Staphylococcus aureus colonisation in patients from a primary regional hospital

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Abstract. *Staphylococcus aureus* (SA or *S. aureus*) is a common pathogen that leads to local and systemic infections in communitarian and hospitalised patients. *Staphylococcus* colonizing nasal or pharyngeal sites can become virulent and cause severe infections. In this study, we collected 322 pharyngeal exudates and 142 nasal exudates from hospitalised and outpatients for screening purposes. The carriage rates in the pharynx were 27.06% for *S. aureus*, 11.55% for methicillin-resistant *S. aureus* (MRSA) and 5.61% for methicillin-oxacillin resistant *S. aureus* (MORSA). The carriage rates in the nose were 35.38% for *S. aureus*, 18.46% for MRSA and 13.85% for MORSA. The median multiple antibiotic resistance (MAR) index of SA was 33.33%. The MAR of MRSA was significantly higher than that of methicillin-susceptible strains (MSSA) (45.45% vs. 18.75%, P<0.0001) and the MAR of MORSA was 57.14%. Hierarchical clustering analysis revealed differences in the resistance of methicillin-sensitive, MRSA and MORSA strains. On the whole, our study demonstrates the pattern of distribution of nasal and pharyngeal colonisation with SA, MRSA and MORSA in adults vs. children, inpatients vs. outpatients, ICU patients vs. non-ICU patients, and females vs. males, which can be used for adjusting the screening and decontamination protocols in a hospital. SA is a pervasive pathogen with constantly changing trends in resistance and epidemiology and thus requires constant monitoring in healthcare facilities.

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Introduction

Staphylococcus aureus (SA or *S. aureus*) is a common pathogen that causes local and systemic infections in patients in the community and hospitalised patients, due to its large array of virulence factors. It is the most common germ found in the pharynx and nasal cavities in screening samples. Although the nasal cavities are considered the primary carriage site for SA, data suggest that the pharynx can equally contribute to carrier status (1,2).

In many cases of hospitalised patients, *Staphylococcus* colonizing nasal or pharyngeal sites can become virulent and can cause severe and even fatal infections in cases of: endocarditis, meningitis, blood stream infections, surgical site infections (3), allogenic transplant (4), acquired vitamin K coagulopathies (5), parapneumonic pleurisy (6). In the hospital environment, SA strains initially sensitive to methicillin [methicillin-susceptible strains (MSSA)] can transform into methicillin-resistant SA (MRSA). Fundamental differences have been found between community-acquired MRSA (CA-MRSA) and hospital-acquired MRSA (HA-MRSA) (2), which exhibits an increased drug resistance due to antibiotic selective pressure. The increase in the resistance of MRSA strains has a significant impact on patient care and also influences all the components of the infection control system (7). Mult-iresistant MRSA strains are defined as strains resistant to three or more non-β lactam drugs. These strains are designated as methicillin-oxacillin resistant SA (MORSA) and are associated

with treatment failure. From these reasons, it is clear that there is a need to monitor the incidence and antibiotic resistance of MRSA strains on a regular basis (8). There is also a need for the discovery of novel molecules that may have antibacterial activity against SA strains. Some progress has been made with testing the essential oil of propolis from the Cerrado biome, as well as anhydrofusarubin and methyl ether of fusarubin extracted from the endorphytic fungus, *Cladosporium* sp., isolated from the leaves of *Rauwolfia serpentina* (L.) Benth. ex Kurz. (family, Apocyanaceae); these tests have yielded promising results against SA strains (9,10); however, further studies are required to confirm these findings.

The current study aimed to evaluate the prevalence of colonisation with SA in a hospital environment and in the Oltenia province in Romania where our hospital is located, and to compare the risk factors for colonisation with multi-resistant strains of SA. We also aimed to characterise the antibiotic resistance phenotypes of SA strains circulating in the Oltenia province in order to orient the preventive antibiotic therapy.

Materials and methods

This cross-sectional study was conducted between January-December 2016 and included a total of 329 patients (167 males and 162 females) aged between 6 months and 94 years; 210 patients were hospitalised in the County Clinical Emergency Hospital of Craiova (Craiova, Romania) and 119 were outpatients. We collected 322 pharyngeal exudates and 142 nasal exudates for screening purposes [active surveillance cultures (ASC)]. In total, 2 pharyngeal exudates were collected from 19 patients, and 2 nasal exudates were collected from 12 patients. The reason for the collection of 2 exudates was the fact that the first exudate culture was negative, despite the clinical symptoms, and the physician ordered the collection of a second sample.

This study was carried out in accordance with the Helsinki Declaration of 1975, and was approved by the Review Ethics Board of the University Medicine and Pharmacy of Craiova and of the County Clinical Emergency Hospital of Craiova, Romania. All patients involved in this study signed a full informed consent prior to obtaining the samples. We collected both pharyngeal and nasal exudates from 135 patients, only pharyngeal exudates from 187 patients and only nasal exudates from 7 patients. One swab was taken from the nostrils which was rotated gently in both nostrils, and one swab was taken from the pharynx by sweeping both tonsils. We used rayon-tipped swab with Amies charcoal transport medium (Copan Diagnostics Inc., Brescia, Italy).

The germs were identified by classical microbiological diagnosis, as previously described (11). We plated both swabs directly on selective media for SA (ChromID *S. aureus*) and MRSA (ChromID MRSA; both from Biomerieux, Marcy-l'Étoile, France). Antibiotic susceptibility testing was performed according to the Clinical Laboratory Standards Institute (CLSI) guidelines released in 2015 (12), using the Kirby-Bauer method. From isolated colonies on selective medium for SA (ChromID *S.aureus*, Biomerieux) and MRSA (ChromID MRSA, Biomerieux), we performed an inoculum in liquid broth (Biomerieux) which we adjusted to 0.5 McFarland turbidity with a Densimat instrument (Biomerieux). The inoculum was poured into Muller Hinton agar plates (Biomerieux). After drying the plates for 3 min at 37˚C, we placed the antibiotic disks (Oxoid Ltd., Basingstoke, UK) in an equally spaced fashion, using a maximum of 6 disks per plate. The plates were then incubated at 37˚C for 18 h and the following day the inhibition zone diameters were measured using an electronic caliper for maximum precision of the measurement. For the quality control of the Muller Hinton agar plates and antibiotic disks, we used the Kirby-Bauer method with the SA control strains, ATCC 25923 and ATCC 43300 (Liofilchem s.r.l, Teramo, Italy).

Statistical analysis. Consecutive samples collected from the same patient after an interval of <7 days were excluded from the analysis. For data entry and all statistical calculations, we used Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and Stata (StataCorp LLC, College Station, TX, USA). Numerical variables are expressed as the means ± standard deviation. We divided the patients into categories [adults (age, >18 years) and children (age, ≤18 years)]. Categorical variables were expressed as proportions. For differences between resistance indexes of different patient groups, we used the Student's t-test when the values distribution was normal (as assessed by the Kruskal-Wallis rank test when the values distribution was not normal (normality distribution was tested by the Shapiro-Walk method). For differences between proportions of SA, MRSA and MORSA in the various groups, we used the Chi-square test the test on the equality of proportions with Normal distribution. A value of $P<0.05$ was considered to indicate a statistically significant difference.

The statistical method hierarchical clustering was used in order to construct an inheritance tree of the isolates based on the antibiotic resistance pattern. As the strains that transmit from a patient to another will probably suffer mutations in the genes of antibiotic resistance according to the administered antibiotic treatment, the relatedness by the antibiotic resistance pattern can be used as an indication of the genetic relatedness of the SA strains. We measured the diameters of inhibition areas around antibiotic disks on a Petri dish and used them to perform hierarchical clustering analysis in STATA software with the option of Ward's minimum variance clustering. The assignment of isolates to clusters was based upon inhibition zone diameters.

Results

From the 322 pharyngeal exudates, 104 (32.30%) were positive, whereas from the 142 nasal exudates, 48 (33.80%) were positive. The species isolated consisted mostly of *S. aureus* (67.21% in pharyngeal swabs and 75.41% in nasal swabs), coagulase negative staphylococci (0.82% in pharyngeal swabs and 4.92% in nasal swabs), *Klebsiella* spp. (21.31% in pharyngeal swabs and 9.84% in nasal swabs) and in smaller percentages, *Escherichia coli*, *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp. and glucose non-fermenters Gram-negative rods (Table I and Fig. 1). The prevalence in the two types of swabs differed only for *Klebsiella* (Chi-square test, $P=0.0540$) and for coagulase-negative staphylococci, without reaching statistical significance ($P=0.0739$). The prevalence in the nasal cavity of coagulase negative staphylococci was greater in females compared with males (12.00 vs. 0.00%, $P=0.0330$). The prevalence of *S. aureus* was significantly ($P<0.0001$) greater in outpatients (91.84%) that in inpatients (50.68%) (Table I).

Table I. The bacterial species isolated from pharyngeal and nasal swabs, broken down by patient sex, age group (adults/children) and hospitalisation status (inpatient/outpatient).

Species	Pharyngeal swabs (n=122)	Nasal swabs (n=61)	P-value	Pharyngeal swabs			Nasal swabs			Pharyngeal swabs			Nasal swabs			Pharyngeal swabs			Nasal swabs		
				Males (n=68	Females (n=54)	P-value	Males (n=36)	Females (n=25)	P-value	Adults (n=112)	Children (n=10)	P-value	Adults (n=61)	Children (n=0)	P-value	Inpatients (n=73)	Outpatients (n=49)	P-value	Inpatients (n=58)	Outpatients (n=3)	P-value
S. aureus	82 (67.21%)	46 (75.41%)	0.2543	44 (64.71%)	38 (70.37%)	0.5083	29 (80.56%)	17 (68.00%)	0.2627	75 (66.96%)	7 (70.00%)	0.8447	46 (75.41%)	-	-	37 (50.68%)	45 (91.84%)	<0.001[a]	43 (74.14%)	3 (100.00%)	0.3104
Coagulase-negative staphylococci	1 (0.82%)	3 (4.92%)	0.0739	0 (0.00%)	1 (1.85%)	0.2601	0 (0.00%)	3 (12.00%)	0.0330[a]	1 (0.89%)	0 (0.00%)	0.7641	3 (4.92%)	-	-	1 (1.37%)	0 (0.00%)	0.4107	3 (5.17%)	0 (0.00%)	0.6862
E. coli	3 (2.46%)	1 (1.64%)	0.7207	2 (2.94%)	1 (1.85%)	0.6993	1 (2.78%)	0 (0.00%)	0.4008	3 (2.68%)	0 (0.00%)	0.6003	1 (1.64%)	-	-	1 (1.37%)	2 (4.08%)	0.3441	1 (1.72%)	0 (0.00%)	0.8186
Klebsiella sp.	26 (21.31%)	6 (9.84%)	0.0540[a]	16 (23.53%)	10 (18.52%)	0.5021	3 (8.33%)	3 (12.00%)	0.6363	24 (21.43%)	2 (20.00%)	0.9158	6 (9.84%)	-	-	25 (34.25%)	1 (2.04%)	<0.001[a]	6 (10.34%)	0 (0.00%)	0.5574
Proteus sp.	1 (0.82%)	1 (1.64%)	0.6151	1 (1.47%)	0 (0.00%)	0.3710	0 (0.00%)	1 (4.00%)	0.2263	1 (0.89%)	0 (0.00%)	0.7641	1 (1.64%)	-	-	1 (1.37%)	0 (0.00%)	0.4107	1 (1.72%)	0 (0.00%)	0.8186
Enterobacter sp.	1 (0.82%)	1 (1.64%)	0.6151	1 (1.47%)	0 (0.00%)	0.3710	1 (2.78%)	0 (0.00%)	0.4008	1 (0.89%)	0 (0.00%)	0.7641	1 (1.64%)	-	-	1 (1.37%)	0 (0.00%)	0.4107	1 (1.72%)	0 (0.00%)	0.8186
Pseudomonas sp.	5 (4.10%)	2 (3.28%)	0.7852	3 (4.41%)	2 (3.70%)	0.4294	2 (5.56%)	0 (0.00%)	0.2308	4 (3.57%)	1 (10.00%)	0.3259	2 (3.28%)	-	-	4 (5.48%)	1 (2.04%)	0.3476	2 (3.45%)	0 (0.00%)	0.7436
Glucose non-fermenters Gram-negative rods	3 (2.46%)	1 (1.64%)	0.7207	1 (1.47%)	2 (3.70%)	0.4294	0 (0.00%)	1 (4.00%)	0.2263	0 (0.00%)	0 (0.00%)	1.0000	1 (1.64%)	-	-	3 (4.11%)	0 (0.00%)	0.1508	1 (1.72%)	0 (0.00%)	0.8186

The numbers represent the number of patients infected with the species. Percentages represent the ratio between the number of patients infected with the species and the number of patients in the category. [a]Significant difference (P<0.05). *S. aureus*, *Staphylococcus aureus*, *E. coli*, *Escherichia coli*.

Table II. Carriage rates in the pharynx and nose for the strains of *S. aureus*, MRSA and MORSA.

Strain	Pharyngeal carriage (303 patients screened)	Nasal carriage (130 patients screened)	P-value	Double carriage (104 patients screened)	Global carriage (329 patients screened)
S. aureus colonisation	82 (27.06%)	46 (35.38%)	0.0820	10 (9.62%)	118 (35.87%)
MRSA colonisation	35 (11.55/42.68%)[a]	24 (18.46/52.17%)[a]	0.0547	4 (3.85/40.00%)[a]	55 (16.72/46.61%)
MORSA colonisation	17 (5.61/48.57%)[b]	18 (13.85/75.00%)[b]	0.0040[c]	1 (0.96/25.00%)[b]	34 (10.33/61.81%)
Not infected with *S. aureus*	221 (72.94%)	84 (64.62%)	0.0820	94 (90.38%)	211(64.13%)

[a]MRSA prevalence is expressed both as a ratio of MRSA-infected patients from the total number of patients and ratio between MRSA-infected patients and patients infected with *S. aureus*. [b]MORSA prevalence is expressed both as a ratio of MORSA-infected patients from the total number of patients and ratio between MORSA-infected patients and patients infected with MRSA. [c]Significant difference (P<0.05). *S. aureus*, *Staphylococcus aureus*, MRSA, methicillin-resistant *Staphylococcus aureus*; MORSA, methicillin-oxacillin resistant *Staphylococcus aureus*.

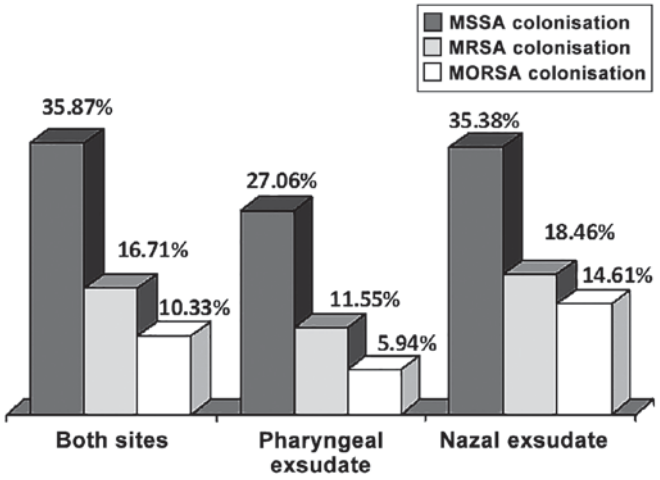

Figure 1. Colonisation rates with *Staphylococcus aureus* in the nose and pharynx. MSSA, methicillin-susceptible strains; MRSA, methicillin-resistant *Staphylococcus aureus*; MORSA, methicillin-oxacillin resistant *Staphylococcus aureus*.

In addition, 3 strains of *Candida albicans* were isolated only from inpatients from two pharyngeal swabs and one nasal swab (0.91% of patients) (data not shown).

The absolute *S. aureus* carriage was 35.87%, as 118 out of the 329 patients had *S. aureus* either in the pharynx or in the nose. In total, 82 patients (27.06%) out of the 303 patients with screened pharyngeal swabs had *S. aureus* in the throat and 46 patients (35.38%) out of the 130 patients with screened nasal swabs had SA in the nose (Table II). A total of 10 patients had SA both in the throat and nose. Thus, the nasal carrier rate was marginally significantly higher than that in the pharynx (proportion's test, P=0.0820). The absolute MRSA prevalence was 16.72% (55 out of the 329 patients). MRSA was present in the pharyngeal exudates in 35 patients out of the 303 screened patients (11.55%) and in the nasal exudates in 24 screened patients, out of 130 (18.46%). In total, 4 patients (3.85%) had MRSA carriage both in the nose and pharynx. When the MRSA prevalence was expressed as the proportion of staphylococcal isolates, the global rate was then 46.61%, the rate in pharyngeal exudate was 42.68% and that in the nasal exudate was 52.17% (proportion's test, P=0.0547). MORSA strains were isolated from 34 patients (10.33%), and the prevalence rates were 5.61% in the pharyngeal exudates and 13.85% in the nasal exudates (proportion's test, P=0.040). In total, 1 patient (0.96%) had MORSA present both in the nose and pharynx (Table II). Thus, MORSA strains were clearly more prevalent in the nasal swabs, compared with the pharyngeal swabs. It should be noted that all the 7 nasal exudates collected from children were negative (Table I).

The prevalence of *S. aureus* colonisation was marginally higher (Chi-square, P=0.1024) in males (40.12%) compared with females (31.48%), and significantly higher (Chi-square, P=0.0225) in adults (38.01%) vs. children (18.92%). The *S. aureus* colonisation rates did not differ significantly between outpatients and inpatients (Chi-square, P=0.3015) (Table III).

A marked difference in MRSA prevalence in adults was observed, as this was >3-fold higher than that in children, with a significant difference (P=0.0225). In addition, MRSA was more frequent in inpatients, compared with outpatients (P=0.0458). No significant difference was observed in MRSA prevalence between intensive care unit (ICU) patients and patients in other wards of the hospital (P=0.2664) (Table III).

The MORSA prevalence as a proportion of SA isolates was 61.81% of the isolated *S. aureus* strains (Table II). A marked difference in MORSA prevalence was observed in adults (11.30%), which was almost 2-fold higher than that in children (2.70%), although the differene was not statistically significant (P=0.1055). In additoin, MORSA prevalence was significantlymore frequent (P=0.0458) in inpatients (12.86%), compared with outpatients (5.88%). No significant difference was observed in MORSA prevalence between ICU patients and patients in other wards of the hospital (P=0.2742) (Table III).

Resistance of SA strains. The median multiple antibiotic resistance (MAR) index of the SA strains was 33.33% (Table IV). As expected, the median MAR of MRSA was higher than that of MSSA (45.45 vs. 18.75%) and the median MAR of MORSA was even higher (57.14%), as was expected (Table IV). The median MAR of the inpatients was clearly higher than the median MAR of the outpatients (42.86 vs. 30.77%, P=0.0006). In addition, ICU patients had a higher median MAR that non-ICU patients (42.41 vs. 33.56%, P=0.0410). No statistically significant differences in the median MAR were observed between adults and children (35.71 vs. 21.43%, P=0.2484) or between females and males (30.77 vs. 38.46%, P=0.3707) (Fig. 2). We observed an increased MAR in the inpatients compared with the outpatients, both for MRSA strains (53.33% vs. 30.77%, P=0.0024) and MORSA strains (61.25% vs. 50.00%, P=0.0250) (Table IV).

Table III. Prevalence rates of colonisation with *S. aureus*, MRSA and MORSA by age, hospitalisation status (inpatient/outpatient), ward type and sex.

Strain	Adults (292 patients)	Children (37 patients)	P-value	Inpatients (210 patients)	Outpatients (119 patients)	P-value	ICU (99 patients)	Non-ICU (230 patients)	P-value	Males (167 patients)	Females (162 patients)	P-value
S. aureus colonisation	111 (38.01%)	7 (18.92%)	0.0225	71 (33.81%)	47 (39.50%)	0.3015	35 (35.35%)	83 (35.93%)	0.8988	67 (40.12%)	51 (31.48%)	0.1024
MRSA colonisation	54 (18.49/48.65%)[a]	1 (2.70/14.29%)[a]	0.0153[c]	38 (18.09/53.52%)[a]	17 (14.29/36.17%)[a]	0.3736	20 (20.20/57.14%)[a]	35 (15.15/42.17%)[a]	0.2664	37 (22.16/55.22%)[a]	18 (11.11/35.29%)[a]	0.0730
MORSA colonisation	33 (11.30/29.73%)[b]	1 (2.70/14.29%)[b]	0.1055	27 (12.86/38.03%)[b]	7 (5.88/14.89%)[b]	0.0458[c]	13 (13.13/37.14%)[b]	21 (9.09/25.30%)[b]	0.2742	23 (13.77/34.33%)[b]	11 (6.79/21.57%)[b]	0.0375[c]
Not infected with *S. aureus*	181 (61.99%)	30 (81.08%)	0.0225[c]	139 (66.19%)	72 (60.50%)	0.3015	64 (64.65%)	146 (64.07%)	0.8988	100 (59.88%)	111 (68.52%)	0.1024

[a]MRSA prevalence is expressed both as a ratio of MRSA-infected patients from the total number of patients and ratio between MRSA-infected patients and patients infected with *S. aureus*. [b]MORSA prevalence is expressed both as a ratio of MORSA-infected patients from the total number of patients and ratio between MORSA infected patients and patients infected with MRSA. [c]Significant difference (P<0.05). *S. aureus*, *Staphylococcus aureus*, MRSA, methicillin-resistant *Staphylococcus aureus*; MORSA, methicillin-oxacillin resistant *Staphylococcus aureus*.

Table IV. The median multiple antibiotics resistance index of the isolated strains of *Staphylococcus aureus*, MRSA and MORSA by age group, hospitalization status, ward type and sex.

	All 329 patients	Adults (292 patients)	Children (37 patients)	P-value	Inpatients (210 patients)	Outpatients (119 patients)	P-value	ICU (99 patients)	Non-ICU (230 patients)	P-value	Males (167 patients)	Females (162 patients)	P-value
S. aureus	33.33%	35.71%	21.43%	0.2484	42.86%	30.77%	0.0006[a]	42.86%	33.33%	0.0410[a]	38.46%	30.77%	0.3707
MRSA	45.45%	44.60%	62.50%	-[b]	53.33%	30.77%	0.0024[a]	44.16%	46.15%	0.1256	43.75%	47.73%	0.5821
MSSA	18.75%	18.75%	19.05%	0.3456	24.05%	18.18%	0.2370	15.39%	24.05%	0.5662	22.42%	18.75%	0.4353
MORSA	57.14%	55.49%	62.50%	-[b]	61.25%	50.00%	0.0250[a]	69.05%	53.33%	0.1050	55.24%	60.00%	0.7080

[a]Significant difference (p<0.05). [b]The statistical test could not be performed as there was only one children with colonization by a MORSA strain that it was also a MRSA strain. MRSA, methicillin-resistant *Staphylococcus aureus*; MORSA, methicillin-oxacillin resistant *Staphylococcus aureus*; MSSA, methicillin susceptible strains; ICU, Intensive Care Unit; *S. aureus*, *Staphylococcus aureus*.

Table V. Results of the multivariate logistic regression analysis on the resistance index of MRSA strains, and risk of acquiring MRSA and MORSA.

	Risk factor					
	Resistance index analysis		Chance to acquire MRSA		Chance to acquire MORSA	
	Coefficient	P-value	Odds ratio	P-value	Odds ratio	P-value
Sex						
Males vs. females	0.017	0.681	2.16	0.050[a]	0.611	0.467
Age group						
<30 years	-0.376	0.027[a]	3.040	0.243	1	-
30-39 years	0.152	0.158	2.096	0.463	0.082	0.209
40-49 years	-0.024	0.829	1.682	0.620	1	-
>50 years	-0.072	0.255	3.382	0.048[a]	0.323	0.368
Patient type						
Inpatients vs. outpatients	0.292	0.008	0.746	0.622	18.92	0.025[a]
Ward type						
ICU vs. non-ICU	0.004	0.937	1.141	0.784	0.487	0.379
Constant	0.257	0	0.297	0.003	1.184	0.807

[a]Significant difference (P<0.05). MRSA, methicillin-resistant *Staphylococcus aureus*; MORSA, methicillin-oxacillin resistant *Staphylococcus aureus*; ICU, intensive care unit.

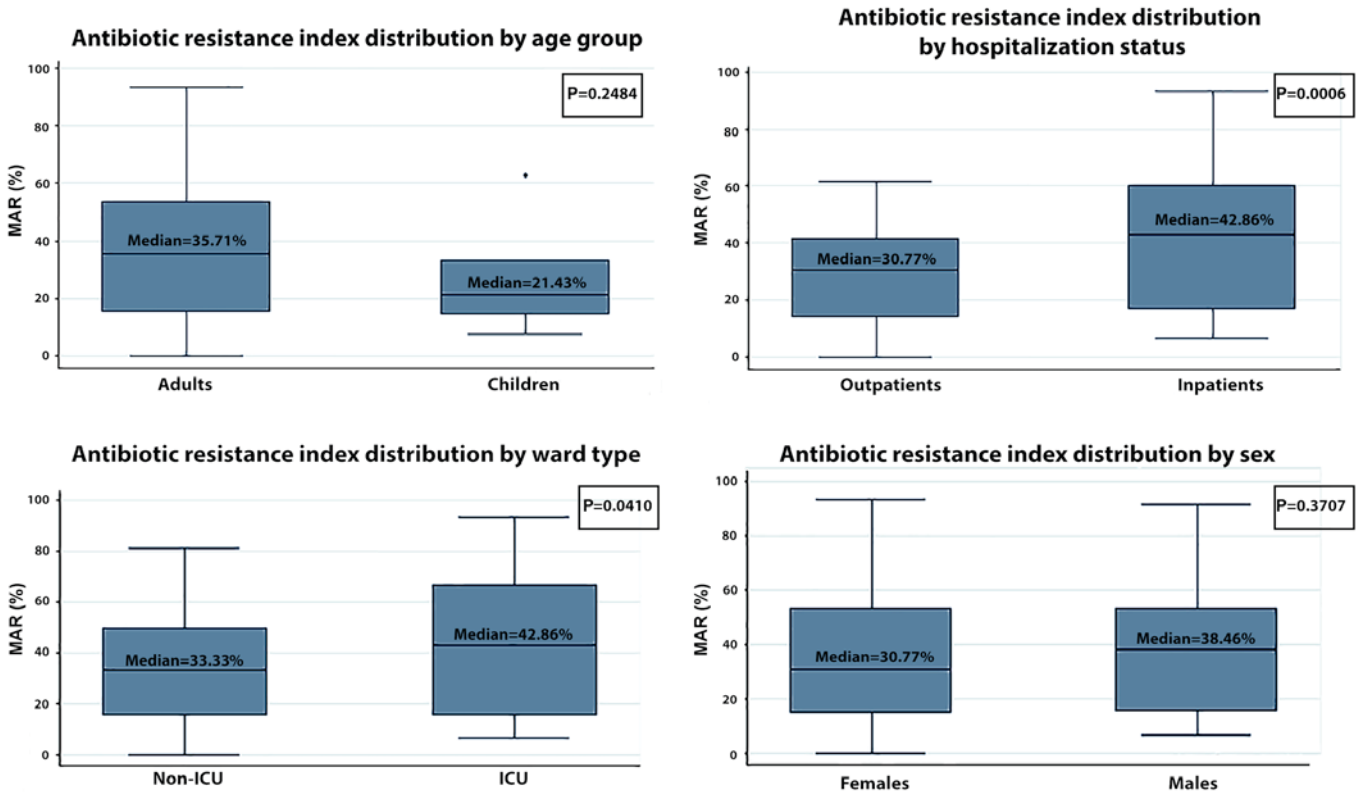

Figure 2. Differences in antibiotic resistance according to age, patient status, ward type and sex. MAR, multiple antibiotic resistance; ICU, intensive care unit.

The multivariate analysis of MRSA infection (Table V) revealed a higher risk for males (OR=2.16, P=0.050) and patients aged >50 years (OR=3.38, P=0.048). Surprisingly hospitalisation in the ICU ward or the patient type (ambulatory or inpatient) had no significant influence on the rate of MRSA colonisation.

Table VI. Antibiotic resistance of *Staphylococcus aureus* strains.

Antibiotic	Global (128 strains)	Adults (111 strains)	Children (7 strains)	P-value	Inpatients (71 strains)	Outpatients (47 strains)	P-value	ICU (35 strains)	Non-ICU (83 strains)	P-value	Males (67 strains)	Females (51 strains)	P-value
Ciprofloxacin	24.79%	25.00%	14.28%	0.5216	37.33%	4.35%	<0.001[a]	32.50%	20.99%	0.1836	25.37%	24.53%	0.9169
Clarithromycin	59.02%	60.87%	28.57%	0.0920	59.21%	58.70%	0.9560	62.50%	57.32%	0.6015	64.29%	51.92%	0.1760
Clindamycin	56.91%	57.76%	42.86%	0.4401	58.44%	54.35%	0.6606	52.50%	59.04%	0.5121	58.90%	54.00%	0.5945
Erythromycin	61.34%	61.61%	57.14%	0.8138	64.86%	55.56%	0.3102	62.50%	60.76%	0.8593	63.77%	58.00%	0.5239
Gentamycin	18.55%	18.64%	14.28%	0.7727	27.63%	4.17%	0.0012[a]	31.71%	12.05%[a]	0.0109[a]	19.18%	17.65%	0.8321
Oxacillin	62.50%	57.14%	100%	0.0250[a]	74.65%	51.06%	0.0084[a]	100%	50.60%	<0.001[a]	74.62%	50.98%	0.0079[a]
Penicillin	91.60%	92.86%	71.43%	0.0476[a]	90.67%	93.18%	0.6291	92.50%	91.14%	0.8084	95.59%	86.27%	0.0712
Rifampin	19.00%	20.43%	0.00%	0.1833	28.36%	0.00%	0.0001[a]	25.71%	15.38%	0.1863	22.03%	14.63%	0.3084
Sulfamethoxazole/ trimethoprim	36.79%	36.00%	57.14%	0.2619	46.88%	21.43%	0.0050[a]	43.33%	34.21%	0.3484	43.33%	28.26%	0.0927
Tetracycline	58.00%	58.95%	42.86%	0.4029	64.18%	45.45%	0.0444[a]	65.79%	53.23%	0.2082	70.91%	42.22%	0.0017[a]

[a]Significant difference (P<0.05). ICU, intensive care unit.

Only the state of hospitalised patients greatly increased the MORSA rate (OR=18.92%, P=0.025) (Table V). The sex and age of the patients had no influence in this case.

The regression of the resistance index of MRSA revealed that a young age (<30 years) (beta coefficient=-0.376, P=0.027) and hospitalisation (beta coefficient=0.292, P=0.008) had a significant impact on the antibiotic resistance of MRSA (Table V).

The resistances to individual antibiotics presented significant differences between the categories of patients in a few cases. When comparing the antibiotic resistances in adults vs. children, these were increased in adults for clarithromycin (60.87 vs. 28.57%; Chi-square test, P=0.0920) and increased in children for oxacillin (57.14 vs. 100%, P=0.0250). The antibiotic resistance was markedly increased in inpatients compared to outpatients for ciprofloxacin (37.33 vs. 4.35%, P<0.0001), gentamycin (27.63 vs. 4.17%, P=0.0012), rifampin (28.36 vs. 0%, P<0.0001), oxacillin (75.00 vs. 50.00%, P=0.084 and sulfamethoxazole/trimethoprim (46.88 vs. 21.43%, P=0.0050). The antibiotic resistances of strains isolated from ICU patients were higher compared with those isolated from non-ICU patients for gentamycin (31.71 vs. 12.05%; proportion's test, P=0.0109) and oxacillin (100 vs. 50.00%; proportion's test, P<0.0001) (Table VI).

We also analysed the resistance phenotypes, based upon resistance to key antibiotics (Table VII). For MSSA, the most prevalent phenotype was that resistant only to penicillin, followed by a phenotype resistant to penicillin, clindamycin, clarithromycin, doxycycline, erythromycin and tetracycline. For MRSA, the most prevalent phenotype was that resistant only to penicillin and cefoxitin, followed by a phenotype with an additional resistance to clindamycin.

We also performed a hierarchical clustering analysis of the strains based upon the diameters of inhibition zones in the Kirby-Bauer antibiotic susceptibility testing method (Fig. 3). We observed 3 main groups: One very sensitive that was hypothesised to be the MSSA strains, one with intermediate resistance could be the 'sensitive MRSA' strains that are generally community-acquired, which was the largest group, and the third group with the greatest resistance that could be regarded as HA-MRSA.

Discussion

Due to the high prevalence rate of SA colonisation in the pharynx and nasal cavity in the general population, the ratio between the number of multidrug-resistant strains of SA over the total number of SA strains is used in the literature as a more accurate measure of colonisation with resistant staphylococci. In patients with facial acne, these can become infected with the Staphylococci from the pharynx and nasal cavity and this could lead to a form resistant to treatment (13). In some patients, these cases of resistance strains may be associated with non-alcoholic fatty liver disease (14).

It should be noted that although the SA carriage rates did not differ significantly between the pharyngeal and nasal cavities, the MRSA and MORSA rates were significantly higher in the nasal cavity. The MORSA carriage rate in the nasal cavity was 13.85%, almost 3-fold higher than the carriage rate in the pharynx (5.61%). Our results revealed that the MRSA nasal carriage rate (18.46%) was higher than the pharyngeal carriage rate (11.55%). This ratio is similar with rates recorded in hospitals from the United States (15). A surprisingly low number of patients (10; 9.62%)

Table VII. Resistance phenotypes in MSSA and MRSA.

A, MSSA resistance patterns

MSSA resistance profile	No. (%)
PEN	16 (23.53)
CLI CLR DOX ERY PEN TCY	8 (11.76)
ERY PEN	3 (4.41)
CLR ERY	2 (2.94)
CIP CLI CSL DOX MFX PEN SXT TCY	2 (2.94)
CLR TCY	2 (2.94)
CLI CLR ERY PEN	2 (2.94)
PEN SXT	2 (2.94)
Wild-type	2 (2.94)
CLR ERY SXT TCY	1 (1.47)
CLI PEN	1 (1.47)
CHL CLI CLR DOX ERY PEN SXT	1 (1.47)
CLI CLR ERY PEN RIF	1 (1.47)
CIP CLI CLR DOX ERY MFX PEN RIF TCY	1 (1.47)
CIP CLI CLR DOX ERY PEN SXT TCY	1 (1.47)
CIP CLI CLR DOX ERY SXT TCY	1 (1.47)
PEN RIF SXT	1 (1.47)
CIP CLI CLR DOX PEN TCY	1 (1.47)
CLI CLR PEN	1 (1.47)
CLR CSL ERY PEN SXT	1 (1.47)
CHL CIP CLI CLR DOX ERY MFX PEN TCY	1 (1.47)
CIP CLI MFX SXT	1 (1.47)
DOX ERY PEN SXT TCY	1 (1.47)
DOX TCY	1 (1.47)
CIP CLR ERY MFX PEN SXT	1 (1.47)
PEN TCY	1 (1.47)
CHL CLI	1 (1.47)
CIP PEN RIF SXT TCY	1 (1.47)
CLI CLR ERY PEN SXT	1 (1.47)
TCY	1 (1.47)
CHL CLI CLR DOX ERY PEN RIF TCY	1 (1.47)
DOX ERY SXT	1 (1.47)
CHL CIP CLI DOX ERY MFX PEN TCY	1 (1.47)
CHL CLI CLR ERY PEN TCY	1 (1.47)
CLI CLR DOX ERY PEN	1 (1.47)
PEN RIF	1 (1.47)
CLI CLR DOX ERY PEN SXT TCY	1 (1.47)
SXT	1 (1.47)
Total	68 (100)

B, MRSA resistance patterns

MRSA resistance profile	No. (%)
FOX PEN	5 (8.33)
CLI FOX PEN	4 (6.67)
CLI CLR DOX ERY FOX PEN TCY	3 (5.)
CLI CLR DOX ERY FOX PEN SXT TCY	3 (5.)
CLI CLR CSL DOX ERY FOX PEN TCY	2 (3.33)
CLR ERY FOX PEN	2 (3.33)
CIP CLI CLR CSL DOX ERY FOX PEN RIF SXT TCY	2 (3.33)
CIP CLI CLR DOX ERY FOX PEN SXT TCY	2 (3.33)
CLI CLR ERY FOX PEN	2 (3.33)
CIP CLI CLR CSL DOX ERY FOX PEN SXT TCY	1 (1.67)
CIP CLI CLR CSL DOX FOX MFX PEN RIF TCY	1 (1.67)
CHL CIP CLI CLR DOX ERY FOX PEN SXT	1 (1.67)
CLR DOX FOX PEN	1 (1.67)
DOX FOX PEN	1 (1.67)
CHL CIP CSL ERY FOX MFX PEN RIF SXT	1 (1.67)

Table VII. Continued.

MRSA resistance profile	No. (%)
CIP CLI CLR ERY FOX MFX PEN SXT	1 (1.67)
CIP CLI CLR ERY FOX PEN	1 (1.67)
CHL CLI CLR DOX ERY FOX SXT TCY	1 (1.67)
CIP CLR CSL DOX ERY FOX MFX PEN RIF SXT TCY	1 (1.67)
CIP CLR DOX ERY FOX MFX PEN TCY	1 (1.67)
CIP CLI CLR CSL DOX ERY FOX MFX PEN SXT TCY	1 (1.67)
CIP CSL ERY FOX PEN	1 (1.67)
CLI CLR ERY FOX	1 (1.67)
CIP DOX FOX PEN SXT	1 (1.67)
CLI CLR CSL DOX ERY FOX PEN	1 (1.67)
CHL CLI CLR CSL DOX ERY FOX MFX RIF SXT TCY	1 (1.67)
CLI CLR CSL DOX ERY FOX PEN RIF TCY	1 (1.67)
CLR CSL ERY FOX PEN	1 (1.67)
CLI CLR CSL DOX ERY FOX PEN SXT TCY	1 (1.67)
CLR DOX ERY FOX PEN TCY	1 (1.67)
CHL CIP CLI CSL DOX ERY FOX MFX PEN RIF SXT TCY	1 (1.67)
CHL CLI CLR CSL DOX ERY FOX PEN RIF	1 (1.67)
CLI CLR CSL DOX ERY FOX RIF SXT TCY	1 (1.67)
CLR ERY FOX PEN TCY	1 (1.67)
CLI CLR CSL ERY FOX PEN	1 (1.67)
CLI CLR DOX ERY FOX	1 (1.67)
DOX FOX PEN TCY	1 (1.67)
ERY FOX PEN TCY	1 (1.67)
CHL CIP CLI DOX FOX PEN	1 (1.67)
CHL CIP CLI CLR CSL DOX ERY FOX MFX PEN RIF SXT TCY	1 (1.67)
CHL CIP CLI CSL DOX FOX MFX PEN RIF SXT	1 (1.67)
CLI CLR DOX FOX PEN RIF TCY	1 (1.67)
CLI CLR DOX FOX PEN TCY	1 (1.67)
CLI CLR CSL DOX FOX PEN TCY	1 (1.67)
Total	60 (100)

PEN, penicilin; CLI, clindamycin; CLR, clarithromicin; DOX, doxicycline; ERY, erythromycin; FOX, cefoxitin; TCY, tetracycline; CIP, ciprofloxacin; CSL, cefoperasone/sulbactam; MFX, moxifloxacin; SXT, sulfamethoxazole/thrimethoprim; CHL, cloramfenicol; RIF, rifampin; MSSA, methicillin-susceptible strains; MRSA, methicillin-resistant *Staphylococcus aureus*.

had SA carriage in both sites, which in our opinion, can partly be explained by the lower number of nasal swabs collected and by the application of decolonisation procedures to patients admitted to our hospital. Nevertheless, the failure of nasal decolonization procedures with clorhexidin and mupirocin has been reported in patients that also have pharyngeal colonization with SA. A probable explaination for this is that pharyngeal strains become resistant to agents used for decolonization (that are detected in low concentrations in the pharynx after nasal application) (16) and then re-colonise the nasal cavities (17).

The pharynx also constitutes a SA reservoir. Pharyngeal colonisation can be cleared only by oropharyngeal decolonisation applied concomitantly with nasal decolonisation or systemic antibiotherapy. Recolonisation has been reported with the same SA strain after decolonisation (17). Probably, the sources for recolonisation are other carriage sites, such as the throat, or the patient's environment. The elimination of *S. aureus* from extranasal sites has been proposed in order to increase the efficiency of future treatment regimens. Repeated

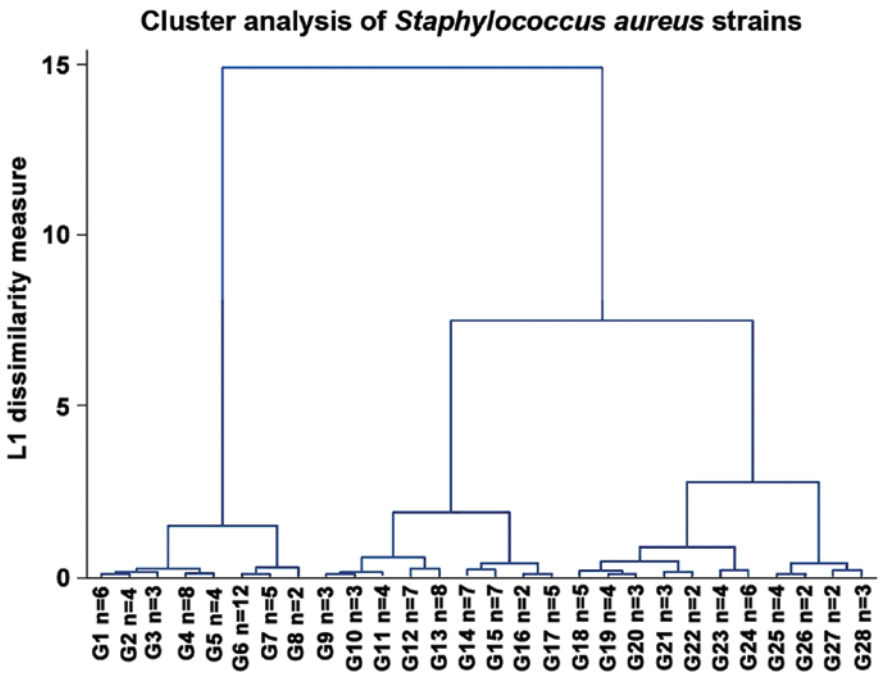

Figure 3. Hierarchical cluster analysis of *Staphylococcus aureus* strains based upon inhibition zone diameters.

treatments have as a consequence the development of resistance to mupirocin (18).

There are growing concerns about the routine use of antibiotherapy in hospitalised patients. In this study, 3 inpatients had fungal infections with *Candida* spp., 2 in the pharynx and one in the nasal cavity. The prevalence of fungal infections obtained by us (0.91%) (data not shown) was surprisingly low compared with a previous study (19). This may be explained by the fact that screening samples were used, and the majority of the patients did not suffer from major conditions that can lower the immunity in order to favorise fungal infections.

The acquired resistance of *S. aureus* has been the focus of several publications, especially after penicillin began to be used in the middle of the past century, regarding MRSA epidemiology and its resistance to penicillin. Transmission mainly occurs in hospitals (20-22). The excessive use of antibiotics in hospitals is considered a major risk in the guidelines of the Society for Healthcare Epidemiology of America (SHEA) (23). A revised infection control guideline from 2013 (24) to prevent MRSA expansion includes the limited use of glycopeptides, cephalosporins and fluoroquinolones.

As regards surveilance, a complex aspect is the fact that, as regards MRSA, it has been demonstrated that hospitals are the main place of occurrence for multi-resistant *S. aureus*, which is now known as MRSA (25). International studies over the past 20 years have shown the rising prevalence of MRSA (26 and refs therein). The theory that the highest occurrence occurs in patients that are drug abusers or persons that undergo hemodialysis has been refuted. Initially, the first reports of MRSA were in large hospitals (>500 beds) in 1980 (27). However, MRSA was also later found in smaller ones.

Future studies are warranted in order to determine the factors that lead to the transition from MSSA to MRSA. The shift from MSSA to MRSA occurs very rapidly (within 24-48 h) in patients that are hospitalised. Thus, both the particulars of the organism and the onset of the infection contradict the cross-transmission as the first main cause for the appearance of MRSA in the hospital environment. Another factor that argues against cross-transmission is the large number of different strains discovered (28). The effect of specific antibiotics on MRSA strains has been previously analysed (29). It was shown that the resistance level of MRSA in patients who received antibiotic therapy was 2-fold compared to that in those who did not undergo antibiotic treatment (30). It has also been shown that the higher risk was associated with the use of quinolones, seconded by the use of glycopeptides, cephalosporins and other β-lactams (31).

In conclusion, the present study demonstrates the pattern of distribution of nasal and pharyngeal colonisation with SA, MRSA and MORSA in various categories of patients, which can be used for adjusting the screening and decontamination protocols in our hospital. The antibiotic resistance pattern of SA strains demonstrated a high resistance of MRSA and MORSA strains, probably driven by antibiotic use. Resistance to erythromycin, tetracycline, clindamycin and clarithromycin was high and consequently, these drugs are not recommended for the empirical therapy of *S. aureus* infections. *S. aureus* is a pervasive pathogen with constantly changing trends in resistance and epidemiology, and thus requires constant monitoring in healthcare facilities.

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