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Brief Communication

Epidemiology of *Staphylococcus aureus* infections and nasal carriage at the Ibn Rochd University Hospital Center, Casablanca, Morocco

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A B S T R A C T

Infections caused by *Staphylococcus aureus* are a major problem in hospitals. The multidrug resistance and the nasal carriage of *S. aureus* play a key role in the epidemic of these infections. In this prospective study, 160 *S. aureus* strains were isolated from pathological samples of patients (79 cases) and nasal swabs (81) of cases and controls from January to July 2007. The susceptibility to 16 antibiotics, including cefoxitin, was determined by the agar diffusion method, and methicillin resistance was confirmed by amplifying the *mecA* gene by polymerase chain reaction (PCR). The prevalence of methicilin-resistant *S. aureus* (MRSA) was high in the burns (57.7%) and dermatology (39.4%) wards, and the MRSA strains isolated were extremely multi-resistant, but all of them were still susceptible to vancomycin. The rate of *S. aureus* nasal carriage was high in both cases and controls, in state, MRSA nasal carriage was more common among people infected with *S. aureus*.

Introduction

S. aureus infections constitute a major health care problem because this pathogen has developed resistance to most antibiotics introduced in antibiotherapy.[1] Moreover, the nasal carriage of *S. aureus*/methicilin-resistant *S. aureus* (MRSA) is recognized as a risk factor for the acquisition of an endogenous infection and plays an important role in the spread of this pathogen not only in the hospital care units but in the community as well.[2]

Data exist for different countries regarding *S. aureus*/MRSA infections, but in Africa and developing countries only a few reports are available. This study was conducted in order to better understand the epidemiology of *S. aureus*/MRSA at the University Hospital Center Ibn Rochd (UHCIR) by determining the rate of MRSA in different risk departments, establishing the resistance profile of *S. aureus* against various antibiotic families, and studying the nasal carriage of *S. aureus* in infected patients, as well as its role in the acquisition of *S. aureus* infection through a case-control study.

Materials and methods

This study was established at the UHCIR Casablanca, a 1,609-bed teaching hospital with three major branches (administrative, clinical, and pediatric). There are eight intensive care units (ICUs), with 83 beds. The UHCIR receives approximately 100,000

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patients for consultations and 32,000 admissions per year, with an estimated 210,000 patient/days.

This prospective study was conducted over a period of six months (January-July 2007). During this period, all strains of *S. aureus* isolated from various samples received from the target units (pediatric, dermatology, burns ICUs) at the UHCIR were collected. Only one strain per patient was chosen (the most resistant one, and isolated from the most invasive sample).

The study focused on the services of pediatrics, dermatology, burns, and ICUs. These services were of interest because they recorded the highest prevalence of MRSA in UHCIR during the last three years, according to the laboratory database.

For each case patient (hospitalized in the target units and from whom an *S. aureus* strain was isolated), a control, present in the same service at the same date and with no *S. aureus* infection was selected. Patient cases and controls were subjected to a nasal swab to search for nasal carriage of *S. aureus* after their consent.

Patients without completed standardized questionnaire and informed consent for the nasal carriage were excluded from the study.

Isolation and identification of S. aureus *strains*

The isolation and identification of *S. aureus* were performed according to the conventional methods of bacteriology: seeding on Columbia nalidixic acid (CNA) agar, colonial morphology, Gram stain, catalase, and tube coagulase test.

Antibiogram

The antibiogram was performed by the agar diffusion method according to the guidelines of the CASFM (Antibiogram Committee of the French Microbiology Society, 2007 edition). Resistance to methicillin was evaluated through a cefoxitin disk loaded at 30 µg. The strains with diameter less than 25 mm were subjected to polymerase chain reaction (PCR) of *mec*A gene.

Resistance phenotypes associated to isolated strains were determined by other antibiotics: kanamicin 30 IU, tobramicin 10 µg, gentamicin 15 µg, erythromycin 15 IU, spiramycin 100 µg, lincomycin 15 µg, pristinamycin 15 µg, pefloxacin 5 µg, tetarcycline 30 IU, minocycline 30 IU, fusidic acid 10 µg, rifampin 30 µg, fosfomycin 50 µg, trimethoprim sulfamethoxazol (cotrimoxazol) 1.25 (23.75) µg, and vancomycin 30 µg.

The automated reading and interpretation was performed using Osiris (Osiris PLC Biorad®). Intermediate strains are considered resistant.

Quality control was carried out through the *S. aureus* strain ATCC 25923 provided by the laboratory of the National Reference Center for *Staphylococci* (CNRS) in Lyon, France.

Detection of mecA *gene*

Deoxyribonucleic acid (DNA) of *S. aureus* strains was extracted from a young culture (brain heart broth beef incubated for 18-24h at 37°C) by phenol/chloroform method.[3] The extracted DNA was used as template for searching *mec*A gene using the protocol described by Murakami and al.[4]

Results

Staphylococcus aureus *infections*

Prevalence and epidemiology

During the period of the study, the laboratory isolated 194 strains of *S. aureus* from patients in the target units. Only 40.7% of those patients met the inclusion criteria; as a result, 79 strains of the isolated pathogen *S. aureus* were studied. The distribution of these strains was as follows: 41.8% from patients in the dermatology department, 32.9% in the burns service, 21.5% in ICUs, and 3.8% in the pediatric ward. In the majority of cases (70.9%) strains were isolated from skin samples and surgical wounds; in 16.4%, from respiratory secretions; and in 8.9%, from bacteraemia. Only two strains were isolated from catheter and one from pleural fluid (Table 1).

The overall prevalence of MRSA in the hospital in 2007 was 18.4%.

Sensitivity to antibiotics

MRSA

Among the 79 strains isolated from pathological samples received from target units, 28 were resistant to methicillin (35.4%). These strains showed a multidrug-resistant profile; in fact, 27 of them (96.4%) were resistant to aminoglycosides, fluoroquinolones, and tetracycline. Moreover, 25 strains (89.3%) were also resistant to rifampin (Table 2).

MRSA strains were also characterized by the phenotypes kanamycin-tobramycin-gentamycin resistant (KTG) and macrolides-lincosamides-streptogamines B (MLSB). MLSB phenotypes divided the resistance profiles into two major profiles; the inducible MLSB profile observed in 18 strains (64.3%), and the lincosamides (L) phenotype observed in nine strains (32.1%) (Table 3).

Methicillin-sensitive S. aureus *(MSSA)*

MSSA strains represented 64.5% (51 strains) of total pathogen *S. aureus* studied. The wild profile was observed in only 6% of the total strains, while strains with isolated resistance to penicillin G was the most frequently observed, with a rate of 55%. The overall rate of resistance to penicillin G was 90.2%, and only 6% for erythromycin, pefloxacin, and kanamycin. Relatively higher resistance rates were observed for tetracycline (29.5%), minocycline (15.7%), and fusidic acid (13.7%).

A statistical comparison of rates of antibiotic resistance between MRSA and MSSA showed a significant difference ($p < 0.01$) for all the antibiotics tested, except for pristinamycin, vancomycin, and fosfomycin, which were 100% active against the two groups of *S. aureus* (Table 2).

Nasal carriage of S. aureus *in patients (cases and controls)*

Rate of S. aureus *and MRSA carriage*

The number of patients (cases) was 79, and 47 patients met the inclusion criteria as controls. Nasal carriage was conducted among these 126 patients (cases and controls). A total of 81

Table 1 - Number (%) of *S. aureus* strains isolated from various pathological samples and nasal swabs (nasal carriage rate)

	Pathological samples n = 79						Nasal swabs n = 81		
	Cutaneous and surgical wound	Respiratory secretions	Bacteraemia	Catheter	Pleural fluid	Total	Cases n = 79	Controls n = 47	p
S. aureus	56 (70.9)	13 (16.4)	7 (8.9)	2 (2.5)	1 (1.3)	79 (100)	56 (70.9)	25 (53.2)	NS
MSSA	34	13	3	-	1	51 (64.5)	27 (34.2)	18 (38.3)	NS
MRSA	22	-	4	2	-	28 (35.4)	29 (36.7)	7 (14.9)	< 0.01

NS, not significant; MSSA, methicilin-sensitive *S. aureus*; MRSA, methicilin-resistant *S. aureus*.

Table 2 - Resistance rate associated to MRSA and MSSA isolated from pathological samples received from target units at the university hospital Ibn Rochd in Casablanca, Morocco

Antibiotics	Number of strains (%)		p
	MRSA n = 28	MSSA n = 51	
Penicillin G	28 (100)	46 (90.2)	< 0.01
Kanamycin	27 (96.4)	2 (3.9)	< 0.01
Tobramycin	27 (96.4)	0	< 0.01
Gentamycin	27 (96.4)	0	< 0.01
Erythromycin	18 (64.3)	3 (5.9)	< 0.01
Lincomycin	9 (32.1)	0	< 0.01
Fusidic acid	18 (64.3)	7 (13.7)	< 0.01
Pefloxacin	27 (96.4)	3 (5.9)	< 0.01
Tetracycline	28 (100)	15 (29.4)	< 0.01
Minocycline	26 (96.4)	8 (15.7)	< 0.01
Rifampin	25 (89.3)	0	< 0.01
Trimethoprim sulfamethoxazol (cotrimoxazol)	19 (67.9)	0	< 0.01
Pristinamycin	0	0	-
Fosfomycin	0	0	-
Vancomycin	0	0	-

-, indeterminate value; MSSA, methicilin-sensitive *S. aureus*; MRSA, methicilin-resistant *S. aureus*.

Table 3 - Resistance profiles of MRSA strains from pathological samples and nasal swabs of patients in dermatology and burns wards at the University Hospital Ibn Rochd in Casablanca, Morocco

Antibiotype profile	Pathological samples n = 28	Nasal carriage n = 36	Total n = 64 (%)
P-K-T-G-E*-Pef-Te-Mno-Rif-Tsx-FuA	14	16	30 (46.9)
P-K-T-G-L**-Pef-Te-Mno-Rif	8	13	21 (32.8)
P-E*-Pef-Te-Mno-Rif-Tsx-FuA	1	3	4 (6.3)
P-K-T-G-E*-Pef-Te-Rif-Tsx-FuA	2	1	3 (4.7)
P-K-T-G-E*-Pef-Te-Mno-Tsx-FuA	1	1	2 (3.1)
P-K-T-G-L**-Pef-Te-Rif	1	1	2 (3.1)
K-T-G-Te-Mno-Tsx	1	1	2 (3.1)

P, penicillin G; K, kanamycin; T, tobramycin; G, gentamycin; L, lincomycin; E, erythromycin; Pef, pefloxacin; Te, tetracycline; Mno, minocycline; Rif, rifampin; Tsx, trimethoprim sulfamethoxazol (cotrimoxazole); FuA, fusidic acid; MRSA, methicilin-resistant *S. aureus*. * inductible macrolides-lincosamides-streptogamines B (MLSB) phenotype, ** L phenotype.

strains of *S. aureus* were isolated (from nasal swab of cases and controls), including 36 strains of MRSA (44.4%).

The carriage rate of *S. aureus* was high: 70.9% for cases versus 53.2% for controls. For MSSA nasal carriage rate, the difference between cases (34.2%) and controls (38.3%) was not significant ($p > 0.05$). Interestingly, the MRSA nasal carriage rate was significantly higher among cases: 36.7% versus 14.9% in controls ($p < 0.01$) (Table 1).

Sensitivity to antibiotics

The study of different profiles of resistance related to MRSA strains isolated from nasal swab samples of case and control patients showed multidrug resistance to the different antibiotics tested. This result matches with the finding concerning MRSA strains isolated from pathological samples. The frequency and distribution of the major resistance profiles of nasal carriage *S. aureus* strains are also comparable with those from the pathological samples (Table 3).

Discussion

In the present study, many reasons led to the exclusion of some case patients, such as incomplete standardized questionnaire, refusal of some patients to sign the informed consent for nasal swab, and discharge of many patients from the target units. For controls, there were some difficulties in finding patients hospitalized in the same period as case patients, which justifies the number of control patients included in this study.

The prevalence of MRSA at the UHCIR of Casablanca has increased from 14.4% to 23.4% between 2000 and 2002.[5,6] This increase is mainly due to high rates of MRSA reported in the ICUs in 2002. In 2007 this rate decreased to 18.4%, due to better application of the hospital hygiene measures recommended by the Committee for the Fight Against Nosocomial Infections (CLIN). The rate of MRSA reported at the UHCIR is comparable to the rates reported in Tunisia, and it is still low in comparison with the rates in Algeria, Egypt, and Jordan.[7]

In this hospital, the rate of MRSA was higher in the dermatology and burns departments than in the ICUs. This finding is a result – as mentioned before – of an efficient hygiene policy in the ICUs. The prevalence in the dermatology and burns wards was 39.4% and 57.7%, respectively. Comparable high values have been reported in the literature for burn patients in several countries, notably in African countries such as Tunisia, Libya, and South Africa.[8-10] The damaged cutaneous barrier of these patients contributes to the development of such infections.

Susceptibility pattern of S. aureus

MRSA

The mechanism of resistance to methicillin in *S. aureus* is mainly due to the production of the modified protein PBP2a (penicillin-binding protein 2a) encoded by the *mecA* gene which is carried in the mobile genetic element SCCmec (Staphylococcal cassette chromosome mec).[11] Apart from this main mechanism, there are others that remain far less common, such as the hyperproduction of beta-lactamase observed in borderline-resistant *S. aureus* (BORSA), and the presence of a modified PBP in the modified-resistant *S. aureus* (MODSA).[12] In this series, all MRSA strains carried the *mecA* gene and were resistant via PBP2a production.

Multidrug resistance of MRSA strains were well documented several years ago;[1] however, the situation in Europe, Asia, and America is changing, with the emergence of MRSA strains with reduced susceptibility to antibiotics since the 1990s.[13] Interestingly, in this hospital all MRSA strains had the former multi-resistant character. The strains were resistant at least to six antibiotics other than beta-lactams, and several resistance phenotypes have been identified, namely: KTG phenotype, inducible MLSB phenotype, and L phenotype.

The MLSB inducible strains were isolated mostly in the dermatology ward, but also in the burns ward. They were associated with resistance to cotrimoxazole, rifampin, and fusidic acid, unlike L phenotype strains, which were sensitive to these antibiotics. The MLSB phenotype was widely reported in several countries such as South Africa, India, and Poland.[14-16]

In the present study, the rate of resistance to cotrimoxazole, fusidic acid, and rifampin are high compared with values reported previously in Morocco.[6,17] However, the same values have been reported in some African countries such as Ivory Coast, Senegal, and Cameroon.[5] Contrariwise, fosfomycin, pristinamycin, and glycopeptides remain as active molecules against the MRSA strains.

The multiresistant character of MRSA observed in the present study limits the choice of antibiotics for treatment. In hospitals, vancomycin remains the suitable antibiotic for the treatment of MRSA infections. However, dissemination of glycopeptide resistant strains is a concern, especially with the isolation of some strains with reduced susceptibility to vancomycin.[18] Given this situation, it is interesting to point out the importance of the introduction and marketing of other active molecules against MRSA in Morocco, such as fosfomycin.[19]

MSSA

The rate of resistance to penicillin of the MSSA strains isolated in the present study was at least 90%, this finding is consistent with data from Lowy et al., where over 90% of strains of *S. aureus* have become resistant to penicillin by penicillinase production.[20]

Resistance to tetracycline was 29.4%, and 13.7% for fusidic acid. Fusidic acid resistance was especially high in the dermatology ward, reaching 30%. This relatively high value can be explained by the large spectrum of this antibiotic and its widespread use in dermatology units.[21]

Nasal carriage of S. aureus

Nasal carriage plays an important role for the transmission, autoinfection, and cross infection in hospital and also in the community. Since the 1950s, several authors have demonstrated the role of *S. aureus* carriage in the acquisition of infection with *S. aureus*/MRSA.[22]

This study showed that the carriage rate of MSSA through patients is comparable to that seen in control patients (non-significant difference). This can be explained by the high rate of carriage of *S. aureus* in both case patients and controls in this hospital. On the other side, the analysis of MRSA nasal carriage between case patients and controls showed that carrying MRSA can be considered as a risk factor for developing *S. aureus* infection (significant difference); this result is consistent with the literature data.[23,24]

This carriage study shows also a significant movement of *S. aureus* in both controls and infected patients with *S. aureus*, while the carriage of MRSA is more common among people infected with *S. aureus*. These patients constitute a potential source of contamination, and the risk of endogenous infections is significant for this population. This is supported by the similarity between the resistance patterns of strains isolated in pathological samples and those of nasal carriage. The study of the nasal carriage in the nursing staff and the application of molecular typing methods such as the SCCmec typing, pulsed field gel electrophoresis (PFGE) and multi locus sequence typing (MLST) are also important to better understand the role of nasal carriage in infection.

Conclusion

The rate of MRSA in the burns ward and in the dermatology ward at the UHCIR was high, and the strains isolated were characterized by their multi-resistance to antibiotics and the differential repartition of MLSB phenotype between these two wards. Even if the rate of nasal carriage of MSSA is high, it does not represent a risk factor for *S. aureus* infection. However, MRSA is recognized as a potential risk factor for developing *S. aureus* infection in this hospital. It is important in this case to establish a policy to fight against the spread of this pathogen (MRSA), taking into account the important role of nasal carriage of MRSA in this epidemiology.

Conflict of interest

All authors declare to have no conflict of interest.

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