans and hedgehogs in the Helsinki metropolitan area in Finland. *One Health* 2023;**16**:100516. doi:10.1016/j.onehlt.2023.100516.

[31] Kyaw WK, Aung MS, San T, Maw WW, Mu KK, Mon WLY, et al. Molecular epidemiological characterization of *Staphylococcus aureus* and *Staphylococcus argenteus* clinical isolates from a national tertiary Care Hospital in myanmar: co-isolation of multiple clones and identification of novel staphylocoagulase genotype. *Microb Drug Resist* 2023;**29**:127–37. doi:10.1089/mdr.2022.0191.

[32] Edslev SM, Westh H, Andersen PS, Skov R, Kobayashi N, Bartels MD, et al. Identification of a PVL-negative SCC*mec*-IVa sublineage of the methicillin-resistant *Staphylococcus aureus* CC80 lineage: understanding the clonal origin of CA-MRSA. *Clin Microbiol Infect* 2018;**24**:273–8. doi:10.1016/j.cmi.2017.06.022.

[33] Schouls LM, Veldman K, Brouwer MSM, Dierikx C, Witteveen S, van Santen-Verheuvel M, et al. *cfr* and *fexA* genes in methicillin-resistant Staphylococcus aureus from humans and livestock in the Netherlands. *Commun Med (Lond)* 2022;**2**:135. doi:10.1038/s43856-022-00200-w.

[34] Youenou B, Martins Simoes P, Tristan A, Farfour E, Beauruelle C, et al. Linezolid resistance: detection of the *cfr(B)* gene in French clinical MRSA strains. *J Antimicrob Chemother* 2023;**78**:445–9. doi:10.1093/jac/dkac411.

[35] Wang XM, Zhang WJ, Schwarz S, Yu SY, Liu H, Si W, et al. Methicillin-resistant *Staphylococcus aureus* ST9 from a case of bovine mastitis carries the genes *cfr* and *erm(A)* on a small plasmid. *J Antimicrob Chemother* 2012;**67**:1287–9. doi:10.1093/jac/dks028.