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Antibiotic resistance profile and molecular characterization of *Staphylococcus aureus* strains isolated in hospitals in Kabul, Afghanistan

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Abstract

The aim of this study was to investigate the molecular features and the antibiotic resistance profile of 98 clinical *Staphylococcus aureus* isolates collected during 6 months in two hospitals of Kabul, Afghanistan. For all isolates, antimicrobial resistance patterns were determined by the disc diffusion method (including methicillin resistance which was detected using cefoxitin). The presence of the *mec*A/*mec*C genes was detected by PCR. Strains were then extensively characterized using microarray analysis. Of the 98 *S. aureus* isolates, methicillin-resistant *S. aureus* (MRSA) prevalence was high at 66.3%. Antibiotic susceptibility testing also revealed a high resistance rate to penicillin (100%), erythromycin (66.3%), ciprofloxacin (55.1%), and cotrimoxazole (40.8%). Resistance to tobramycin was detected in 25.5%, to gentamicin in 16.3%, to chloramphenicol in 34.7%, and to doxycycline in 23.5% of the isolates. All the MRSA isolates were *mec*A-positive and none of them harbored *mec*C. Isolates were grouped into twelve clonal complexes and twenty-seven distinct clones. The most frequently detected clones were the Southwest Pacific clone (CC30-MRSA-IV PVL+) (21/65 MRSA, 32.3%), the CC22-MRSA-IV TSST-1+ clone (11/65 MRSA, 16.9%), and the Bengal Bay clone (ST772-MRSA-V PVL+) (11/65 MRSA, 16.9%). The PVL genes were found in 59.2% (46/65 MRSA and 12/33 methicillin-susceptible *S. aureus*, MSSA) and *tst1* gene in 16.3% of isolates. This molecular study highlights the high prevalence of MRSA and the large genetic diversity of the *S. aureus* isolates circulating and detected in two hospitals of Kabul, with the presence of multiple virulence and antibiotic resistance genes.

Keywords *Staphylococus aureus* · MRSA · Antibiotic resistance · Molecular typing · Kabul · Afghanistan

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Introduction

Staphylococcus aureus is a major human pathogen responsible for serious infections in both community and hospital settings [1]. *S. aureus* can cause a wide range of infections, from minor skin infections, such as carbuncles, folliculitis, and impetigo, to deep-seated (abscesses or cellulitis) or life-threatening infections such as pneumonia, osteomyelitis, bacteremia, toxic shock syndrome, or endocarditis. The global emergence and spread of methicillin-resistant *S. aureus* (MRSA) in hospitals since the 1960s are a public health concern and limit the therapeutic arsenal for severe staphylococcal infections. Indeed, healthcare-associated (HA-) MRSA are usually resistant to most antimicrobial agents. In the case of severe infections due to these multi-resistant strains, glycopeptides are often the drugs of last resort; however, isolates with reduced susceptibility to vancomycin are frequently reported [2], and some vancomycin-resistant *S. aureus* (VRSA)

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strains harboring the *van*A gene from enterococci have also been described but are very rare [3]. On the other hand, the emergence and spread of community-acquired (CA-) MRSA clones expressing the Panton-Valentine leukocidin (PVL) in the last two decades are an additional cause for concern and have changed the epidemiology of MRSA [4, 5]. These strains are generally associated with recurrent skin and soft tissue infections (SSTIs) but can also cause necrotizing pneumonia or destructive bone and joint infection [6]. Interestingly, some CA-MRSA clones predominate in geographically restricted areas while others have achieved a pandemic level.

In central Asia, including Afghanistan, studies about MRSA have mainly focused on the prevalence of MRSA, but few data are available about the circulating clones. In this part of the world, *S. aureus* infections in healthcare settings show a high prevalence of MRSA [7]. For example, in Pakistan, a multicentric study in four hospitals showed a MRSA rate of 41.9% in 2006–2008 [8]. In India, two multicentric studies described comparable MRSA rates of 41% and 45% in 2008–2009 and 2011, respectively [9, 10]. In Iran, a systematic review reported a mean MRSA rate of 43.0% between 2000 and 2016 [11] and several VRSA isolates were described [12]. More specifically, in Afghanistan, a recent study concerning *S. aureus* infections diagnosed in Kabul hospitals highlighted a high rate of methicillin resistance (56.2%) and an increase in the prevalence of multidrug-resistant (MDR)-MRSA compared to the neighboring countries [13]. In parallel, an overuse of antimicrobials was described during the last decade in primary healthcare clinics of five major provinces of Afghanistan, including Kabul [14] and, in 2014, some authors raised the question of over prescription of antibiotics in a district hospital in Kabul where more than 50% of out-patients were prescribed at least one antibiotic [15]. This widespread use of antimicrobial therapies could have contributed to the increase of MRSA prevalence in Afghanistan and the selection of MDR clones.

Nevertheless, although molecular typing data for *S. aureus* and MRSA are abundantly available for Western Europe, North America, or Australia, few studies have been conducting to describe *S. aureus* epidemiology in the rest of the world, including Central Asia and more specifically, Afghanistan. In this context, the aim of this work was to investigate the molecular features of *S. aureus* strains isolated from clinical samples in two hospitals of Kabul, as well as their antibiotic resistance profile.

Materials and methods

Strains collection

From January to June 2017, all *S. aureus* isolates cultured from in- and out-patient samples in two hospitals located in the center of Kabul (Maiwand and Ibn-Sina hospitals) were collected. During the 6-month study period, a total of 98 isolates of *S. aureus* obtained from 98 individual patients were characterized. The strains were isolated from various types of clinical samples: pus (skin and soft tissue infections; 65.3%), ear pus (18.4%), blood (7.1%), urine (3.1%), vaginal swab (3.1%), tracheal aspirations (2%), and semen (1%). Out of the 98 patients, 33 (33.7%) were women and 65 (66.3%) were men. The mean age was 19 years ([1–75] years, median 12 years).

Phenotypic assays

Standard microbiological procedures were conducted on clinical samples with minimum delay for culture, confirmatory tests, and antibiotic susceptibility testing (AST). Presumptive *S. aureus* isolates were subcultured for 18–24 h at 37 °C onto blood agar base medium (Oxoid, Basingstoke, UK) supplemented with 5% sheep blood. Confirmatory tests were carried out for the identification of *S. aureus* isolates, by performing Pastorex Staph Plus (Bio-Rad, Marnes-la-Coquette, France) and coagulase assay. AST was performed on Mueller-Hinton agar (Oxoid) by Kirby Bauer disc diffusion method according to the 2017 guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [16]. The following panel of antibiotics was tested: penicillin G, cefoxitin (used to detect methicillin/oxacillin resistance), tobramycin, gentamicin, erythromycin, clindamycin, ciprofloxacin, chloramphenicol, doxycycline, rifampicin, and cotrimoxazole.

Detection of *mec*A/*mec*C genes and *agr* typing by multiplex PCR

Cellular DNA was obtained from *S. aureus* colonies grown overnight on blood agar plates using DNA Extraction Kit (Promega, USA) in accordance with the manufacturer's instructions. Detection of the *mec*A and *mec*C genes and *agr* typing were performed by multiplex PCR using primers already published [17–19].

Molecular characterization of strains by DNA microarray

DNA was extracted and purified using commercial extraction kits (DNeasy kit and Promega instrument; Qiagen, Hilden, Germany), according to the manufacturer's protocol. The DNA microarray Identibac *S. aureus* genotyping® (Alere Technologies, Jena, Germany) was used as previously described [20]. This microarray allows the detection of 336 different target sequences corresponding to 185 genes and their allelic variants. The assignation of isolates to clonal complexes (CCs) was determined by comparison of the hybridization profiles to previously typed multi-locus sequence typing reference strains [20].

Statistical analysis

Statistical analysis was done using SPSS 21 (IBM Inc., Chicago). Binary logistic regression was used to determine the association between MRSA or MSSA status, gender, and age. Chi-square test was used to compare the resistance profiles and the presence of virulence genes between MRSA and MSSA (methicillin-susceptible *S. aureus*). A *p* value less than 0.05 was considered statistically significant.

Results

Antibiotic susceptibility testing

Out of the 98 *S. aureus* isolates, all were resistant to penicillin and 65 (66.3%) were methicillin-resistant (Table 1). Twenty-five (25.5%) were resistant to tobramycin, 16 (16.3%) to gentamicin, 65 (66.3%) to erythromycin, 11 (11.2%) to clindamycin, 54 (55.1%) to ciprofloxacin, 34 (34.7%) to chloramphenicol, 23 (23.5%) to doxycycline, 3 (3.1%) to rifampicin, and 40 (40.8%) to cotrimoxazole. MRSA isolates were statistically more frequently resistant to gentamicin (*p* = 0.002), erythromycin (*p* < 0.001), ciprofloxacin (*p* < 0.001), and cotrimoxazole (*p* = 0.017) than MSSA (Table 1). The difference of MRSA distribution was not statistically significant according to gender (*p* = 0.39) and age (*p* = 0.25).

Molecular characterization of *S. aureus* isolates

Various resistance genes were detected in the 98 collected isolates: *bla*Z in almost all isolates (97/98, 99%), *msr*A and *mph*C in 43/98 (43.9%), *erm*C in 16/98 (16.3%), *aph*A3 in 44/98 (44.9%), *aad*D in 22/98 (22.4%), *aac*A-*aph*D in 16/98

Table 1 Antibiotic resistance of the 98 *S. aureus* isolates collected during 6 months, from January to June 2017, in two hospitals in Kabul (MSSA, *n* = 33, and MRSA, *n* = 65)

Antibiotic	All isolates *n* (%)	MRSA *n* (%)	MSSA *n* (%)	*p* value
Penicillin G	98 (100)	65 (100)	33 (100)	-
Tobramycin	25 (25.5)	19 (29.2)	6 (18.2)	0.236
Gentamicin	16 (16.3)	16 (24.6)	0 (0)	*0.002*
Erythromycin	65 (66.3)	51 (78.5)	14 (42.4)	*< 0.001*
Clindamycin	11 (11.2)	9 (13.8)	2 (6.1)	0.249
Ciprofloxacin	54 (55.1)	45 (69.2)	9 (27.3)	*< 0.001*
Chloramphenicol	34 (34.7)	24 (36.9)	10 (30.3)	0.515
Doxycycline	23 (23.5)	16 (24.6)	7 (21.2)	0.707
Rifampicin	3 (3.1)	2 (3.1)	1 (3.0)	0.99
Cotrimoxazole	40 (40.8)	32 (49.2)	8 (24.2)	*0.017*

Significant *p* values are shown in italic

(16.3%), and *tet*K in 39/98 isolates (39.8%) (Table 2). No strain harbored the resistance genes *van*A, *qac*A, *qac*C, *fus*B, *mup*R, and *cfr*. The presence of *msr*A, *mph*C, *aac*A-*aph*D, *aph*A3, *sat*, and *dfr*A was higher in MRSA than in MSSA whereas the presence of *lnu*A, *tet*K, and *tet*Efflux was higher in MSSA. Out of the 65 isolates phenotypically resistant to methicillin, all harbored the *mec*A gene and no isolate was positive for *mec*C. Concerning the staphylococcal chromosome cassette *mec* (SCC*mec*) element of these MRSA isolates, 47 (72.3%) harbored SCC*mec* type IV, 17 (26.2%) harbored SCC*mec* type V, and one (1.5%) harbored SCC*mec* type III.

Regarding the molecular typing of the isolates by microarray, a wide clonal diversity was observed. The 98 isolates belonged to twelve different clonal complexes (CCs) and were assigned to 27 distinct clones (Tables 3 and 4). Three CCs (CC1, CC22, and CC30) dominated and accounted for 65.3% of the isolates; four other CCs (CC8, CC15, CC121, and CC398) accounted for 24.5%.

The most frequent detected clone was the Southwest Pacific clone (CC30-MRSA-IV PVL+), which accounted for 21.4% (21/98) of all isolates and 32.3% (21/65) of MRSA. It was mostly isolated from skin and soft tissue infections. Isolates of this clone were resistant to multiple antibiotics, particularly oxacillin (100%), erythromycin (95.2%), ciprofloxacin (95.2%), and cotrimoxazole (57.1%). Various resistance genes were detected in these isolates: *bla*Z operon, *msr*A, *mph*C, *fos*B and *tet*Efflux in all isolates; *aph*A3 and *sat* in 19/21 (90.5%) isolates; *aad*D and *tet*K in 1/21 (4.8%). The enteroxin gene cluster *egc* (*seg*, *sei*, *sem*, *sen*, *seo*, and *seu*) was detected in all these isolates.

The second most frequent detected clone was the Bengal Bay clone (ST772-MRSA-V PVL+), which accounted for 11.2% (11/98) of all isolates and 16.9% (11/65) of MRSA. Isolates of this clone were also resistant to multiple antibiotics, particularly oxacillin (100%), tobramycin (63.6%), gentamicin (54.6%), erythromycin (100%), ciprofloxacin (100%), chloramphenicol (63.6%), and doxycycline (63.6%). Various resistance genes were detected in these isolates: *bla*Z operon, *msr*A, *mph*C, *fos*B, and *tet*Efflux in all isolates; *tet*K in 6/11 (54.6%); and *aac*A-*aph*D in 5/11 (45.5%). Nine enterotoxin genes (*sea*, *sec*, *sel*, and the *egc*) were detected in almost all of these isolates (Table 3). Another clone, the CC22-MRSA-IV TSST1+ (toxic shock syndrome toxin-1) clone, accounted for 11/98 (11.2%) of all isolates and 11/65 (16.9%) of MRSA isolates. Isolates of this clone were less resistant to antibiotics: oxacillin (100%), erythromycin (27.3%), doxycycline (27.3%), rifampicin (18.2%), and cotrimoxazole (36.4%). Various resistance genes were detected in these isolates: *bla*Z operon in 10/11 (90.9%), *erm*C in 2/11 (18.2%), *dfr*A in 11/11 (100%), and *tet*K in 3/11 (27.3%). The *egc* cluster was detected in all of these isolates. All other clones of MRSA were detected in 6 or less isolates (< 7%) and presented a high diversity (Table 3).

Table 2 Presence of resistance genes in 98 *S. aureus* isolates collected during 6 months, from January to June 2017, in two hospitals in Kabul (MSSA, *n* = 33, and MRSA, *n* = 65).

	Gene	All isolates n (%)	MRSA n (%)	MSSA n (%)	p value
Beta-lactam resistance	*bla*Z	97 (99.0)	64 (98.5)	33 (100)	0.47
	*mec*A	65 (66.3)	65 (100)	-	-
	*mec*C	0 (0)	0 (0)	-	-
Macrolide/lincosamide resistance	*erm*A	1 (1.0)	1 (1.5)	0 (0)	0.47
	*erm*B	1 (1.0)	0 (0)	1 (3.0)	0.16
	*erm*C	16 (16.3)	16 (24.6)	5 (15.2)	0.82
	*lnu*A	7 (7.1)	0 (0)	7 (21.2)	*< 0.001*
	*msr*A	43 (43.9)	38 (58.5)	5 (15.2)	*< 0.001*
	*mph*C	43 (43.9)	38 (58.5)	5 (15.2)	*< 0.001*
Aminoglycoside resistance	*aac*A-*aph*D	16 (16.3)	16 (24.6)	0 (0)	*0.002*
	*aad*D	22 (22.4)	13 (20.0)	9 (27.3)	0.42
	*aph*A3	44 (44.9)	41 (63.1)	3 (9.1)	*< 0.001*
	sat	44 (44.9)	41 (63.1)	3 (9.1)	*< 0.001*
Cotrimoxazole resistance	*dfr*A	16 (16.3)	16 (24.6)	0 (0)	*0.002*
Fusidic acid resistance	*fus*B	0 (0)	0 (0)	0 (0)	-
Tetracycline resistance	*tet*K	39 (39.8)	21 (32.3)	18 (54.8)	*0.03*
	*tet*M	2 (2.0)	1 (1.5)	1 (3.0)	0.62
	*tet*Efflux	80 (81.6)	48 (73.8)	32 (97.0)	*0.005*
Choramphenicol resistance	*cat*	1 (1.0)	0 (0.0)	1 (3.0)	0.16
	cfr	0 (0)	0 (0)	0 (0)	-
	*fex*A	0 (0)	0 (0)	0 (0)	-
Mupirocin resistance	*mup*R	0 (0)	0 (0)	0 (0)	-
Fosfomycin resistance	*fos*B	65 (66.3)	46 (70.8)	19 (57.6)	0.19
Vancomycin resistance	*van*A	0 (0)	0 (0)	0 (0)	-

Significant *p* values are shown in italic

Out of the 33 MSSA isolates, 6 (18.2%) belonged to the CC15 and did not harbor genes for PVL, TSST-1, and enterotoxins. Five MSSA isolates (15.2%) belonged to the CC121-MSSA PVL+ clone and harbored the resistance genes *bla*Z, *fos*B, *tet*K, and *tet*Efflux, and virulence genes encoding PVL and enterotoxins (*seb*, and *egc*). Five MSSA isolates (15.2%) belonged to the ST291/813 and four (12.1%) belonged to the CC30-MSSA PVL+ clone. All other clones of MSSA were detected in three or less isolates (4% of all strains) but showed high diversity (Table 4).

The overall rate of PVL-positive isolates was 59.2% (58/98), these isolates belonging to 8 MRSA and 5 MSSA clones. The prevalence of PVL genes was higher in MRSA than in MSSA (70.8% vs 36.4%, *p* = 0.001). The arginine catabolic mobile element (ACME) locus was present in only one strain of MRSA belonging to the USA300 clone (ST8-MRSA-IV PVL+), isolated from an ear pus. Contrary to PVL, few isolates harbored the TSST-1 (16/98, 16.3%). Table 5 shows the prevalence data for virulence genes in the *S. aureus* isolates collected.

Discussion

This epidemiological study investigated the antibiotic resistance profile and performed the molecular characterization of MRSA and MSSA clinical isolates at two main health facilities in Kabul. The overall prevalence of MRSA was high at 66.3% (65/98). A very high genetic diversity was detected using microarray analysis, with 27 distinct clones among the 98 isolates collected during a 6-month period.

This study is the first molecular study of *S. aureus* clinical isolates in Afghanistan and reports the various clones circulating in its capital. The prevalence of MRSA in this study was higher compared to findings of similar studies conducted in neighboring countries such as Pakistan [21], Iran [22], Saudi Arabia [23], and Turkey [24]. In this study, a wide clonal diversity was detected with twelve different clonal complexes, along with 13 distinct MRSA and 14 MSSA clones. The typing results revealed a predominance of three MRSA clones, representing 44% of the isolates: Southwest Pacific (CC30-MRSA-IV PVL+), Bengal

Table 3 Distribution and characteristics of the 65 MRSA isolates collected from January to June 2017 in two main hospitals in Kabul

CC (number of isolates)	Clone assignment (Alere)[a]	n	% of isolates	agr type	SCCmec type	PVL	TSST	Enterotoxin genes	Antibiotic resistance genes (other than mecA)	Antibiotic resistance associated with methicillin resistance[b]
CC1 (12)	CC1-MRSA-IV PVL+, USA400	1	1.5	3	IV	Pos	Pos	sea, seh, sek, seq	blaZ, ermC, aadD, tetK, tetEfflux	Tob, Ery, Dox, Sxt
	ST772-MRSA-V PVL+, Bengal Bay Clone	11	16.9	2	V	Pos	Neg	sea, sec, seg (10/11), sei, sel, sem, sen (10/11), seo, seu	blaZ, msrA, mphC, aacA-aphD (5/11), fosB, tetK (6/11), tetEfflux	Tob (7/11), Gen (6/11), Ery, Cli (1/11), Cip, Chl (7/11), Dox (7/11), Sxt (3/11)
CC8 (7)	CC8-MRSA-IV, USA500	4	6.2	1	IV	Neg	Neg	seb, sek, seq	blaZ, ermC (3/4), aacA-aphD, aadD, aphA3, sat, fosB, tetEfflux	Tob, Gen, Ery, Cli (3/4, Cip, Chl (3/4), Sxt
	CC8-MRSA-IV, UK-EMRSA-14/WA MRSA-5	1	1.5	1	IV	Neg	Neg		blaZ, aacA-aphD, aadD, fosB, tetEfflux	Tob, Gen, Cip, Sxt
	ST8-MRSA-[IV + ACME] PVL+, USA300	1	1.5	1	IV	Pos	Neg	sek, seq	blaZ, msrA, mphC, aphA3, sat, fosB, tetEfflux	Ery, Cip
	ST239-MRSA-[III + SCCmer+ccrC], Vienna/Hungarian/Brazilian Clone	1	1.5	1	III	Neg	Neg	sea, sek, seq	blaZ, ermA, aacA-aphD, aphA3, tetK, tetM, fosB, tetEfflux	Tob, Gen, Cip, Chl, Sxt
CC22 (16)	CC22-MRSA-IV TSST-1+	11	16.9	1	IV	Neg	Pos	seg, sei, sem, sen, seo, seu	blaZ (9/11), ermC (2/11), dfrA, tetK (3/11)	Tob (1/11), Ery (3/11), Cip (1/11), Chl (2/11), Dox (3/11), Rif (2/11), Sxt (4/11)
	CC22-MRSA-IV PVL+	5	7.7	1	IV	Pos	Neg	seg, sei, sem, sen, seo (4/5), seu	blaZ, ermC (4/5), aacA-aphD, aadD, dfrA	Tob, Gen, Ery (4/5), Cli (1/5), Cip (2/5), Chl (3/5), Sxt (4/5)
CC30 (28)	CC30-MRSA-IV PVL+, Southwest Pacific clone	21	32.3	3	IV	Pos	Neg	sea (1/21), seg, sei, sem, sen, seo, seu	blaZ, msrA, mphC, aadD (1/21), aphA3 (19/21), sat (19/21), tetK (1/21), fosB, tetEfflux	Tob (1/21), Ery (20/21), Cli (3/21), Cip (20/21), Chl (6/21), Dox (1/21), Sxt (12/21)
	CC30-MRSA-V/VT PVL+, WA MRSA-124	6	9.2	3	V	Pos	Neg	seg, sei, sem, sen, seo, seu	blaZ, ermC (1/6), msrA (5/6), mphC, aphA3 (5/6), sat, tetK, fosB, tetEfflux	Tob (1/6), Ery (5/6), Cip (4/6), Chl (1/6), Dox (3/6), Sxt (1/6)
	CC30-MRSA-IV PVL+/TSST-1+	1	1.5	3	IV	Pos	Pos	seg, sei, sem, sen, seo, seu	blaZ, msrA, mphC, aadD, tetK, fosB, tetEfflux	Tob, Ery, Sxt
CC88 (1)	CC88-MRSA-IV PVL+	1	1.5	3	IV	Pos	Neg	sep	blaZ, tetK, tetEfflux	None
CC509 (1)	CC509-MRSA-IV	1	1.5	3	IV	Neg	Neg	sem, seo	blaZ, tetK, tetEfflux	None

[a] MLST and clone assignments correspond to the comparison of the hybridization profiles on the DNA microarray to previously typed MLST reference strains [17]

[b] Tob, tobramycin; Gen, gentamicin; Ery, erythromycin; Cli, clindamycin; Cip, ciprofloxacin; Chl, chloramphenicol; Dox, doxycycline; Rif, rifampicin; Sxt, cotrimoxazole

Table 4 Distribution and characteristics of the 33 MSSA isolates collected from January to June 2017 in two main hospitals in Kabul

CC (number of isolates)	Clone assignment (Alere)[a]	n	% of isolates	agr type	PVL	Exfoliative toxin	TSST	Enterotoxin genes	Antibiotic resistance genes	Antibiotic resistance[b]
CC1 (2)	CC1-MSSA PVL+	1	3.0	3	Pos	Neg	Pos	sea, seh, sek, seq	blaZ, tetEfflux	Pen
	ST848-MSSA-[ccrAB1]	1	3.0	2	Neg	Neg	Neg	sec, seg, sei, sel, sem, sen, seo, seu	blaZ, fosB, tetEfflux	Pen
CC5 (1)	CC5-MSSA	1	3.0	2	Neg	Neg	Pos	sec, seg, sei, sel, sem, sen, seo, seu	blaZ, msrA, mphC, aphA3, sat, tetK, fosB, tetEfflux	Pen, Ery, Sxt
CC15 (6)	CC15-MSSA	6	18.2	2	Neg	Neg	Neg	sec, seg, sei, sel, sem, sen, seo, seu	blaZ, ermC (3/6), lnuA (4/6), aadD (4/6), tetK (4/6), fosB, tetEfflux	Pen, Tob (4/6), Ery (3/6), Clin (1/6), Cip (2/6), Chl (1/6), Dox (2/6), Sxt (2/6)
CC22 (1)	CC22-MSSA	1	3.0	1	Neg	Neg	Neg	sec, seg, sei, sel, sem, sen, seo, seu	blaZ, ermC, tetK	Pen, Ery
CC30 (5)	CC30-MSSA	1	3.0	3	Neg	Neg	Neg	seg, sei, sem, sen, seo, seu	blaZ, msrA, mphC, aphA3, sat, fosB, tetEfflux	Pen, Ery, Cip
	CC30-MSSA PVL+	4	12.1	3	Pos	Neg	Pos (1/4)	sea (1/4), sec (1/4), seg, sei, sel (1/4), sem, sen, seo, seu	blaZ, lnuA (1/4), aadD (3/4), tetK (3/4), fosB, tetEfflux	Pen, Tob (2/4), Ery (2/4), Clin (1/4), Chl (1/4), Sxt (1/4)
CC45 (2)	CC45-MSSA	2	6.1	1	Neg	Neg	Neg	sec (1/2), seg, sei, sel (1/2), sem, sen, seo, seu	blaZ, msrA (1/2), mphC (1/2), tetEfflux	Pen, Ery (1/2), Cip (1/2), Chl (1/2)
CC88 (1)	CC88-MSSA PVL+	1	3.0	3	Pos	Neg	Neg	sep	blaZ, tetEfflux	Pen
CC121 (6)	CC121-MSSA	1	3.0	4	Neg	Neg	Neg	seg, sei, sem, sen, seo, seu	blaZ, fosB, tetEfflux	Pen
	CC121-MSSA PVL+	5	15.2	4	Pos	Neg	neg	seb (4/5), seg, sei, sem, sen, seo, seu	blaZ, tetK (3/5), fosB, tetEfflux	Pen, Ery (1/5), Cip (1/5), Chl (2/5), Dox (1/5), Sxt (2/5)
CC398 (5)	ST291/813-MSSA	5	15.2	1	Neg	Pos	Neg	sen, seo, seu	blaZ, ermB (1/5), ermC (1/5), aphA3 (1/5), sat (1/5), tetK (3/5), tetM (1/5), tetEfflux	Pen, Ery (1/5), Cip (3/5), Chl (3/5), Dox (1/5)
CC1021 (1)	CC1021-MSSA-[ccrAB2+] PVL+	1	3.0	3	Pos	Neg	Neg	sea, seb, sei, sek, sem, sen, seo, seq, seu	blaZ, tetK, tetEfflux	Pen, Ery, Rif, Sxt
ST1278 (3)	ST1278-MSSA-[ccrA1]	3	9.1	3	Neg	Neg	Neg	seh	blaZ, lnuA (2/3), msrA (2/3), mphC (2/3), aadD (2/3), tetK (2/3), tetEfflux	Pen, Ery (2/3), Cip (1/3), Chl (2/3), Dox (2/3)

[a] MLST and clone assignments correspond to the comparison of the hybridization profiles on the microarray to previously typed MLST reference strains [17]

[b] Pen, penicillin G; Tob, tobramycin; Gen, gentamicin; Ery, erythromycin; Cli, clindamycin; Cip, ciprofloxacin; Chl, chloramphenicol; Dox, doxycycline; Rif, rifampicin; Sxt, cotrimoxazole

Table 5 Presence of virulence genes in 98 *S. aureus* isolates collected during 6 months (January–June 2017) in two hospitals in Kabul (MSSA, *n* = 33, and MRSA, *n* = 65)

Group	Gene	All isolates *n* (%)	MRSA *n* (%)	MSSA *n* (%)	*p* value
Toxic shock syndrome toxin gene	*tst1*	16 (16.3)	13 (20.0)	3 (9.1)	0.167
Enterotoxin genes	*sea*	17 (17.3)	14 (21.5)	3 (9.1)	0.124
	sep	2 (2.0)	1 (1.5)	1 (3.0)	0.622
	seb	9 (9.2)	4 (6.2)	5 (15.2)	0.145
	sec	16 (16.3)	11 (16.9)	5 (15.2)	0.823
	seg	70 (71.4)	54 (83.1)	16 (48.5)	*0.001*
	seh	5 (5.1)	1 (1.5)	4 (12.1)	*0.024*
	sei	72 (73.5)	55 (84.6)	17 (51.5)	*< 0.001*
	sek	9 (9.2)	7 (10.8)	2 (6.1)	0.446
	sel	16 (16.3)	11 (16.9)	5 (15.2)	0.823
	sem	73 (74.5)	56 (86.2)	17 (51.5)	*< 0.001*
	sen	71 (72.4)	54 (83.1)	17 (51.5)	*0.001*
	seo	72 (73.5)	55 (84.6)	17 (51.5)	*< 0.001*
	seq	9 (9.2)	7 (10.8)	2 (6.1)	0.446
	seu	72 (73.5)	55 (84.6)	17 (51.5)	*< 0.001*
Panton-Valentine leukocidin	*lukFPVL-lukSPVL*	58 (59.2)	46 (70.8)	12 (36.4)	*0.001*
Exfoliative toxins	*etd*	5 (5.1)	0 (0.0)	5 (15.2)	*0.001*
Miscellaneous virulence genes or loci	ACME	1 (1.0)	1 (1.5)	0 (0.0)	0.474
	sak	79 (80.6)	53 (81.5)	26 (78.8)	0.745
	chp	73 (74.5)	46 (70.8)	27 (81.8)	0.236
	scn	97 (99.0)	64 (98.5)	33 (100)	0.474

Significant *p* values are shown in italic

Bay (ST772-MRSA-V PVL+), and CC22-MRSA-IV TSST-1+ clones. The 24 other clones were present in one to 6 isolates.

The main limitation of this study is that only *S. aureus* isolates collected in two hospitals of Kabul were included. Therefore, even if these hospitals perform most of the bacteriological analyses in Kabul, the results do not reflect the global epidemiology in Afghanistan and are only representative of *S. aureus* epidemiology for infections diagnosed in hospitals. Nevertheless, the huge diversity of strains highlighted by the typing results illustrates the position of Kabul as a center of intense international exchanges.

Three different MRSA and two different MSSA clones were identified within CC30. The Southwest Pacific clone (CC30-MRSA-IV PVL+, USA1100) was the most prevalent clone, detected in 32.3% of MRSA isolates. This MDR and PVL+ clone is widely distributed in the Pacific islands and was first identified in New Zealand among the Samoan immigrant population [25]. It is a widespread CA-MRSA clone in Australia and has been found in several European countries as well as in Hong Kong, Taiwan, the USA, Saudi Arabia, the United Arab Emirates, and Iran [25–27]. The major occurrence of this clone in Afghanistan could possibly be linked to the large migratory movements of Afghans to Australia, Saudi Arabia, Iran, European countries, and some of the southwest Pacific

countries, especially Indonesia, and Malaysia. The WA MRSA-124 clone (CC30-MRSA-V/VT PVL+), detected in 9.2% of MRSA isolates, is another virulent and MDR clone and was isolated mainly from SSTIs. The same lineage has already been described in Egypt [28] and in a patient living in the German/Polish border region and presenting a SSTI [4].

Two MSSA and two MRSA clones were identified within the CC1. Within this clonal complex, the Bengal Bay clone (ST772-MRSA-V PVL+), detected in 19.6% of MRSA isolates, is nowadays the dominant clone in India [29, 30]. As the Southwest Pacific clone, this one is also PVL+ and MDR. Studies from Pakistan, Nepal, and Australia also reported the predominance of this clone [31–33] which has become endemic in these regions. The presence of this clone in Kabul may illustrate an epidemiological link between Afghanistan and the neighboring countries, especially India and Pakistan where many Afghan families travel for medical treatment, and many young students go to pursue their higher education. This clone appears to be an increasing public health threat in several countries in the region, as it can be encountered in hospital as well as in community settings [31]. One PVL+ isolate, collected in Kabul from a SSTI, belonged to the USA400 pulsotype, one of the first-described PVL+ CA-MRSA. This one was reported to cause fatal infections in healthy children in the late 1990s in the USA [34] and then sporadically described in Australia, Germany, and

the UK. Prior to this study, USA400 had not been reported in the neighboring countries such as Pakistan and Iran.

Two MRSA and one MSSA clones were identified within the CC22. This is a common and widespread clonal complex, and different MRSA lineages have emerged from this genetic background. The CC22-MRSA-IV TSST-1+ clone, detected in 19.6% of all MRSA isolates, has been mostly reported in Saudi Arabia and the Middle East region [25]. Its presence in Afghanistan could be related to the large movements of Afghans to Saudi Arabia for pilgrimage each year. Seven MRSA isolates were assigned to the CC22-MRSA-IV PVL+ . This clone has been described in Germany, Australia, England, Ireland, Hong Kong, and the United Arab Emirates [4, 35]. Both clones were reported in hospital-acquired infections from Pakistan as well [31].

Four different MRSA clones were identified within the CC8. Among them, the USA500 clone (CC8-MRSA-IV PVL-) was predominant. This clone has been identified in the USA, Australia, the UK, and Ireland, and sporadically in Germany [4, 36]. Interestingly, only one isolate was identified as the well-known USA300 (CC8-MRSA-IV PVL+) clone. This clone of CA-MRSA, mainly reported in the USA, was also reported in numerous countries in Europe or Asia such as Saudi Arabia, Qatar, and Pakistan [4, 25, 32, 37]. One isolate was also identified as belonging to the Vienna/Hungarian/ Brazilian clone (ST239-MRSA-III). This HA-MRSA lineage is probably the oldest pandemic MRSA strain and has been reported in many European countries [4, 38], as well as in Asia including Saudi Arabia [25], Iran [27], and Pakistan [32].

The overall prevalence of PVL among *S. aureus* isolates collected in Kabul was 59.2%, these isolates belonging to 8 MRSA and 5 MSSA clones, with a higher prevalence in MRSA. This high prevalence and the diversity of PVL+ isolates must be highlighted and could be linked to the import of different strains via international exchanges. Contrary to other countries, we did not observe the predominance of one single CA-MRSA clone such as the USA300 in North America. In studies in neighboring countries, the prevalence of PVL was generally lower: from 0 to 12.7% in Turkey [39], from 7.4 to 55.6% in Iran according to the studies [40], and around 9% of *S. aureus* isolates responsible for community-acquired infections in West Bengal of India [41].

In conclusion, the prevalence of MRSA in *S. aureus* isolates circulating in Kabul was quite high (66.3%) when compared with the prevalence rates obtained from other similar studies conducted in neighboring countries. Most of *S. aureus* isolates, especially MRSA, were multidrug-resistant (generally resistant to beta-lactams, macrolides, quinolones, and cotrimoxazole and less often to aminoglycosides, chloramphenicol, and tetracyclines). This may be related to the overuse or the inappropriate use of antimicrobials which was recently reported in Afghanistan [14, 15]. A great diversity of *S. aureus* clones was brought to light using microarray

analysis, with a predominance of the Southwest Pacific, the CC22-MRSA-IV TSST-1, and the Bengal Bay clones. This diversity and the clones identified reflect epidemiological links, including travels, migrations, commerce, and international military actions, with South West Pacific, Indian, and Middle Eastern/Arabian Gulf regions, as well as Western Europe, the USA, and Africa. In the future, major efforts have to be made on the monitoring, distribution, and spread of multidrug-resistant bacteria to control their diffusion and reduce the prevalence of MRSA in Afghanistan.

Authors' contribution Frédéric Laurent and François Vandenesch were involved in the conception of the study, and in analysis and interpretation of data. Haji Mohammad Naimi was involved in conducting the study, statistically analyzing the data, and drafting the manuscript. Camille André was involved in antibiotic susceptibility testing. Céline Dupieux was involved in interpretation of data and revising and editing the manuscript. Michèle Bes, Anne Tristan, and Claude-Alexandre Gustave were involved in the interpretation of DNA microarray. Qand Agha Nazari conducted preliminary identifications of strains. All authors read and approved the final manuscript.

Data Availability All relevant materials and data supporting the findings of this study are contained within the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval The approval of an ethics committee was not necessary, because the clinical samples of our study were taken as part of standard patient care.

Consent to participate Not applicable.

Consent for publication Not applicable.

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