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1 **Molecular characterization and antimicrobial resistance of nasal**

2 ***Staphylococcus aureus* in the community of Kabul**

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15 **ABSTRACT**

16 **Objectives:** This study aimed to investigate the prevalence of methicillin-resistant
17 *Staphylococcus aureus* (MRSA) nasal carriage in the community among students at Kabul
18 University and their molecular characteristics.

19 **Methods:** Nasal swabs were collected from anterior nares of 150 healthy non-medical students in
20 Kabul University. Antimicrobial susceptibility testing was performed on all *S. aureus* isolates,
21 and all detected MRSA isolates were then confirmed by *mecA/mecC* PCR and characterized
22 using DNA microarray.

23 **Results:** A total of 50 *S. aureus* strains were isolated from the anterior nares of the 150
24 participants. The prevalence of *S. aureus* and MRSA nasal carriage among Kabul students was
25 33.3% and 12.7%, respectively. Seven (36.8%) MRSA isolates and 8 (25.8%) methicillin-
26 susceptible *Staphylococcus aureus* (MSSA) isolates were multidrug resistant (MDR; i.e.,
27 resistant to at least 3 different antimicrobials tested). All MRSA isolates (n=19) were susceptible
28 to linezolid, rifampicin and fusidic acid. Seven MRSA clones, belonging to 4 clonal complexes
29 (CCs), were identified. The most commonly identified clone was CC22-MRSA-IV TSST-1-
30 positive, which accounted for 63.2% (12/19) of MRSA isolates. The SCC*mec* typing showed that
31 most MRSA strains harbored SCC*mec* type IV (94.7%). Thirteen (68.4%) MRSA isolates carried
32 the TSST-1 and 5 (26.3%) the PVL genes.

33 **Conclusion:** Our findings revealed the relatively high prevalence of MRSA nasal carriers in the
34 community in Kabul, with the predominance of the CC22-MRSA-IV TSST-1-positive clone, and
35 the frequent multidrug resistance among these isolates.

36

37 **Keywords.** Nasal carriage; MRSA clones; antibiotic resistance; molecular typing; Kabul.

38 **1. Introduction**

39 Since methicillin-resistant *Staphylococcus aureus* (MRSA) was first identified in 1961, it has
40 been associated with higher mortality rates and increased lengths of hospital stays as well as
41 associated health care costs (1). MRSA infections are a serious risk for patients in healthcare
42 settings and a challenge for public health, whereas infections due to community-acquired MRSA
43 (CA-MRSA) seem to be increasing among people in different areas of the world (2). Nasal
44 carriage of *S. aureus* has been shown to play a key role in the pathogenesis of *S. aureus*
45 infections, in particular in patients undergoing surgery, dialysis, and intensive care unit (ICU)
46 patients (3), but it also constitutes a risk factor for the development of skin and soft tissue
47 infections in non-hospitalized and non-diseased subjects (4). MRSA strains that reside in the
48 anterior nares constitute a reservoir which predisposes the host to future infections (5): in a
49 systematic review, patients colonized with MRSA were four times more likely to develop
50 invasive infection than patients colonized with MSSA (6). Therefore, determining the prevalence
51 of *S. aureus* nasal carriage, as well as antimicrobial resistance profiles and molecular typing of
52 nasal MRSA isolates in healthy populations is useful for understanding the epidemiology of
53 community-acquired infections and supporting infection control measures.

54 In central Asia, including Afghanistan, studies of MRSA have mainly focused on the prevalence
55 of MRSA, but few data are available about the circulating clones. A recent study concerning
56 *S. aureus* infections diagnosed in Kabul hospitals highlighted a high rate of methicillin resistance
57 (66.3 %) and the presence of multiple virulence and antibiotic resistance genes in *S. aureus*
58 isolates, with a predominance of CC30-MRSA-IV PVL-positive, CC22-MRSA-IV TSST-1-
59 positive and ST772-MRSA-V PVL-positive clones (7). However, no data are available about the
60 prevalence of MRSA carriage and the clones circulating in the community.

61 The present study aimed to investigate the prevalence of MRSA nasal carriage, and the molecular
62 characteristics of MRSA isolates in the community of Kabul through *S. aureus* nasal screening in
63 non-medical students at Kabul University.

64

65

66 **2. Materials and Methods**

67

68 **2.1. Study population**

69 A total of 150 healthy student volunteers from non-medical faculties of Kabul University were
70 enrolled in this study from March to July 2018. Informed consent was obtained from all
71 participants included, according to Afghanistan ethical rules. Every student with a history of
72 antibiotic consumption, hospitalization, admission to a nursing home, or surgery during the last 2
73 months were excluded.

74

75 **2.2. Identification of *S. aureus* isolates and antimicrobial susceptibility testing**

76 Samples were collected from both anterior nares of volunteers with sterile moistened swabs.
77 Standard microbiological procedures were conducted on nasal swabs with minimum delay for
78 culture. Samples were cultured for 18–24 hours at 37 °C onto blood agar base medium (Oxoid,
79 Basingstoke, United Kingdom) supplemented with 5% sheep blood. Then presumptive *S. aureus*
80 isolates were identified by carrying out confirmatory tests, consisting of Pastorex Staph Plus latex
81 agglutination test (Bio-Rad, Marnes-la-Coquette, France) and tube coagulase assay (EDTA-rabbit
82 plasma; Difco Laboratories, Detroit, MI, US).

83 Antimicrobial susceptibility testing (AST) was performed on Mueller Hinton agar (Oxoid) by

84 Kirby Bauer disc diffusion method (antibiotic discs from i2a, Montpellier, France) according to

85 the 2019 guidelines of the European Committee on Antimicrobial Susceptibility Testing
86 (EUCAST) (8). The following antimicrobial agents were tested: penicillin G, ceftiofur (used to
87 detect methicillin resistance), erythromycin, clindamycin, norfloxacin, kanamycin, tobramycin,
88 gentamicin, cotrimoxazole, linezolid, fusidic acid, chloramphenicol, fosfomycin, and rifampicin.
89

90 **2.3. Detection of *mecA/mecC* genes and *agr* typing by multiplex PCR**

91 Cellular DNA was obtained from *S. aureus* colonies grown overnight on blood agar plates using
92 DNA Extraction Kit (Promega, Madison, WI, US) in accordance with manufacturer's
93 instructions. The detection of the *mecA* and *mecC* genes and *agr* typing were performed by
94 multiplex PCR using primers already published (9-11).
95

96 **2.4. Molecular characterization of strains by DNA microarray**

97 The DNA microarray Identibac *S. aureus* genotyping® (Alere Technologies, Jena, Germany) was
98 used for MRSA isolates as previously described (12). This microarray allows the detection of 336
99 different target sequences corresponding to 185 genes and their allelic variants, including both
100 virulence and resistance genes. The assignment of isolates to clonal complexes (CCs) and
101 SCC*mec* types was determined automatically by comparison of the hybridization profiles to
102 previously typed reference strains (12).
103

104 **2.5. Statistical analysis**

105 Statistical analysis was done using SPSS 21 (IBM Inc. Chicago). Chi-square test was used to
106 compare the significance resistance profile between MRSA and MSSA. Binary logistic
107 regression was used to determine the association between MRSA nasal carriage, gender and age.
108 A *P-value* less than 0.05 was considered as statistically significant.

109

110 3. Results

111 During the study period, a total of 50 *S. aureus* isolates were isolated from the anterior nares of
112 the 150 volunteers included in the study, resulting in a nasal carriage rate of 33.3%. Out of the 50
113 *S. aureus* isolates, 36 (72.0%) were obtained from men and 14 (28.0%) from women. The mean
114 age of carriers was 22.2 years (SD 2.8; [19-38] y, median 22 y). Nineteen of the 50 *S. aureus*
115 isolates (38.0%) were identified as MRSA: 14/36 (38.9%) in men and 5/14 (35.7%) in women.
116 Therefore, the prevalence of nasal MRSA carriage among Kabul University students was 12.7%
117 (19/150). The difference of MRSA frequency was not statistically significant according to gender
118 ($p=0.84$) and age ($p=0.18$).

119 The antimicrobial resistance rates of MRSA, MSSA, and *S. aureus* isolates are presented in Table
120 1. Most *S. aureus* isolates were resistant to penicillin (96%). The rates of resistance for other
121 antibiotics were 32% for erythromycin, 8% for clindamycin, 18% for norfloxacin, 30% for
122 kanamycin, 22% for tobramycin, 6% for gentamicin, 18% for cotrimoxazole, 4% for fusidic acid,
123 2% for chloramphenicol, and 8% for fosfomicin. The resistance rates of MRSA isolates to
124 common antimicrobials were not significantly different from those of MSSA isolates (Table 1).
125 Moreover 36.8% (7/19) of MRSA isolates and 25.8% (8/31) of MSSA isolates were multidrug
126 resistant (MDR), i.e. resistant to at least 3 classes of antibiotics. All MRSA isolates were
127 susceptible to linezolid, rifampicin and fusidic acid.

128 Various resistance genes were detected in the 19 MRSA isolates. *blaZ*, *dfrA* and *tetK* were the
129 most frequently detected genes in 89.5%, 52.6% and 47.4% of MRSA isolates, respectively. No
130 strain harbored the resistance genes to fusidic acid (*fusB*), to chloramphenicol (*cat*, *fexA*), to
131 linezolid (*cfr*), to mupirocin (*mupA*), and to vancomycin (*vanA*). Out of the 19 isolates
132 phenotypically resistant to methicillin, all harbored the *mecA* gene and no isolate was positive for

133 *mecC* (Table 2). Concerning the staphylococcal chromosome cassette *mec* (SCC*mec*) element of
134 these MRSA isolates, 18 (94.7%) harbored SCC*mec* type IV, and one (5.3%) harbored SCC*mec*
135 type V.

136 Regarding the molecular typing of MRSA isolates, a high clonal diversity was observed. The 19
137 MRSA isolates belonged to four different clonal complexes (CCs) and were assigned to seven
138 distinct clones. One CC (CC22) dominated and accounted for 79% (15/19) of the isolates, and
139 three other CCs (CC5, CC30 and CC88) accounted for 21% (Table 3).

140 The most frequently detected clone was CC22-MRSA-IV carrying the toxic shock syndrome
141 toxin (TSST-1) gene, which accounted for 63.2% (12/19) of MRSA isolates. Three resistance
142 genes were detected in these isolates: the *blaZ* operon in 10/12 (83.3%), *dfrA* in 8/12 (66.7%)
143 and *tetK* in 6/12 (50%). The enterotoxin gene cluster (*egc*; enterotoxins *seg*, *sei*, *sem*, *sen*, *seo* and
144 *seu*) was detected in all these isolates. The only other clone detected in at least 2 isolates was
145 CC22-MRSA-IV positive for Panton-Valentine leukocidin genes, which accounted for 10.5%
146 (2/19) of MRSA isolates. These two isolates were resistant to multiple antibiotics including
147 aminoglycosides and erythromycin and harbored various resistance genes: the *blaZ* operon,
148 *ermC*, *aacA-aphD* and *dfrA*.

149 Only single isolates from 5 other MRSA clones completed this list, which represented 26.5% of
150 all MRSA isolates and included well-known clones such as the Paediatric clone CC5-MRSA-IV
151 TSST-1-positive, the Southwest Pacific clone CC30-MRSA-IV PVL-positive and the Barnim
152 clone CC22-MRSA-IV UK-EMRSA-15 (Table 3).

153 Overall, 5/19 MRSA isolates (26.3%) were PVL-positive and 13/19 (68.4%) TSST-1-positive.

154

155

156

157 **4. Discussion**

158 This original study is the first molecular characterization of MRSA isolates in Afghan
159 community and reports the various clones circulating among young healthy individuals. A wide
160 clonal diversity of MRSA was detected with 4 different clonal complexes, along with 7 distinct
161 clones. Molecular analyses showed a predominance of one MRSA clone, representing 63.2% of
162 the isolates: the CC22-MRSA-IV TSST-1-positive. The 6 other clones detected were present only
163 in 1 or 2 isolates.

164 The main limitation of this study is that only MRSA isolates collected from nasal carriers among
165 Kabul university students were included. Therefore, the results do not reflect the global
166 epidemiology of MRSA nasal carriage in Afghanistan. Nevertheless, students are a population
167 without risk factors and the population in our study came from different parts of Afghanistan,
168 therefore represent quite well the Afghan community. In addition, the diversity of strains
169 highlighted by the typing results illustrates the position of Kabul as a center of intense
170 international exchanges, as already described (7).

171 The results of the present study show that the epidemiology of MRSA carriage in Kabul
172 community is quite similar to the epidemiology of MRSA clinical isolates in Kabul hospitals. Out
173 of the 7 MRSA clones detected here, 5 clones that accounted for 89.5% of the isolates, were also
174 reported in Kabul hospitals (7). This suggests direct transfers between community and
175 nosocomial MRSA clones in Kabul and diffusion of MRSA between these two settings.

176 The prevalence of nasal colonization with *S. aureus* in our study was 33.3%, a higher prevalence
177 than that observed (19.6%) among healthy students of medical and non-medical universities in
178 Urmia, Iran (13); however, the prevalence of nasal carriage of MRSA were similar (around 13%).
179 Another study in China among medical students showed a *S. aureus* nasal colonization rate of

180 15.4% and a prevalence of MRSA carriage of 3% (14), which is lower than our findings. In our
181 study, the frequency of MRSA nasal carriage was not significantly different between male and
182 female students, which shows that gender may not be a risk factor for MRSA nasal colonization,
183 while in a study in Iran, (13) authors reported that male gender was a risk factor and argued that it
184 may be related to personal hygiene especially by the female sex. As previously reported, these
185 data indicate that factors influencing MRSA colonization vary significantly, from country to
186 country. Taken together, these results demonstrate that local epidemiological data are needed to
187 guide national public health policies.

188 In the present study, three different MRSA clones were identified within CC22. This is a
189 common and widespread clonal complex, and different MRSA lineages have emerged from this
190 genetic background. The CC22-MRSA-IV TSST-1-positive was the most prevalent clone,
191 representing more than half of all MRSA isolates. In a previous study about MRSA prevalence in
192 clinical samples in Kabul health facilities, this clone was less frequent in hospital infections, with
193 16.9% of MRSA isolates (7). This strain is mostly reported in Saudi Arabia and the Middle East
194 region (15). Its presence in Afghanistan could be related to the large movement of Afghans to
195 Saudi Arabia for pilgrimage each year. The second most frequent MRSA clone was the CC22-
196 MRSA-IV PVL-positive, which accounted for 7.7% of MRSA clinical isolates in our previous
197 study in Kabul (7). This clone has also been described in clinical samples from Western Europe
198 (Germany, England, Ireland), Australia, Hong Kong and the United Arab Emirates (16). Only
199 one isolate (5.3%) belonged to the CC22-MRSA-IV UK-EMRSA-15 Barnim clone; this is a
200 common and pandemic strain of MRSA that has been found mainly in Western Europe, but also
201 in other parts of the world including some Persian Gulf countries (17).

202 Two MRSA clones were identified within CC30: the CC30-MRSA-IV PVL-positive Southwest
203 Pacific clone, and the CC30-MRSA-V/VT PVL-positive clone. These lineages were also reported

204 from Iran, United Arab Emirates, Saudi Arabia, Taiwan, Egypt and Europe, both from the
205 community and hospitals (15,16). One MRSA clone was identified within CC5, the CC5-MRSA-
206 IV TSST-1-positive Paediatric clone. This MDR clone which also harbored the epidermal cell
207 differentiation inhibitor gene (*edinA*) was first reported in hospitals in neonatal patients in
208 Portugal (18), and in neonates and adults in Brazil (19) and Turkey (20). Finally, one clone was
209 identified within CC88, the CC88-MRSA-IV PVL-positive, which harbored enterotoxin genes
210 and resistance genes only to penicillins and tetracyclines; this clone has been already reported in
211 healthcare-associated infections in the region of Afghanistan (7), Saudi Arabia (15) and Kuwait
212 (21).

213 In this study, PVL genes were found in 26.3% of MRSA isolates (n=5), which is similar to
214 another study among healthy adults in Iran (22). This quite high prevalence of PVL genes among
215 MRSA isolates should require more attention. The transmission of such virulent strains in the
216 universities and other crowded places where people are often in close physical contact can
217 contribute to the transmission and the spread of these strains among the community.

218 In conclusion, the relatively high frequency of MRSA nasal carriage, especially the virulent
219 TSST-1 and PVL positive clones, among healthy population in Kabul is a reason for concern,
220 since individuals who harbor these isolates can act as reservoirs for outbreaks of MRSA
221 infections. Supporting policies for preventing the hospital-community spread of MRSA and
222 reducing nasal carriage with continued efforts to enhance hygiene among students are necessary.

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225 National Reference Center for staphylococci for technical assistance.

226

227

228 **DECLARATIONS**

229

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233

234 **Competing interests**

235 None declared.

236

237 **Ethical approval**

238 This study was approved by the Academic Council of the Faculty of Pharmacy and Research
239 Committee of Kabul University with approval numbers: FoP.124, 12/02/2018 and KURC.115,
240 20/02/2018. Written consent from all participants was obtained.

241

242 **Authors' contribution**

243 Haji Mohammad Naimi was involved in conducting the study, statistically analyzing the data and
244 drafting the manuscript. Céline Dupieux was involved in interpretation of data, revising and
245 editing the manuscript. Frédéric Laurent and François Vandenesch were involved in the
246 conception of the study, and in analysis and interpretation of data. Michèle Bes and Anne Tristan

247 were involved in the interpretation of DNA Microarray. Qand Agha Nazari conducted
248 preliminary identifications of strains. All authors read and approved the final manuscript.

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311

312 **Table 1.** Antimicrobial resistance rates of *S. aureus*, MRSA and MSSA nasal isolates among
 313 non-medical students in Kabul, Afghanistan, between March and July 2018 (n=50).

Antibiotic	Resistance rate (%)			<i>P</i> value
	<i>S. aureus</i> (n=50)	MRSA (n=19)	MSSA (n=31)	
Penicillin G	96	100	93.5	0.26
Erythromycin	32	36.8	29	0.57
Clindamycin	8	10.5	6.5	0.61
Norfloxacin	18	26.3	12.9	0.23
Kanamycin	30	26.3	32.3	0.66
Tobramycin	22	10.5	29	0.13
Gentamicin	6	10.5	3.2	0.29
Cotrimoxazole	18	15.8	19.4	0.75
Linezolid	0	0	0	-
Fusidic acid	4	0	6.5	0.26
Chloramphenicol	2	5.3	0	0.2
Fosfomicin	8	5.3	9.7	0.58
Rifampicin	0	0	0	-

314

315

316

317 **Table 2.** Antimicrobial resistance genes identified in MRSA isolates from nasal carriers among
 318 non-medical students in Kabul, Afghanistan, between March and July 2018 (n=19).

Antibiotic class	Resistance gene	All isolates n (%)
Beta-lactam	<i>blaZ</i>	17 (89.5)
	<i>mecA</i>	19 (100)
	<i>mecC</i>	0 (0)
Macrolide/lincosamide	<i>ermA</i>	0 (0)
	<i>ermB</i>	0 (0)
	<i>ermC</i>	2 (10.5)
	<i>lnuA</i>	0 (0)
	<i>msrA</i>	3 (15.8)
	<i>mphC</i>	3 (15.8)
	Aminoglycoside	<i>aacA-aphD</i>
<i>aadD</i>		1 (5.3)
<i>aphA3</i>		3 (15.8)
<i>sat</i>		3 (15.8)
Cotrimoxazole	<i>dfrA</i>	10 (52.6)
Fusidic acid	<i>fusB</i>	0 (0)
Tetracycline	<i>tetK</i>	9 (47.4)
	<i>tetM</i>	0 (0)
Chloramphenicol	<i>cat</i>	0 (0)
	<i>cfr</i>	0 (0)
	<i>fexA</i>	0 (0)
Mupirocin	<i>mupA</i>	0 (0)
Fosfomycin	<i>fosB</i>	3 (15.8)
Vancomycin	<i>vanA</i>	0 (0)
Multidrug (efflux pump)	<i>sdrM</i>	4 (21.1)

319

320 **Table 3.** Distribution and characteristics of the 19 MRSA isolates collected from anterior nares of non-medical students in Kabul
 321 University between March and July 2018 (n=19).

Clonal									
complex (number of isolates)	Clone assignment (Alere) ^a	n	% of isolates	agr type	PVL	TSST	Enterotoxin genes	Antibiotic resistance genes	Antibiotic resistance ^b
CC5 (1)	CC5-MRSA-IV TSST-1+, Paediatric clone	1	5.3	II	Neg	Pos	<i>sec, seg, sei, sel, sem, sen, seo, seu</i>	<i>blaZ, msrA, mphC, aphA3, sat, fosB, sdrM</i>	PEN, FOX, ERY, KAN
CC22 (15)	CC22-MRSA-IV, UK-EMRSA- 15/Barnim EMRSA-like MRSA	1	5.3	I	Neg	Neg	<i>seg, sei,sem, sen, seo, seu</i>	<i>blaZ, tetK</i>	PEN, FOX, NOR
	CC22-MRSA-IV PVL+	2	10.5	I	Pos	Neg	<i>seg, sei,sem, sen, seo, seu</i>	<i>blaZ, ermC, aacA-aphD, aadD (1/2), dfrA</i>	PEN, FOX, ERY, KAN, TOB, GEN, NOR (1/2), FOF (1/2)
	CC22-MRSA-IV TSST-1+	12	63.2	I	Neg	Pos	<i>seg, sei,sem, sen, seo, seu</i>	<i>blaZ (10/12), dfrA (8/12), tetK (6/12)</i>	PEN, FOX, ERY (2/12), CLI (1/12), NOR (1/12), SXT (2/12)
CC30 (2)	CC30-MRSA-IV PVL+, Southwest Pacific Clone	1	5.3	III	Pos	Neg	<i>seg, sei,sem, sen, seo, seu</i>	<i>blaZ, msrA, mphC, aphA3, sat, fosB, sdrM</i>	PEN, FOX, ERY, CLI, KAN, NOR, CHL
	CC30-MRSA-V/VT PVL+, WA MRSA-124	1	5.3	III	Pos	Neg	<i>seg, sei,sem, sen, seo, seu</i>	<i>blaZ, msrA, mphC, aphA3, sat, tetK, fosB, sdrM</i>	PEN, FOX, ERY, KAN, NOR, SXT
CC88 (1)	CC88-MRSA-IV PVL+	1	5.3	III	Pos	Neg	<i>sep, sek, seq</i>	<i>blaZ, tetK, sdrM</i>	PEN, FOX
Total		19	100.0						

322 ^a MLST and clone assignments correspond to the comparison of the hybridization profiles on the DNA microarray to previously typed MLST reference strains
 323 (12).

- 324 ^b PEN, penicillin; FOX, cefoxitin; ERY, erythromycin; CLI, clindamycin; KAN, kanamycin; TOB, tobramycin; GEN, gentamicin; NOR, norfloxacin; FOF,
325 fosfomycin; SXT, trimethoprim-sulfamethoxazole; CHL, chloramphenicol.
326 TSST-1: toxic shock syndrome toxin 1; PVL: Panton-Valentine Leukocidin.