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ORIGINAL ARTICLE

Staphylococcus aureus nasal carriage among homeless population in Lisbon, Portugal

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage is a major risk factor for infection, namely among populations in the community with inherent prompting factors, such as the homeless. In Portugal, there are no data on *S. aureus*/MRSA nasal carriage among the homeless community. A total of 84 homeless individuals living in Lisbon (34 with no permanent address and 50 living in shelter) were nasally screened for *S. aureus*/ MRSA. All isolates were characterized to determine antimicrobial susceptibility and clonal type. A total of 43 (51.2%) *S. aureus* carriers were identified, including a single individual colonized with MRSA (1.2%). *S. aureus* carriage rate was higher among individuals with no permanent address (58.8% versus 46%), younger (45.7 ± 12.7 versus 52.5 ± 10.8 years), and with diagnosis of asthma (9% versus 0%). The single MRSA belonged to the EMRSA-15 clone (PFGE D, ST15-SCC*mec* IVh, and *spa* type t790). Almost half of the methicillin-susceptible *S. aureus* (MSSA) isolates (41.9%, $n = 18$) belonged to two major clones, ST398-t1451 ($n = 13$) and ST30-t399/t11980/t12808 associated with PFGE I ($n = 5$). A high proportion of isolates showed non-susceptibility to mupirocin (64%), erythromycin (45%), and fusidic acid (20%) and induced resistance to clindamycin (39%). None of the isolates harboured PVL. Our results suggest that the homeless population of Lisbon does not constitute a reservoir of MRSA in the community, but harbour the highly transmissible ST398-t1451 MSSA lineage.

Keywords MRSA · Nasal carriage · Homeless · Portugal

Introduction

Staphylococcus aureus is not only an ubiquitous colonizer of the human anterior nares and skin, but also a major pathogen, remaining a leading cause of human infections worldwide, in both hospitals and community largely due to its methicillin-resistant form (MRSA) [1, 2].

Homelessness is one of the main societal problems and an important challenge across many countries. A Social Protection report from the European Union showed that in 2016, 23.5% of Europeans were at risk of poverty or social exclusion [3]. Data from Portugal were similar to the European mean, with 25.1% of people at risk of poverty, namely in metropolitan areas of Lisbon and Oporto [3]. In the capital (Lisbon), it is estimated that 852 individuals live without physical accommodation, and are described as being absolutely homeless [4].

Compared with the general population, homeless people are at higher risk for morbidity and mortality, with higher rates of chronic physical health conditions, including almost all type of infections that frequently lead them to emergency services [5]. Moreover, individuals who are homeless were reported to have an increased risk for MRSA colonization and subsequent infection, since they share crowded and often unsanitary living conditions, typically manifest poor underlying

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health such as HIV infection and tuberculosis or diabetes, and have limited access to hygiene facilities and healthcare services [6–8].

Although the burden caused by MRSA infections is widely recognized and its prevention and control have been identified as public health priorities in the European Union, the situation in Portugal remains troublesome: (i) in 2017, Portugal showed a MRSA nosocomial prevalence of 39.2% that largely exceeds the European mean (16.9%) [9]; (ii) the first European vancomycin-resistant *S. aureus* (VRSA) was isolated in Portugal in 2013 [10]; (iii) prevalent MRSA lineages in Portuguese hospitals have been detected in the community [11, 12]. However, there are no studies on MRSA carriage among homeless people in Portugal, which may constitute a MRSA reservoir in the community. Furthermore, people living in shelters in Lisbon have shown higher significant prevalence of respiratory infections and obstructive lung disease. Homeless shelters may facilitate virus transmission and respiratory infections [13]. The contribution of *S aureus* carriage to these adverse outcomes is uncertain.

The aim of the present study was to determine MRSA colonization rates among the homeless in Lisbon, determine major risk factors for *S. aureus* carriage, and characterize *S. aureus* clonal population, antibiotic resistance, and virulence.

Methods

Homeless community setting

For the present study, two groups of homeless people were considered accordingly to the European Observatory for Homelessness [14, 15]:

1. individuals living in a roofless condition or in secure housing with no permanent address, in the public space or in a precarious place (from now on referred as "with no permanent address");
2. people living without a house, being in temporary accommodation on homeless shelters designed for that purpose, which promotes their reintegration (from now on referred as "living in shelters").

A total of 84 individuals, meeting the eligibility criteria (≥ 18 years old, living without facilities for cooking, and with a nasal mucosa without signs of injury) and representing 10.3% of the entire homeless population identified in Lisbon [4], were included in the study (Table 1). The protocol was approved by the Ethics Committee of Escola Superior de Saúde da Cruz Vermelha Portuguesa and a written informed consent was obtained from all participants.

Nasal screening and bacterial isolates

Sampling was performed by trained doctors and nurses, between November 2016 and January 2018. Samples were taken by swabbing both nares of each individual with a sterile cotton swab, which was stored in Stuart transport medium until processing in the laboratory as previously described [16]. MRSA was confirmed by PCR amplification of the *nuc* and *spa* genes for species identification [17, 18], and the detection of the *mecA* gene for methicillin resistance [19].

Phenotypic and molecular characterization

Antimicrobial susceptibility testing was performed by the disk diffusion method, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST_http://www.eucast.org/), for cefoxitin, ciprofloxacin, chloramphenicol, clindamycin, erythromycin, fusidic acid, gentamicin, linezolid, mupirocin, penicillin, quinupristin-dalfopristin, rifampin, teicoplanin, tetracycline, and trimethoprim-sulfamethoxazole (SXT). Strain *S. aureus* ATCC29213 was used as quality control. Vancomycin minimum inhibitory concentration (MIC) was determined by Etest.

The isolates were characterized by pulsed-field gel electrophoresis (PFGE) [20], *spa* typing [18], multilocus sequence typing (MLST) [21], SCC*mec* typing [22, 23], and by detection of the Panton Valentine leucocidine (PVL) gene [24], as previously described.

Statistical analysis

Categorical variables were compared using the χ^2 or Fisher's exact test when appropriate, while continuous variables were tested using Student's *t* test, through the SPSS Statistics software version 21 (IBM Portugal, Lisbon, Portugal). *p* values of ≤ 0.05 were considered statistically significant.

Results

Prevalence of *S. aureus* and methicillin resistance

Eighty-four homeless individuals (34 with no permanent address and 50 living in shelters) were nasal screened for *S. aureus*/MRSA, out of which 43 (51.2%) were *S. aureus* carriers. A single individual with no permanent address was colonized by an MRSA (1.2%). The prevalence of *S. aureus* carriage in individuals with no permanent address was higher than among people living in shelters ($n = 20$, 58.8% versus $n = 23$, 46%) although the difference was not statistically significant ($p = 0.2739$).

Table 1 Distribution of individuals screened by geographic region and homeless condition

Geographic location	Individuals (No./total no.)	Homeless condition (no.)[a]	
		No permanent address	Shelter
Northeast	9/177	8	1
Centre	5/113	5	–
South	24/82	21	3
Shelter 1	31/210	–	31
Shelter 2	15/67	–	15
Total	84/818	34/431	50/387
%	10.3%	7.9%	12.9%

[a] Four individuals, sampled at Northeast (Gare 2, $n = 1$) and South (Gare 1, $n = 3$), have been attending homeless shelters for night accommodation and therefore were considered as shelter attendees, but identification of the attended shelter was not available

For homeless people with no permanent address, the highest *S. aureus* prevalence (66.6%; $n = 14$) was detected among people from Gare 1, followed by city hall (60%, $n = 3$) and by Gare 2 (37.5%, $n = 3$). The MRSA carrier was detected in Gare 1. Similar *S. aureus* carriage rates were detected among people living in the two shelters sampled (Shelter 1, $n = 12$, 38.7% versus Shelter 2, $n = 7$, 46.6%, $p = 0.7515$).

A single individual was colonized with two different *S. aureus* isolates, therefore a total of 44 *S. aureus* isolates (20 from homeless with no permanent address and 24 from shelters) were recovered for further characterization.

Subject demographics and risk factors for MRSA carriage

S. aureus carriage was higher among younger individuals (45.7 ± 12.7 versus 52.5 ± 10.8 years, $p = 0.009$) and people with diagnosis of asthma (9% versus 0%, $p = 0.04$). However, no significant difference was detected neither in *S. aureus* carriage between male and female individuals (50.8%, $n = 32$ and 52.4%, $n = 11$ respectively, $p = 0.90$), nor in other conditions commonly associated with increased MRSA carriage rates (Table 2): smoking ($p = 0.28$), alcohol ($p = 0.12$), and illicit drug use ($p = 0.19$).

Antibiotherapy in the last 6 months preceding the screening showed to be a protective factor for *S. aureus* colonization in this population (0% versus 10%, $p = 0.04$).

Antimicrobial resistance

The *mecA* gene was detected in a single *S. aureus* isolate showing resistance to beta-lactams, rifampicin, erythromycin, and induced resistance to clindamycin.

The MSSA isolates showed a non-multiresistant profile (Table 3), with 29.5% ($n = 13$) of the isolates presenting penicillin resistance only, and 45% ($n = 20$) resistance to erythromycin, out of which 85% ($n = 17$) showed also induced resistance to clindamycin. Moreover, 20% ($n = 9$) of the isolates showed resistance to fusidic acid and less than 10% showed resistance to ciprofloxacin (7%, $n = 3$), chloramphenicol (5%, $n = 2$), and tetracycline (5%, $n = 2$). Non-susceptibility to mupirocin and to rifampicin was detected in 64% ($n = 28$) and 18% ($n = 8$) of the isolates, respectively. All isolates were susceptible to vancomycin (MIC < 1.5 mg/L).

Antimicrobial resistance profiles detected were similar in both groups, individuals with no permanent address and living in shelter (Table 3), except for mupirocin that was higher among people attending shelters (mupirocin: 45% versus 79%, $p = 0.02$). However, 50% ($n = 6$) of the isolates with resistance to three or more antibiotics were recovered from the population with no permanent address.

S. aureus clonal diversity

The single MRSA isolate belonged to PFGE type D and was characterized by *spa* type t790, sequence type (ST) 22 and carried SCC*mec* cassette type IVh (Table 4). This isolate was recovered from a foreign man from the Middle East, screened at Gare 1, and not having a permanent address.

Almost half of the MSSA isolates (41.9%, $n = 18$) were distributed into two major clones (Table 4): ST398-*spa* type t1451 ($n = 13$) and ST30-*spa* types t399/t11980/t12808 associated with PFGE I ($n = 5$). The remaining MSSA belonged to 10 minor clones out of which four were represented by single isolates only (Table 4). The major MSSA lineage ST398-t1451 was prevalent among both homeless with no permanent address and shelter populations ($p = 1.000$). Noteworthy, seven of the 12 MSSA lineages were exclusively found in one of the groups (Table 4).

None of the isolates carried PVL.

Table 2 Demographic and health associated characteristics of the homeless individuals included in the study

Variable	Total ($n = 84$) (%)	*S. aureus* carriers ($n = 43$) (%)	No *S. aureus* carriers ($n = 41$) (%)	p value
Demographic characteristics				
Age (years)	49.0 ± 12.2	45.7 ± 12.7	52.5 ± 10.8	*0.009*
Gender				
Male	63 (75)	32 (74)	31 (76)	0.900
Female	21 (25)	11 (26)	10 (24)	
Housing status at recruitment				
No permanent address	34 (40)	20 (46)	14 (34)	0.250
Living in shelter	50 (60)	23 (54)	27 (66)	
Has a primary care provider				
Yes	29 (35)	13 (30)	16 (39)	0.400
No	55 (65)	30 (70)	25 (61)	
Emergency room observation (< 2 years)	10 (12)	4 (9)	6 (15)	0.450
Recent antibiotic therapy (< 6 months)	4 (5)	–	4 (10)	*0.040*
Addiction	54 (64)	29 (67)	25 (61)	0.540
Smoker	44 (52)	25 (58)	19 (46)	0.280
Alcohol abuse	26 (31)	10 (23)	16 (39)	0.120
Injection drug use	15 (18)	10 (23)	5 (12)	0.190
Vascular disease risk factors	18 (21)	9 (21)	9 (22)	0.910
Hypertension	12 (14)	7 (16)	5 (12)	0.590
Hypercholesterolaemia	5 (6)	1 (2)	4 (10)	0.150
Diabetes	7 (8)	4 (9)	3 (7)	0.740
Respiratory disease	53 (56)	29 (61)	24 (59)	0.400
Chronic bronchitis	53 (63)	29 (67)	24 (59)	0.400
Chronic obstructive pulmonary disease	5 (6)	2 (5)	3 (7)	0.610
Asthma	4 (5)	4 (9)	–	*0.040*
Pulmonary infection (< 2 years)	23 (27)	11 (26)	12 (29)	0.700
Heart disease	8 (10)	4 (9)	4 (9)	0.940
Ischemic cardiac disease	3 (4)	2 (5)	1 (2)	0.580
Infectious disease*	12 (14)	5 (12)	7 (17)	0.480
HIV infection	5 (6)	3 (7)	2 (5)	0.680
HBV infection	2 (2)	–	2 (5)	0.140
HCV infection	5 (5)	2 (5)	3 (7)	0.610
Gastroenterologic disease	24 (29)	13 (30)	11 (27)	0.730
Viral hepatitis	5 (6)	2 (5)	3 (7)	0.610
Alcoholic hepatitis	7 (8)	2 (5)	5 (12)	0.210
Neurologic disease	12 (14)	7 (16)	5 (12)	0.590
Epilepsy	4 (5)	4 (9)	–	0.050
Previous stroke	7 (8)	4 (9)	3 (7)	0.740
Psychiatric disease	20 (24)	10 (23)	10 (24)	0.900
Depression or hopelessness	14 (17)	6 (14)	8 (20)	0.490
Schizophrenia	2 (2)	2 (5)	–	0.160

p values ≤ 0.05 were considered statistically significant and are italicized. *HIV* human immunodeficiency virus, *HBV* hepatitis B virus, *HCV* hepatitis C virus

*HBV and HCV infections were considered under "infectious disease" based on positive serological detection of the virus in individuals that did not developed hepatitis disease. Viral hepatitis that caused diagnosed disease were included in "gastroenterologic disease"

Table 3 Antibiotic susceptibility profiles of the *S. aureus* isolates recovered from nasal colonization among homeless individuals

Antibiotic	Non-susceptible *S. aureus*, n (%)[a]			
	Total ($n = 44$)	No permanent address ($n = 20$)	Shelters ($n = 24$)	p value
Cefoxitin	1 (2)	1 (5)	0	0.280
Penicillin	32 (73)	16 (80)	16 (67)	0.430
Rifampicin	8 (18)	2 (10)	6 (25)	0.180
Clindamycin	17 (39)	9 (45)	8 (33)	0.320
Erythromycin	20 (45)	9 (45)	11 (46)	0.920
Tetracycline	2 (5)	1 (5)	1 (4)	0.920
Fusidic acid	9 (20)	5 (25)	4 (17)	0.540
Ciprofloxacin	3 (7)	1 (5)	2 (8)	0.640
Mupirocin	28 (64)	9 (45)	19 (79)	*0.020*
Quinupristin-dalfopristin	4 (9)	1 (5)	3 (13)	0.640
Chloramphenicol	2 (5)	1 (5)	1 (4)	0.920

[a] Percentage relative to the total number of *S. aureus* isolated in each condition

p values ≤ 0.050 were considered statistically significant and are italicized. None of the isolates showed resistance to gentamicin, trimethoprim-sulfamethoxazole, teicoplanin, linezolid, and vancomycin

Discussion

We performed the first nasal screening in a population of homeless individuals in Lisbon, Portugal. The *S. aureus* carriage rate (51.2%) detected was higher than rates previously reported in Portuguese healthy populations (20.1 to 46%) [16, 25–27]. However, MRSA was detected in a single individual (1.2%), which is in agreement with the very low MRSA prevalence among the healthy community in Portugal (< 1–1.8%) [16, 26, 27], and also much lower than the rates reported in other homeless communities (2.8 to 25.6%) [6, 28–30].

People living in temporary accommodation on homeless shelters in Lisbon had a significantly greater risk of respiratory infection and diagnosis of asthma and chronic obstructive pulmonary disease (COPD) than people living in a roofless condition [13]. However, our data indicate that *S. aureus* carriage does not contribute to this adverse outcome. Moreover, homeless people colonized with *S. aureus* were younger than individuals not colonized ($p = 0.009$), which could be due to a higher exposition to risk factors such as illicit drug use observed in 31.2% of the individuals with < 40 years; this is in agreement with other European reports [5].

Table 4 Molecular characterization of the *S. aureus* isolates recovered from nasal colonization among homeless individuals

MRSA/MSSA	PFGE	*spa* type	ST	SCC*mec*	Prevalence, *n* (%)		
					Total ($n = 44$)	No permanent address ($n = 20$)	Shelter ($n = 24$)
MRSA	D	t790	22	IVh	1 (100)	1 (100)	0
MSSA	*	t1451	398	–	13 (30.2)	6 (31.6)	7 (29.2)
	I	t399/t11980/t12808	30	–	5 (11.6)	1 (5.3)	4 (16.7)
	B	t084/t346	582	–	4 (9.3)	3 (15.8)	1 (4.2)
	C	t073/t116/t861/t13186	45	–	4 (9.3)	3 (15.8)	1 (4.2)
	E	t127/t267/t693	1	–	4 (9.3)	2 (10.5)	2 (8.3)
	G	t126/t148	72	–	4 (9.3)	2 (10.5)	2 (8.3)
	L	t002/t5333	5	–	3 (7.0)	0	3 (12.5)
	F	t1309	672	–	2 (4.7)	0	2 (8.3)
	D	t005	22	–	1 (2.3)	1 (5.3)	0
	H	t209	109	–	1 (2.3)	1 (5.3)	0
	J	t493	182	–	1 (2.3)	0	1 (4.2)
	K	t5333	5	–	1 (2.3)	0	1 (4.2)

*No PFGE type was associated with ST398-t1451 isolates. SmaI restriction enzyme does not recognize restriction sites due to DNA methylation, a feature typical of ST398 *S. aureus* lineage

Homeless people have poor access to sanitation facilities and are often subjected to crowded living conditions promoting high person-to-person contact, which could support the increased *S. aureus* carriage rate detected, namely among people attending shelters. However, the very low MRSA rate detected in our study suggests that, despite the high prevalence of MRSA in Portuguese hospitals (39% in 2017) [9], the homeless population in Lisbon does not constitute a MRSA reservoir. Most individuals reported no recent contact with hospitals, which could justify the low MRSA carriage rate detected. Moreover, MSSA colonization has been reported as a protective factor against MRSA carriage and infection [31, 32].

A considerable proportion of isolates showed resistance to antibiotics commonly used to treat skin and soft tissue infections such as erythromycin (45%), clindamycin (39%), and fusidic acid (20%) [33, 34] limiting the treatment options among homeless [35]. Moreover, we found a high mupirocin non-susceptibility (64%), which might be driven by the increasing use of mupirocin in Portuguese hospitals for nasal decolonization protocols, where low-level resistance was reported [36].

The single MRSA recovered in the Lisbon homeless population belonged to EMRSA-15, the major clone circulating in Portuguese hospitals, as well as in the community, public buses and respective passengers in Lisbon and Oporto [11, 12, 37].

The majority of the MSSA isolates belonged to ST398-t1451 clonal type. MRSA-ST398 is the most prevalent *S. aureus* lineage associated with livestock [38]. In Portugal, MRSA-ST398 is extremely prevalent among pigs in different farms [39]. MRSA-ST398 is also found in human colonization but less frequently in disease. Contrarily, MSSA-ST398 was rarely reported in livestock, but is increasingly detected among people in the community, not only in asymptomatic carriage but also in disease [40–45]. MSSA-ST398 lineage is easily transmissible among humans, well adapted to the human host, and showed a high capacity to acquire resistance and pathogenic potential [40, 41, 46–50]. Single MRSA and MSSA ST398-t1451 isolates were already detected in the community in Portugal [11, 51], while the related MSSA-ST398-t571 lineage was found in 11% of nursing students in Lisbon [16] and as a minor clone causing bloodstream infection in Portuguese hospitals [51, 52].

Conclusions

We performed the first nasal screening of the homeless population in Portugal and contributed to overcome the lack of information available in Europe regarding this population. The overall *S. aureus* colonization rate was high (51.2%) while the MRSA nasal carriage was very low (1.2%) despite the broad confidence level used. Our results suggest that the homeless population of Lisbon does not constitute a reservoir of MRSA in the community, but a pool of a highly transmissible ST398-t1451 MSSA lineage. *S. aureus* non-susceptibility to mupirocin, fusidic acid, and clindamycin among homeless queries the efficacy of decolonization protocols and skin and soft tissue infection antibiotherapy in this vulnerable population.

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Compliance with ethical standards

The protocol was approved by the Ethics Committee of Escola Superior de Saúde da Cruz Vermelha Portuguesa and a written informed consent was obtained from all participants.

Conflict of interest The authors declare that they have no competing interests.

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