Staphylococcus aureus nasal and pharyngeal carriage in Senegal

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Abstract

Nasal and pharyngeal swabs were collected from 132 patients admitted to the Principal Hospital in Dakar (Senegal), in January and February 2012. The prevalence of *Staphylococcus aureus* carriage was 56.1% (n = 74): 40.2% for pharyngeal samples and 36.4% for nasal samples. None of the isolates was methicillin-resistant. Carriage was independently associated with being female (p <0.01) and large households (≥15 members) (p 0.04). The *luk*-PV genes encoding Panton–Valentine leukocidin (PVL) were present in 26.2% of the isolates. These data highlight the importance of the oropharynx as a site of colonization, and the high prevalence of PVL-positive isolates in Senegal as compared with industrialized countries.

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Staphylococcus aureus remains a major human pathogen [1]. Colonization of the anterior nares is considered to be the most important risk factor for *S. aureus* infection, and decolonization is recommended in certain specific populations at risk of infection in developed countries [2]. Recent studies have identified the oropharynx as an unrecognized and underestimated site of colonization [3–5]. This has potentially major implications for decolonization strategies [2], as topical agents for eradicating nasal colonization are unlikely to affect throat carriage [6]. We report here the prevalence and risk factors of *S. aureus* carriage, antimicrobial drug susceptibility and the molecular characterization of isolates in patients admitted to the emergency department of the Principal Hospital, Dakar, Senegal (West Africa).

Nasal and pharyngeal swabs were collected from patients recruited at random, on 2 days per week, within 2 h of their admission in January and February 2012. None of the patients showed skin and soft tissue infection at inclusion. A standardized specific questionnaire was completed, for the collection of demographic data, medical history over the previous 12 months, and information concerning risk factors for nasopharyngeal carriage (Table 1). The Senegalese national ethics committee approved the study protocol.

The swabs were placed at +4°C immediately after sampling, and were processed within 4 h. They were used to inoculate a pre-enrichment medium consisting of brain–heart broth containing 7.5% NaCl. After incubation overnight at 37°C in aerobic conditions, selective enrichment was carried out in brain–heart broth (BioMérieux, Marcy l'Etoile, France) supplemented with colistin (4 mg/L). We used 25 μL of the selective enrichment broth to inoculate specific agar plates (BBL CHROMagar *S. aureus* and BBL CHROMagar MRSA; Becton Dickinson, Heidelberg, Germany). *S. aureus* identification, antimicrobial susceptibility by the disk diffusion method in accordance with the guidelines of the French Society for Microbiology (see CA-SFM guidelines: http://www.sfm-microbiologie.org), genomic extraction, multiplex PCR amplification of the *agr* locus for confirmation of the identification, screening for *luk*-PV genes, *spa* typing and multilocus sequence typing (MLST) were performed as previously described [7–9]. MLST was performed on one strain randomly selected for each of the *spa* types identified.

The chi-square test and Student's *t*-test were used to compare categorical and continuous variables in univariate analysis, respectively, with R software. Factors with p-values of <0.20 in univariate analysis were retained for multivariate analysis. We considered p-values of <0.05 to indicate significant associations.

In total, 132 patients (63 female/69 male; mean age, 38 years; median age, 41.3 years) of Senegalese origin were included. Seventy-four (56.1%) were carrying *S. aureus* (44 female/30 male; mean age, 39.3 years; median age, 38 years; interval range, 11–84 years; 25th percentile, 25.3 years; 75th percentile, 50.8 years). The overall prevalence of carriage in the nose and/or throat (74/132, 56.1%) and of carriage in the throat (53/132, 40.2%) was consistent with the few studies that

TABLE 1. Risk factors associated with *Staphylococcus aureus* carriage in 132 patients

			Univariate analysis		Multivariate analysis		
	Carriage (*n* = 74)	Non-carriage (*n* = 58)	Crude OR	p-value	Adjusted OR	95% CI	p-value
Mean age in years (95% CI)	39.3 (39.2–39.5)	43.7 (43.6–43.8)	–	0.13	0.98	0.96–1.01	0.23
Female gender, *n* (%)	44 (70)	19 (30)	2.9	0.003	3.0	1.4–6.6	<0.01
Underlying chronic disease, *n* (%)	25 (52)	14 (48)	1.6	0.25	–	–	–
Hospitalization in the last 12 months, *n* (%)	11 (69)	5 (31)	1.8	0.29	–	–	–
Surgery in the last 12 months, *n* (%)	4 (80)	1 (20)	3.3	0.38	–	–	–
Skin and soft tissue infection in the last 12 months, *n* (%)	11 (79)	3 (21)	3.3	0.09	3.5	0.9–14.2	0.08
Antibiotic treatment in the last 3 months, *n* (%)	25 (61)	16 (39)	1.5	0.34	–	–	–
Family healthcare worker, *n* (%)	16 (61)	10 (39)	1.3	0.66	–	–	–
Smoker, *n* (%)	3 (27)	8 (73)	0.27	0.06	0.22	0.03–1.4	0.10
≥15 household members, *n* (%)	16 (73)	6 (27)	2.5	0.09	3.2	1.1–9.9	0.04

have reported and compared nasal and pharyngeal carriage (49.9–57.8% and 34.7–44%, respectively) [3–5]. Nasal carriage of *S. aureus* was reported in 36.4% (48/132) of those tested. This value is in the middle of the range of reported values (14.0–55.1%) [2]. Carriage was exclusively pharyngeal in 26 patients (19.7%), exclusively nasal in 21 (15.9%), and concerned both sites in 27 (36.5%).

Large households (≥15 members) were borderline significantly associated with an enhanced risk of *S. aureus* carriage (p 0.04) in our study, as previously described [2]. Men have repeatedly been reported to have a higher incidence of carriage in several populations and settings [4], whereas we found an association between carriage and being female (p <0.01) (Table 1). Further investigations with a larger cohort of patients are needed to confirm these results.

One colony from each site positive for *S. aureus* was investigated by *spa* typing. Overall, the 101 *S. aureus* isolates belonged to 42 *spa* types, which were separated by the Based Upon Repeat Pattern algorithm into 11 *spa* clonal complexes (CCs) and six singletons (groups represented by a single *spa* type). Each *spa* CC corresponded to a single MLST CC. CC variability in the throat was similar to that in the nose (Table 2). Twenty-one (77.7%) of the 27 patients showing both nasal and pharyngeal colonization had identical isolates at both sites, as demonstrated on the basis of *spa* types. In such cases, only one isolate per patient was retained for further analyses (80 isolates in total). None of the isolates was methicillin-resistant. They showed susceptibility to most of the antibiotics tested, except for penicillin G (87.5% of resistant isolates), co-trimoxazole (50.0%), and tetracycline (27.5%). Seventy-one per cent (*n* = 57) clustered into five main MLST CCs: CC15 (21.3%), CC8 (15.0%), CC5 (16.3%), CC152 (10.0%), and CC45 (8.8%) (Table 2). Seven of the 11 CCs found in our study, including four of the five major CCs, have been identified as successful genetic backgrounds for methicillin-resistant *S. aureus*, suggesting that they may provide a stable genetic environment for *SCCmec* integration. They have been found worldwide (CC1, CC8, CC30, CC45, and CC152) [1] or more specifically in Dakar (CC5) or Africa (CC88) [9]. It is therefore important to continue the

TABLE 2. Molecular characteristics of methicillin-susceptible *Staphylococcus aureus* isolates from nasal and pharyngeal swabs collected from 74 carriers in Dakar, Senegal

MLST		Nasal strains			Pharyngeal strains			Total	
CC	ST	*spa*-types (n)	*luk*-PV (%)	Total	*spa*-types (n)	*luk*-PV n (%)	Total	Strains n (%)	Patients[a] n (%)
15	15	084 (10)	3 (30)	10	084 (9), 279 (1), 491 (1), 774 (1)	3 (25)	12	22 (22)	17 (21)
8	8	1476 (4), 2555 (1), 451 (1)	1 (17)	6	451 (2), 2555 (1), 1476 (2), 451 (1), 8 (1)	0	7	13 (13)	11 (14)
	1404		0	0	1617 (1)	0	1	1 (1)	1 (1)
5	5	311 (8), 062 (1)	1 (11)	9	311 (4)	0	4	13 (13)	13 (16)
45	508	861 (4), 1510 (2), 3986 (2)	0	8	1510 (1), 10429 (1), 10430 (1)	0	3	11 (11)	7 (9)
152	152	4235 (3), 4690 (1)	3 (75)	4	355 (2), 1172 (1), 4235 (1)	4 (100)	4	8 (8)	6 (7)
	377	5129 (1)	1 (100)	1	5129 (1)	1 (100)	1	2 (2)	2 (3)
121	121	314 (3)	3 (100)	3	314 (3)	3 (100)	3	6 (6)	3 (4)
30	30	276 (1), 012 (1)	2 (100)	2	012 (3)	2 (67)	3	5 (5)	4 (5)
97	97	267 (1), 359 (1)	0	2	267 (1)	0	1	3 (3)	2 (3)
80	80		0	0	5802 (1), 131 (1)	0	2	2 (2)	2 (3)
1	852	5500 (1), 127 (1)	0	2			0	2 (2)	2 (3)
88	88		0	0	692 (1)	0	1	1 (1)	1 (3)
[b]		7760 (2), 091 (1), 1458 (1), 10426 (1), 10427 (1)	2 (29)	7	091 (1), 100 (1), 8481 (1)	0	3	10 (1)	9 (11)
[c]			0	0	635 (1), 1991 (1)	2 (100)	2	2 (2)	0
Total			16 (30)	54		15 (32)	47	101 (100)	80 (100)

MLST, multilocus sequence typing; CC, clonal complex; ST, sequence type.
[a]For patients displaying both nasal and pharyngeal colonization, only one of the identical *spa*-type paired isolates was retained for subsequent analysis (in all, 80 strains).
[b]*spa* CCs with no founder.
[c]Singleton *spa* types shorter than five repeats.

monitoring of antimicrobial susceptibility in clinical *S. aureus* isolates.

The *luk*-PV genes encoding Panton–Valentine leukocidin (PVL) were present in 22.6% of the isolates, belonging to 15 (35.7%) of the 42 *spa* types. PVL prevalence in *S. aureus* isolates from carriage is highly variable worldwide [10]. There may be a gradient, with the prevalence of these isolates decreasing from West and Central Africa (Senegal (present study); Mali, >35% [11]; Gabon, 56% [12]) to North Africa (Algeria, 9%) [13] and Europe (0.3–1.4%) [14]. The reasons for such a high prevalence remain unclear, but the high virulence of these *S. aureus* isolates [15,16], together with factors specifically associated with developing countries (e.g. poor access to healthcare services, poor hygiene and sanitary conditions, and overcrowding, etc.), may increase the risk of inter-individual transmission [17].

In conclusion, our data highlight the importance of the oropharynx as a site of colonization, and the high prevalence of PVL-positive isolates in strains from Senegal.

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Transparency Declaration

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