

Descriptive Epidemiology of Nasal Carriage of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* Among Patients Admitted to Two Healthcare Facilities in Algeria

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Aim: To evaluate nasal carriage rate and variables associated with *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in patients admitted in two healthcare facilities. **Results:** *S. aureus* was isolated from 159 (26%) of the enrolled patients. Methicillin-susceptible *S. aureus* was isolated from 150 (24.5%) patients, and MRSA was isolated from 9 (1.5%). Cancer and previous hospitalization were associated with a significantly higher frequency of nasal *S. aureus* carriage among the patients admitted to the general hospital and the nephrology department, respectively. MRSA isolates were heterogeneous with respect to their staphylococcal cassette chromosome *mec* element (SCC*mec*) type, sequence type (ST), and toxin genes (*pvl* and *tst1*) content. Four isolates were attributed with the ST80-MRSA-IV clone, which is known to be predominant in Algeria. **Conclusions:** This is the first assessment of *S. aureus* and MRSA nasal carriage and associated variables in Algeria. Our findings provide also a picture of the MRSA strains circulating in the community in this geographic area. They can be useful as a guide for implementing screening and control procedures against *S. aureus*/MRSA in the Algerian healthcare facilities.

Introduction

STAPHYLOCOCCUS AUREUS IS a remarkably versatile pathogen able to cause several infections, produce a large arsenal of virulence factors, escape the defenses of the human organism, and survive also in harsh conditions.

S. aureus causes a wide range of syndromes, from minor skin and soft tissue infections to life-threatening sepsis and toxic shock syndrome.^{12,36} *S. aureus*, indeed, adheres and invades host epithelial cells using a variety of molecules collectively named MSCRAMM (microbial surface components recognizing adhesive matrix molecules).^{12,29,36} Additional factors are responsible for destruction of tissues and cells, including immune cells. Exotoxins, such as the Pantone-Valentine leukocidin (PVL), play also a determinant role.¹² Indeed, the presence of such a virulence factor can significantly increase the morbidity due to this pathogen, especially in geographical areas where the prevalence of PVL-positive strains is high, as it has been recently reported in Algeria.^{6,7} Other alarming features are the high prevalence of methicillin resistance rates within the *S. aureus* strains in some countries, and the

emergence of community-associated methicillin-resistant *S. aureus* (MRSA) infections in otherwise healthy patients.³

S. aureus is a commensal organism that can colonize several sites without causing infection in its host. The anterior nares are the main ecological niche for *S. aureus*. As much as 20% of individuals are reported to be persistently nasally colonized with *S. aureus*, and a further 30% can be intermittently colonized.^{12,29} Bacteria that reside in anterior nares play a role of both reservoir for the spread of this pathogen and risk condition for subsequent infection.^{3,12,33} It is, well established that carriage increases the infection risk.^{18,36} Habitually, it is indeed the colonizing strain that is involved in the infection, according to a study on bacteremias where blood isolates were identical to the nasal ones in 82% of patients.³³ The carrier status can be promoted by several factors, including patient's age and immunological status, recent hospitalization, antimicrobial drug treatment, and underlying disease.^{18,22,29,36} Most of these factors are frequently encountered in the subjects admitted to hospital.

MRSA and, in particular, the so-called European clone ST80-MRSA-IV, is recognized as a prevalent etiological

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agent of infection in Algeria,^{6,7} however, no study has been previously carried out on nasal carriage in this country. This study was conducted in two healthcare facilities in Bejaia, Algeria, to determine the prevalence and epidemiological and clinical variables associated with carriage of *S. aureus* and MRSA in patients sampled at the time of hospital admission. Molecular typing of the MRSA isolates was also performed.

Materials and Methods

Setting

This study was carried out during a period of 27 months in two healthcare facilities in the region of Bejaia, Algeria, both serving about one million population: the first one was the Frantz-Fanon nephrology department with a capacity of 32 beds, comprising also a hemodialysis ward, and the second was the Amizour 240-bed acute general hospital, including wards of pediatrics, surgery, oncology, internal medicine, emergency, and intensive care unit.

Subjects and data collection

All patients who were admitted from April 1, 2010 to June 30, 2012, and gave their consent to nasal swabbing within 48 hours from their admission, were enrolled. Patient data were collected from hospital charts. They included demographics, ward of admission, underlying disease, reason for admission, history of previous hospitalization and antibiotic treatment during the last 6 months, presence of a family member working in healthcare setting, recent surgery, and smoking habits.

Microbiological methods

Specimens were taken with sterile nasal swabs introduced in each nostril to a depth of ~1 cm and rotated five times. Giolitti-Cantoni broth was used as the transport medium. The samples were quickly sent to the laboratory, where mannitol-salt agar plates were inoculated and incubated at 37°C for 48 hours. Identification of *S. aureus* was based on morphology, Gram stain, catalase test, mannitol fermentation, and coagulase test with rabbit plasma. MRSA isolates were detected by plating *S. aureus* colonies onto Mueller-Hinton agar screen plates containing 6 µg/ml oxacillin with 4% NaCl. The presence of the *mecA* gene was confirmed by PCR.

Antibiotic susceptibility determination

Susceptibility of the MRSA isolates was tested against 12 antimicrobial agents using the disk diffusion method according to the guidelines of the Clinical Laboratory Standards Institute.⁴ The antibiotics tested were cefoxitin (30 µg), ciprofloxacin (5 µg), tobramycin (10 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), linezolid (30 µg), rifampicin (5 µg), tetracycline (30 µg), teicoplanin (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), and vancomycin (30 µg). *S. aureus* American Type Culture Collection (ATCC) 29213 was used as a quality control strain.

Molecular typing

All methicillin-resistant isolates were examined by the following molecular methods:

SCCmec typing and SCCmec type IV subtyping. Multiplex PCR was performed to identify the staphylococcal

cassette chromosome *mec* element (SCCmec) types I to VI,²⁴ or by a second method if not typeable by the first one,¹⁹ and results were interpreted according to the guidelines of International Working Group on the Staphylococcal Cassette Chromosome elements (www.sccmec.org). PCR products were then visualized on 3% Tris-Borate-EDTA agarose gels. To distinguish between the different SCCmec IV subtypes, a further multiplex PCR was performed as previously described.²³ An ST22-MRSA-IVa, which had been previously isolated in Palermo, Italy, and had been fully characterized at the Staten Serum Institut, Copenhagen, Denmark, was used as a reference strain.¹¹ Identity of SCCmec VII was confirmed by sequencing and comparison with the sequence deposited in the GenBank database by Takano *et al.*³⁰ (www.ncbi.nlm.nih.gov/nucleotide/AB462393.1)

Multiple loci variable number tandem repeat fingerprinting. Multiple loci variable number tandem repeat fingerprinting (MLVF) analysis was performed according to a method previously described to assess possible clonality among strains isolated from different patients.²⁸ Electrophoresis of the PCR products was performed in Tris-borate buffer 0.5 × -2% agarose gel. The banding patterns were compared visually.

Multilocus sequence typing and *spa* typing. To define the sequence types (ST) of MRSA strains, multilocus sequence typing (MLST) was performed as previously and strains were assigned to STs using the MLST database (<http://saureus.mlst.net/>).⁸ *spa* typing was also carried out on the MRSA-ST22-IVa isolates using the Ridom StaphType software (www.ridom.de/staphtype/).

***pvl* and *tst1* genes detection.** The presence of *pvl* (*lukF-PV* and *lukS-PV*) and *tst1* genes was investigated as previously described and the PCR products were analyzed by electrophoresis on 1.5% agarose gel.¹⁵

Ethical considerations

Informed oral consent was obtained from all patients prior to specimen collection. Ethical considerations were taken into account during all steps of the study. The study results were entered and maintained in a secure database.

Statistical analysis

The statistical analysis was performed by calculating the means and frequencies and the significance of differences was assessed by one-way ANOVA test or Kruskal-Wallis, when appropriate, or by the chi-square test or the Fisher's exact test, respectively. The associations between the variables under examination were evaluated using contingency tables. All reported *p*-values were two-sided and *p* < 0.05 was considered significant.

Results

During the 27-month study period, 612 patients were enrolled: 105 patients were admitted to the Frantz-Fanon nephrology department, and 507 to the Amizour acute general hospital. Two hundred thirty-eight patients were male (male/female ratio, 63.6%). No ethnic differences were observed among the studied population. Twenty subjects were 0–14 year-old. The median age was 53.5 years (interquartile range

[IQR] 38.0–67.5 years) with no significant differences between the patients admitted to the two healthcare facilities (Amizur hospital vs. nephrology department [54.0 (IQR, 38.0–68.0) vs. 48.5 (IQR, 36.0–64.0), $p=0.49$]).

One hundred fifty-nine (26%) out of the 612 patients under investigation were found to carry *S. aureus*. In particular, a significantly higher proportion of patients admitted to the nephrology department proved to be colonized compared to the patients admitted to the acute general hospital (55 out of

105 [52.5%] vs. 104 out of 507 [20.5%], $p<0.001$). In both subgroup of patients, the median age of colonized patients was not significantly different from that of noncolonized patients (Amizur hospital, colonized vs. no colonized patients, 52 years [IQR 38.5–64] vs. 55 years [IQR 38–69], $p=0.38$; nephrology department, colonized vs. no colonized patients, 52 years [IQR 36–64] vs. 46 years [IQR 35–68], $p=0.81$).

Carriage rates, epidemiological and clinical features of the enrolled patients are shown in Table 1. Within patients

TABLE 1. *STAPHYLOCOCCUS AUREUS* CARRIER STATUS OF THE STUDY PATIENTS ADMITTED TO TWO HEALTHCARE INSTITUTIONS IN ALGERIA (APRIL 2010 TO JUNE 2012)

Patient characteristic	<i>S. aureus</i> nasal carriage			
	General hospital n=507	p	Nephrology department n=105	p
Gender				
Male	45 (21.1%)	0.25	25 (58.1%)	0.32
Female	59 (18.9%)		30 (48.4%)	
Antibacterial drug use during the last 6 months				
Yes	41 (22.5%)	0.40	27 (57.5%)	0.34
No	63 (19.4%)		28 (48.3%)	
Hospitalization during the last 6 months				
Yes	81 (21.8%)	0.24	28 (70.0%)	0.005
No	23 (17.0%)		27 (41.5%)	
Recent surgery				
Yes	69 (22.6%)	0.14	21 (63.6%)	0.11
No	35 (17.3%)		34 (47.2%)	
Family member working in healthcare setting				
Yes	21 (20.2%)	0.92	9 (39.1%)	0.15
No	83 (20.6%)		46 (56.1%)	
Smoking habit				
Yes	19 (20%)	0.18	8 (72.7%)	0.15
No	85 (20.6%)		47 (50.0%)	
Skin and soft tissue infection				
Yes	16 (25%)	0.34	7 (50%)	0.84
No	88 (19.9%)		48 (52.7%)	
Pneumonia				
Yes	18 (17.1%)	0.33	7 (70%)	0.40
No	86 (21.4%)		48 (50.5%)	
Urinary tract infections				
Yes	0	—	0	—
No	104 (20.6%)		55 (52.4%)	
Joint disease/osteoarthritis				
Yes	0	—	0	—
No	104 (20.6%)		55 (52.4%)	
Cancer				
Yes	45 (26.6%)	0.016	0	—
No	59 (17.5%)		55 (52.4%)	
Digestive disease				
Yes	0	0.58	0	—
No	104 (20.7%)		55 (52.4%)	
Hypertension				
Yes	19 (17.1%)	0.31	43 (51.2%)	0.62
No	85 (21.5%)		12 (57.1%)	
Heart disease				
Yes	4 (17.4%)	0.70	0	—
No	100 (20.7%)		55 (52.4%)	
Diabetes mellitus				
Yes	13 (17.3%)	0.46	11 (61.1%)	0.41
No	91 (21.1%)		44 (50.6%)	

Data are number (%) of patients.

Bold and underlined figures indicate statistically significant values ($p<0.05$).

TABLE 2. EPIDEMIOLOGICAL AND MOLECULAR CHARACTERISTICS, AND RESISTANCE PATTERN OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM COMMUNITY PATIENTS ADMITTED IN TWO HEALTHCARE INSTITUTIONS IN ALGERIA (APRIL 2010 TO JUNE 2012)

Strain code	Ward	Gender/age (years)	SCCmec type	pvl/tst1 genes	ST	Resistance pattern ^a
25	Internal medicine	F/38	IVc	-/-	5	TOB, GEN
148	Surgery	M/75	IVa	-/-	80	TOB, GEN
165	Internal medicine	F/71	IVa	-/+	22	TOB, SXT
193	Surgery	F/29	IVc	+/-	80	TE
414	Internal medicine	F/37	IVa	-/+	22	TOB, SXT
248	Nephrology	M/67	IVc	+/-	80	ERY
257	Nephrology	M/35	IVc	+/-	80	TOB, GEN
296	Nephrology	F/67	VII	-/-	5	TE
322	Nephrology	F/32	IVh	-/-	535	TOB, TE

^aOnly the resistances to non β -lactam antibiotics are included. ERY, erythromycin; GEN, gentamycin; pvl, genes coding for Panton-Valentine leukocidin (PVL); SCCmec, staphylococcal cassette chromosome mec element; ST, sequence type; SXT, trimethoprim/sulfamethoxazole; TE, tetracycline; TOB, tobramycin.

admitted to the general hospital, *S. aureus* nasal carrier status was significantly associated with cancer, whereas within nephrology patients the carrier status was associated with previous hospitalization (Table 1).

Out of the patients under study nine (1.5%) carried MRSA, whereas the remaining 150 (24.5%) carried methicillin-susceptible *S. aureus* (MSSA). Four MRSA isolates were identified in the patients admitted to the nephrology department and five among those admitted at the Amizour hospital.

Epidemiological, molecular characteristics and resistance pattern of MRSA strains are summarized in Table 2. Resistance to non β -lactam antibiotics was restricted to tetracycline, aminoglycosides, or sulfamethoxazole-trimethoprim. Only one ST80-IVc isolate was resistant to erythromycin.

Eight out of nine isolates carried a SCCmec type IV, with four isolates carrying the subtype IVc. Four isolates belonged to ST80, whereas ST22 and ST5 included two isolates each. The remaining one was assigned to ST535 (clonal complex [CC]30) and carried a SCCmec IVh. Three ST80-MRSA isolates tested positive for the PVL genes. The *tst1* gene was present only in the ST22-MRSA isolates.

The MLVF analysis suggested a possible clonal relation between the two strains (isolates 165 and 414) since no difference in their electrophoretic profiles was observed. *spa* typing assigned both isolates to *spa* type t223. These two isolates were from two elderly patients admitted to the department of internal medicine of Amizour hospital.

Discussion

Scientific data on nasal carriage of *S. aureus* and MRSA in Algeria are erratic and are lacking in the individuals in the community. To our best knowledge, this is the first study exclusively dedicated to assess nasal carriage of MSSA and MRSA in Algeria.

Our *S. aureus* colonization rate was very close to the average rates detected in previous investigations conducted in different countries around the world. Studies on patients recently admitted to different care facilities in various countries have shown colonization rates ranging from 21.5%⁵ or 23.7% in the United States,¹⁴ to 22.1% in Taiwan,³⁵ and 28.9% in Australia.²⁷ These rates were also varying within the same country. For instance, in the United States higher figures than

those previously cited were reported in Hawaii and Georgia with rates as high as 39.9% and 32.4%, respectively.^{16,20} It is possible that differences in patients case mix could account for most of these fluctuations.

According with literature, our study detected remarkably different nasal colonization rates within the two subgroups of patients under investigation, with a higher rate among patients admitted to the nephrology department. Chronic kidney failure is unanimously recognized to be a high risk condition for carriage and disease by *S. aureus*.^{22,32} These patients are, indeed, generally submitted to hemodialysis, a healthcare procedure that is agreed as a major risk factor, mainly because of progressive worsening of general health conditions, application of invasive devices (catheters and fistulas), and frequent underlying disease.^{18,21,22} Moreover, in our experience *S. aureus* carriage proved to be significantly associated in this subgroup of patients with previous hospitalization, a recurrent event in the clinical course of subjects affected by kidney failure.^{22,32} We also found that cancer was associated with colonization by *S. aureus* in the patients admitted to the Amizour general hospital, as a probable consequence of immunological suppression. In recent literature no report is available about this association. Conversely, variables known to behave as risk factors, such as male gender, young age, and smoking habits, did not influence nasal carriage of *S. aureus* in our setting.^{13,16,20}

The rate of MRSA carriage in our study was close to those reported in countries with low incidence of nasal colonization, such as Australia and The Netherlands with 0.7% and 0.03%, respectively.^{27,37} This rate is also lower than that reported from Taiwan,³⁵ France,⁹ and Slovenia³⁴ with rates of 3.8%, 14.6%, and 11.8%, respectively, and in some regions of the United States, as in the states of Texas and Georgia, with rates 3.4% and 7.3%, respectively.^{5,14} The low MRSA carriage prevalence did not allow us to evaluate a possible favoring role of demographic or clinical variables.

MLST analysis determined that four MRSA isolates were ST80, all carrying SCCmec IV, and three encoding PVL genes. This was expected in consideration of the dominant role of this clone in staphylococcal infections in Algeria. In previous studies on MRSA infections among children, newborns, and their mothers, indeed, this clone represented 96.5% of the isolates.^{6,7} This clone is also known to be frequent in North

African and Middle East countries such as Tunisia, Jordan, and Kuwait.^{1,17,31} However, the heterogeneity of the MRSA strains isolated in this study was to some extent a surprising finding compared to the large predominance of ST80-IV in the preceding investigations. Noteworthy, the two *tst1* positive ST22-MRSA-IV strains, spa type *t223*, appeared to be similar to the “Gaza strain,” the predominant MRSA clone in healthy children and their parents living in the Gaza strip.² This CC22-MRSA-IV “Middle Eastern Variant” has been also described in Jordan and Saudi Arabia,^{17,26} and more recently, in newborns and preschool children in Palermo, Italy.^{10,11} The detection of this clone in Algeria confirms its wide dissemination in the Mediterranean and Middle East area. Moreover, within the two strains of ST5-MRSA, one could be identified as belonging to the so-called pediatric clone, which is widespread across America, Europe, Asia, and Australia, but is apparently infrequent in Africa.²⁵ The ST5-MRSA-VII is, on the contrary, an uncommonly identified strain, as reported by Monecke *et al.*²⁵ Finally, the ST535-MRSA-IVh strain belonged to CC30, an important and worldwide spread clonal complex.

This study has some limitations. First, the epidemiological and clinical data about patients were relatively limited, mainly about the previous history of exposure to the healthcare system. Moreover, the descriptive design of the study was unable to support a more accurate analysis of the association of the variables under study with the *S. aureus* carriage. The infection status as a possible outcome was not investigated. However, our study provides for the first time information about *S. aureus* and MRSA nasal carriage in patients at admission to hospital in Algeria and about molecular characteristics of MRSA strains. *S. aureus* carriage, either present at admission to the hospital or acquired during hospitalization, increases the risk for infection. So, the findings of this study could provide helpful hints for more adequate patient management, especially in the case of those suffering from renal failure and cancer, and for the infection control programs in this country. Moreover, the phenotypic and molecular characteristics of the MRSA isolates can contribute to the worldwide map of this pathogen.

Acknowledgments

The authors thank Mr. Karim Bensaid, Ph.D., for his help in statistical analysis.

Disclosure Statement

All authors report no conflicts of interest relevant to this article.

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