

## Original Article

## Nasal carriage of sequence type 22 MRSA and livestock-associated ST398 clones in Tangier, Morocco

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**Introduction:** This study aimed to provide data of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage as well as to determine the genetic lineages of this circulating MRSA in the Tangier community.

**Methodology:** Between 2012 and 2013 two subpopulations consisting of randomly chosen healthy volunteers and outpatients in 11 healthcare facilities were screened. The antibiotic resistance phenotype was determined by disk diffusion. Toxin Panton-Valentin Leukocidin (PVL), toxic shock syndrome toxin-1 gene (*tst*), and *mecA* were detected by polymerase chain reaction (PCR). Nasal swabs were obtained from persons with no identified risk factors for MRSA acquisition. MRSA molecular typing was performed by pulsed-field gel electrophoresis (PFGE), staphylococcal chromosomal cassette *mec*, and *Staphylococcus* protein A (*spa*) typing.

**Results:** A total of 400 subjects (33.3%) were nasally colonized with *S. aureus*, and 17 (1.4%) were nasal carriers of MRSA. The analysis did not identify age, gender, and the two subpopulations as predictors for MRSA colonization. MRSA were more likely to harbor the *tst* gene ( $p < 0.05$ ). This work highlighted a low prevalence of nasal MRSA carriage, with 52.94% belonging to sequence type (ST) ST22. The remaining isolates were distributed as singletons (ST8, ST1, and ST398), whereas approximately one-third of MRSA was not identified, including three novel *spa*-types (t13247, t13248, and t13249).

**Conclusions:** Although we highlighted the current clones present in the Tangier community, they are limited in space and time. Therefore, further studies would be required to obtain a comprehensive picture of the dissemination of MRSA in the community, hospital, and livestock.

**Key words:** *Staphylococcus aureus*; nasal carriage; MRSA; ST22; ST398.

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**Introduction**

*Staphylococcus aureus* is an important bacterial pathogen that causes infections ranging from mild infections of skin and soft tissue to serious invasive diseases [1]. Pathogenicity of *S. aureus* is largely due to its ability to express a broad variety of virulence-associated factors and to colonize humans as well as animals [2]. *S. aureus* nasal carriage is a key source of clinical *S. aureus* infection, though other body sites may also be clinically important [3]. Because methicillin-resistant *S. aureus* (MRSA) was first identified among hospitalized patients and is a major problem in healthcare facilities, it has been referred to as healthcare-associated (HA) MRSA for almost three decades [2]. Since the 1990s, MRSA infections attributed to community-acquired MRSA (CA-MRSA) have been increasingly recognized in the community [4]. Traditionally, CA-MRSA strains colonize or cause infections in persons with no prior history of healthcare-associated risk factors and seem to be more susceptible

to antimicrobial drugs. These strains cause distinct clinical syndromes, have diverse genetic backgrounds, carry smaller SCC*mec* elements, most commonly SCC*mec* type IV or type V, and often harbor the Panton-Valentine leukocidin (PVL) [5]. However, the distinction between HA-MRSA and CA-MRSA remains problematic due to blurred boundaries between them and lack of consistently stable markers [5,6]. Since 2003, MRSA strains from livestock (for example, pigs) or animal products, termed livestock-associated MRSA (LA-MRSA) have emerged worldwide, which reveals the truly zoonotic potential of *S. aureus*/MRSA [7]. Recently, LA-MRSA, notably the sequence type (ST) 398 or related STs clustered in clonal complex (CC) 398, have been found in the community and have spread into hospitals [8].

To date, few studies documenting CA-MRSA in Tangier have been conducted. Therefore, we sought to investigate the prevalence of nasal carriage, the virulence, and the antimicrobial resistance of *S. aureus*,

and to assess the current molecular epidemiology of MRSA in the community setting of Tangier.

## Methodology

### *Study design and patient recruitment*

The present study was conducted over a four-month period, from 10 September 2012 to 16 January 2013. A total of 11 healthcare facilities, the Emergency Department of Tangier's CHR (Centre Hospitalier Régional) and 10 outpatient care centers situated in different regions of the city representing various socio-economic statuses, were selected. The emergency department of CHR and the 10 outpatient care centers act as major referral health facilities in Tangier, and serve a population of one million people. The study involved outpatients and healthy volunteers attending those settings at the time of the study. The healthy population consisted of patients attending for contraception and pre-nuptial check-ups, children coming for vaccinations, and the individuals accompanying them. The current study was approved by the local ethics committee in 2012.

After obtaining consent from patients older than 18 years of age, or from younger patients' parents, 1,200 persons were screened for *S. aureus* carriage. Exclusion criteria for the study population were individuals who were non-residents of Tangier, those who had been hospitalized within the past year, had a device or prosthesis placement, dialysis, close contact with a healthcare worker (family member), factors beyond immunosuppression (diabetes mellitus, malignancy, and current chemotherapy), as well as healthy volunteers who had taken antibiotics in the past two months. Information regarding patient demographics, clinical presentation, residence, and household contacts was collected through standardized questionnaires and medical records.

The standardized questionnaires used in this study include the following data: age, gender, residence area, work status and income, working in a healthcare environment or having a close contact with a healthcare worker (family member), antimicrobial drug therapy history, relationship among various people screened, animal contact, previous institutional admission in the last 12 months (hospital, clinics, day-care attendance), medical procedure in the last 12 months (surgery, dialysis, catheterization, drainage), history of travel, type and length of infection, and history of skin infection (*e.g.*, abscess, furuncles).

### *Isolation and identification of nasal S. aureus isolates*

Samples were collected from both anterior nares by rotating a sterile swab (cotton-tipped polyester swab), which was inoculated into enrichment broth, brain-heart infusion broth (BHI) (Oxoid, Basingstoke, UK), and incubated overnight at 37°C. A loopful of broth was sub-cultured on Mannitol agar (Oxoid, Basingstoke, UK). Suspicious colonies were identified as *S. aureus* by colony morphology and catalase and DNase tests. Presumptive *S. aureus* were confirmed by PCR for 16S rRNA and *nuc* genes, as previously described [9]. *S. aureus* ATCC29213 was used as the positive control.

### *Antibiotic susceptibility testing methods*

Susceptibility testing was performed with the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [10] for cefoxitin (30 µg), erythromycin (5 µg), lincomycin (15 µg), pristinamycin (15 µg), ciprofloxacin (5 µg), gentamicin (10 µg), tobramycin (10 µg), kanamycin (30 UI), tetracycline (30 UI), rifampicin (30 µg), chloramphenicol (10 µg), and co-trimoxazole (23.75 ± 1.25 µg); and according to the recommendations of the Société Française de Microbiologie ([www.sfm-microbiologie.org](http://www.sfm-microbiologie.org)) for fusidic acid (10 µg). Inducible or constitutive lincomycin resistance was determined by the double-disk diffusion test (D-test). The presence of the *mecA* gene was determined by PCR in all cefoxitin-resistant isolates, as previously described [9]. *S. aureus* ATCC 43300 was used as the positive control.

### *Exotoxin gene detection*

All the *S. aureus* isolates were tested for the presence of PVL genes and the *tst* gene by PCR [11]. MW2 (PVL-positive) and FRI913 (*tst*-positive) isolates were used as positive controls for PCR.

### *MRSA molecular typing*

SCC*mec* types were determined by multiplex PCR using previously published primers that identified *mec* and complex genes [12]. Pulsed-field gel electrophoresis (PFGE) was performed by *Sma*I. Restriction digestion and patterns were normalized and compared using the Dice coefficient and unweighted pair group method with arithmetic mean (UPGMA) clustering method with Bio Numerics software version 2.5 (Applied Maths, Sint-Martens-Latem, Belgium). *S. aureus* subspecies NCTC 8325 was used as a control strain, and PFGE EMRSA-15 type L1a (CQ2001-SA05-E) was included for comparison. Strains showing the same PFGE pattern were grouped as a pulsotype and assigned alphabetically (A, B, C, etc.) as previously

described [13,14]. The polymorphic X region of the *spa* gene was amplified using primers *spa*-1113f and *spa*-1514r [15]. Sequences were analyzed using Ridom Staph Type software version 1.4 (Ridom GmbH) and were synchronized with the global *spa* type database via the Staph Type server (www.spaserver.ridom.de). Clustering analysis into *spa* clonal complexes (*spa*-CCs) was carried out using the clustering algorithm based upon repeat pattern (BURP) with default settings. *S. aureus* isolates having fewer than five repeat units were excluded from the clustering analysis [16].

### Statistical analyses

A univariate analysis was used to assess predictors for MRSA carriage among outpatients and healthy volunteers. Comparisons between proportions were drawn with Fisher's exact test and the Chi-squared test. Odds ratio (OR) and 95% confidence intervals (CI) were also calculated. Differences showing a *p* value < 0.05 were considered significant. Calculations were performed using SPSS version 20.0 software (IBM, Armonk, USA).

## Results

### Study population

Among the 1,200 participants screened, 735 (61.2%) were outpatients and 465 (38.8%) were healthy volunteers (Table 1). Carriage rates in outpatients compared with healthy volunteers were 30.2 vs 38.3% (OR = 1.433; 95% CI = 1.122–1.830; *p* < 0.01) for *S. aureus* and 1.08% vs 1.72% (OR = 0.898; 95% CI = 0.339–2.377; *p* > 0.05) for MRSA, respectively. The univariate analysis did not identify gender nor age and the two subpopulations as predictors for MRSA colonization; however, healthy volunteers and male outpatients (OR = 5.952; 95% CI = 3.639–9.737; *p* < 0.001) were at a higher risk of *S. aureus* carriage. The median age of healthy volunteers and colonized outpatients was 25 years (interquartile range [IQR] = 35–9 years).

### Antimicrobial susceptibility profiles

As Table 2 illustrates, MRSA isolates were susceptible or, alternatively, resistant to a limited number of antibiotics. None was resistant to

**Table 1.** Demographics, clinical factors, and univariate analysis of predictors of methicillin-resistant *Staphylococcus aureus* carriage among healthy volunteers and outpatients.

		Healthy volunteers: 178/465				Outpatients: 222/735				P value <sup>3</sup> (healthy / outpatients)	OR <sup>4</sup> (95% CI)
		MRSA (%)	MSSA (%)	P value <sup>1</sup> (MSSA/MRSA)	OR <sup>2</sup> (95% CI)	MRSA (%)	MSSA (%)	P value <sup>1</sup> (MSSA/MRSA)	OR <sup>2</sup> (95% CI)		
		8 (4.49)	170 (95.51)			9 (4.05)	213 (95.95)				
Sex	Male	1 (12.50)	25 (14.70)	0.660	0.828 (0.097–7.027)	4 (44.44)	108 (50.70)	0.488	0.777 (0.203–2.97)	< 0.001	0.167 (0.102– 0.274)
Age / years	0–5	1 (12.50)	21 (12.35)	0.75	NA	3 (33.33)	54 (25.35)	0.552	1.361 (0.218–0.489)	0.02	2.812 (1.108– 7.131)
	06–12	0 (0.00)	7 (4.11)	NA	NA	2 (22.22)	49 (23.00)	0.559	NA	0.3	0.576 (0.164– 2.022)
	13–18	0 (0.00)	5 (2.94)	NA	NA	0 (0.00)	21 (9.85)	0.526	NA	< 0.001	0.106 (0.038– 0.297)
	19–45	5 (62.5)	120 (70.58)	0.231	0.354 (0.063–1.971)	2 (22.22)	54 (25.35)	0.522	0.6481 (0.087–4.816)	< 0.001	4.346 (2.299– 8.22)
	46–80	2 (25)	17 (10)	0.443	0.404 (0.0337–4.853)	2 (22.22)	35 (25.35)	0.657	0.972 (0.154–6.115)	0.284	0.751 (0.358– 1.575)
Toxin gene	<i>tst</i>	4 (50.00)	24 (14.11)	0.022	6.083 (1.425–25.975)	5 (55.55)	29 (13.62)	0.005	7.93 (2.012–1.269)	0.508	1.0321 (0.598– 1.778)
	PVL	0 (0.00)	22 (12.94)	0.34	NA	0 (0.00)	24 (11.26)	0.35	NA	0.371	1.163 (0.628– 2.152)

MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *S. aureus*; CI: confidence interval; NA: not applicable (cannot be calculated due to zero cell); PVL: Pantone-Valentine leukocidin; *tst*: toxic shock syndrome toxin; OR: odds ratio; <sup>1</sup> The differences between MSSA and MRSA in healthy volunteers and between MSSA and MRSA in outpatients; <sup>2</sup> The ratio of risk of being MRSA in the presence of different characteristics; <sup>3</sup> Overall comparison between healthy volunteers and outpatients; statistical significance at *p* < 0.05; <sup>4</sup> The ratio of risk of being outpatients in the presence of different characteristics.

**Table 2.** Comparison of antimicrobial susceptibility profiles of isolates.

Antibiotics	MRSA (17; 4.25%)	MSSA (383; 95.75%)
Erythromycin (5 µg)	1 (5.88)	23 (6.01)
Lincomycin (15 µg)	0 (0.00)	4 (1.4)
Pristinamycin (15 µg)	0 (0.00)	0 (0.00)
Ciprofloxacin (5 µg)	0 (0.00)	4 (1.4)
Gentamicin (10 µg)	1 (5.88)	1 (0.26)
Kanamycin (30 UI)	2 (11.76)	8 (2.09)
Tobramycin (10 µg)	1 (5.88)	3 (0.78)
Tetracycline (30 UI)	7 (41.18)	29 (7.57)
Rifampicin (30 µg)	0 (0.00)	0 (0.00)
Co-trimoxazole (23.75 ± 1.25 µg)	6 (35.29)	2 (0.52)
Fusidic acid (10 µg)	0 (0.00)	6 (1.57)
Chloramphenicol (10 µg)	2 (11.76)	11 (2.87)

MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *S. aureus*.

ciprofloxacin, pristinamycin, rifampicin, and fusidic acid. MRSA strains were resistant when compared to methicillin-sensitive *S. aureus* (MSSA); the difference was statistically significant regarding only tetracycline and co-trimoxazole ( $p < 0.0001$ ).

#### Virulence factor genes

Of the isolates detected, 46 (11.5%) were PVL positive, and 62 (15.5%) were *tst* positive. The distribution of toxin genes in different groups is illustrated in Table 1. A total of 12 isolates harbored both PVL and *tst* genes: 4 strains were from outpatients, and 8 belonged to healthy volunteers.

#### Molecular characterization of MRSA isolates

The results of molecular typing of MRSA strains are presented in Table 3. In the case of the 17 MRSA strains, four different sequence types (STs) were found,

with ST22 being the most prevalent (52.9%). The distribution of ST22 was similar in both subpopulations (OR = 1.25; 95% CI = 0.185–8.444;  $p = 0.6$ ). The three other STs were identified as singletons: one isolate, which exhibited multidrug resistance and was not *SmaI* typeable, was characterized as ST398-MRSA-IV *spa* t011. The second isolate, ST1-IV, showed an inducible lincomycin-resistant phenotype (Table 3). Five MRSA (30%) could not be clustered; among these were three new *spa* types (t13247, t13248, and t13249) and two excluded types (t026 and t502) (Table 3). The banding patterns of MRSA isolates by PFGE exhibited five different pulsotypes (Table 3), with type L as the predominant type. The similarity among isolates within this pulsotype was more than 76.9% by Dice coefficient. PFGE L was related at a cutoff level (CQ2001-SA05-E) by a Dice coefficient of 70%.

**Table 3.** Molecular characterization, antibiotype, and virulence factor genes of MRSA isolates.

Isolate	PFGE	<i>spa</i> type	ST	SCC <i>mec</i>	PVL	<i>tst</i>	Antibiotype
001	L	t13248	Nc	IV	0	0	FOX, SXT
002	Nt	t011	ST398	IV	0	1	FOX, K, TOB, CN, TE, SXT
003	L	t6397	ST22	IV	0	1	FOX
004	L	t11293	ST22	IV	0	1	FOX
005	L	t223	ST22	IV	0	1	FOX
006	L	t223	ST22	IV	0	1	FOX
007	L	t11293	ST22	IV	0	1	FOX, SXT
008	ZB	t13249	Nc	IV	0	0	FOX
009	L	t223	ST22	IV	0	1	FOX, TE
010	L	t223	ST22	IV	0	1	FOX, TE
011	L	t13247	Nc	IV	0	0	FOX, SXT
012	C	t502	Excluded	V	0	0	FOX, TE
013	L	t223	ST22	IV	0	1	FOX, SXT
014	L	t223	ST22	IV	0	0	FOX
015	Nc	t026	Excluded	V	0	0	FOX, TE
016	A	t2658	ST8	V	0	0	FOX, SXT, C, TE
017	XE	t127	ST1	IV	0	0	FOX, E

PFGE: pulsed-field gel electrophoresis; SCC*mec*: staphylococcal cassette chromosome *mec*; ST: sequence type; Nc: not clustered; PVL: Pantone-Valentine leukocidin; *tst*: toxic shock syndrome toxin-1; FOX: cefoxitin; E: erythromycin; CN: gentamicin; TOB: tobramycin; K: kanamycin; TE: tetracycline.

## Discussion

In accordance with several studies, this research shows that nearly one-third of the screened population is colonized by *S. aureus* [2]. MRSA colonization rate (1.4%) appears also to be relatively close to that observed in the global European population [17], yet this rate remains low compared to MRSA carriage (3.3%) in a neighboring Algerian community [18]. The distribution of virulence genes among the two subpopulations was not statistically significant (Table 1), and this statistical insignificance concerned even the presence of the two virulence genes (OR = 2.56; 95% CI = 0.760–8.866;  $p > 0.05$ ). However, it differs between MSSA and MRSA; *tst* was found to be significantly associated with MRSA both in healthy volunteers and outpatients (Table 1), whereas no MRSA was PVL positive. This could be partially explained by the fact that the MRSA *tst* positive isolates belong to the major MRSA clonal sequence type ST22 found (a biased notion).

Furthermore, in sharp contrast to previous studies in North Africa, Europe, the Middle East, and recent sporadic reports in sub-Saharan Africa, the predominant CA-MRSA isolates ST80, which are mainly PVL positive [18,19], were not identified in the current study. In addition, the ST1 and ST8 found differ from USA 400 and USA 300, respectively, in being PVL negative [20].

Distinct ST22 have been found worldwide in hospitals as well as communities; the most common is the epidemic HA-MRSA EMRSA-15 [20,21]. It has been recently reported in healthy individuals (without associated healthcare risk factors) in Italy and the Middle East [22-24]. The origin of ST22-MRSA-IV strains circulating in the Tangier community could be HA-EMRSA-15 types, which are disseminated from hospitals to community environments, as several studies have indicated [6,25]. This hypothesis might find its justification in the fact that the discharged patients continue their wound dressing elsewhere in the outpatient care centers. Nevertheless, the susceptibility pattern of our clone (Table 2) does not match that of HA-EMRSA-15, whose isolates are uniformly resistant to ciprofloxacin and susceptible to co-trimoxazole and minocycline [26]. ST22-MRSA-IV strains could be a variant of UK-EMRSA-15 originating from ST22-MSSA *spa* t223 by means of local evolution, as suggested by Biber *et al.* [23], knowing that the ST22-MRSA-IV strains observed in the present study showed traits similar to the predominant MRSA clone in the Gaza Strip [23] and to the one described in healthy Italian preschool children [22]. This clone mainly lacks

exotoxin PVL, exhibits *spa* t223, and less often carries exotoxin gene *tst*. Nonetheless, the two hypotheses cannot be definitively confirmed since there is no study determining MRSA clones circulating in Tangier's hospital settings or any genetic information regarding the MSSA strain. Importantly, two strains (numbers 4 and 7) colonizing a healthy mother and her infant shared identical PFGE and *spa* types (Table 3). It should be noted that this was the only case in our study relating to household contact carriage. This finding may suggest that this kind of transmission of *S. aureus* could easily occur as previously described [27,28].

The relevant finding of the present study concerns the MRSA-ST398-t011 strain, which was isolated from an outpatient without any history of livestock or pet exposure. Elhani *et al.* [29] isolated one MRSA-ST398-t899 from the nasal sample of a farmer in Tunisia. According to the author, this was the first report of ST398 in humans in Africa. Although colonization of LA-MRSA in African countries seems to be scarce, prudence is needed and further investigations must be undertaken to understand the origin and the dissemination of this clone within our population.

Although our study provides the baseline in the epidemiology of MRSA colonization in Tangier, it has limitations. Firstly, it was limited in space and time. Secondly, surveying only nares and screening participants who are intermittently colonized may result in the underestimation of MRSA prevalence. And finally, five (29.41%) of the MRSA isolates could not be clustered.

## Conclusions

We found a relatively low prevalence of nasal MRSA carriage within persons attending 11 healthcare facilities situated in different regions of the city and representing various socio-economic statuses. The molecular study highlights, for the first time, the current clones present in the Tangier community with a high prevalence of a non-multiresistant, *tst*-positive, PVL-negative t223 ST22-MRSA-IV clone and the presence of one ST 398 clone, and an unclassified third MRSA clone, which require further attention. The number of MRSA isolates available for our investigation was limited and might not reflect the whole epidemiology and MRSA strain types circulating in Tangier. Therefore, to obtain a comprehensive picture of CA-MRSA dissemination, other populations need to be addressed and continuous efforts to understand the epidemiology of this microorganism are necessary, not only in the community but also in the hospital and livestock.

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