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Prevalence of Community Acquired Methicillin Resistant Staphylococcus aureus Nasal Carriage among Children of Consultation: Experience of a **Moroccan University Hospital**

Ed-dyb S^{1,3*}, Aboudourib M^{2,3}, Azzouzi F^{2,3}, Quiddi W^{1,3}, Akhdari N^{2,3}, Amal S^{2,3}, Soraa N^{1,3} and Hocar O^{2,3}

¹Bacteriology-Virology Department, Mohammed VI CHU, 53 Ibn Sina Boulevard, Assif D, 40080 Marrakech, Morocco

²Dermatology Department, Mohammed VI CHU, 53 Ibn Sina Boulevard, Assif D, 40080 Marrakech, Morocco

³Faculty of Medicine and Pharmacy-Cadi Ayyad University, Sidi Abbad, B.P. 7010, 40000 Marrakech, Morocco

*Corresponding author: Saida Ed-dyb, Bacteriology-Virology Department, Mohammed VI CHU, 53 Ibn Sina Boulevard, Assif D, 40080 Marrakech, Morocco, Tel: 00212667504774; E-mail: saeddyb@gmail.com

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Abstract

Background: The carriage of Staphylocoque aureus, including methicillin-resistant S. aureus (MRSA), is a significant risk factor for subsequent staphylococcal infection. The nares are the most consistent sites of colonization. The objective of this study was to determine prevalence for community acquired methicillin-resistant S. aureus (CA-MRSA) nasal carriage among a pediatric population as well as to find out antibiotic susceptibilities of isolated strains.

Patients and methods: We conducted a prospective study from June 2017 to June 2018 on 300 children, who consulted in the different pediatric specialties of Mohamed VI University Hospital of Marrakesh. Nasal swabs were collected from all the consultant children. The identification of nasal carriage of SA was performed in the microbiology laboratory of Mohamed VI University Hospital.

Results: Staphylocoque aureus was isolated from the nares in 49 (16.3%) children, two (4%) isolates were classified as CA-MRSA. The mean children age was 75, 14 months (p=0.05), a male predominance was noticed. The majority of SA carriers were from urban areas, it was noted a high carriage in living patients with five or more people (p=0.024). Antimicrobial susceptibility testing of MRSA stains expressed a 100% resistance to cotrimoxazole, fusidic acid and sensitivity to all other antibiotics.

Conclusion: This study highlights the potential for community MRSA acquisition in our context. These strains, which were initially sensitive to most of antibiotics, begin to become increasingly resistant to nobetalactam antibiotics. This requires continuous monitoring to clarify the factors of antimicrobial resistance and to prevent further spread of community MRSA.

Keywords: Methicillin-resistant *Staphylococcus aureus*; Nasal carriage: Communal: Children

Introduction

Staphylococcus aureus is a commensal bacterium of the cutaneous and mucous flora of man. Permanent nasal carriage is found in 30% of individuals [1,2]. It is one of the most common causes of pediatric infections worldwide, manifesting itself in a range of diseases, ranging from a minor skin infection to a serious and deadly invasive disease [3].

These infections are a real public health problem both by the virulence of the bacteria and the emergence of multiresistant strains to antibiotics. The first reported cases of methicillin-resistant S. aureus infection (MRSA) are more than 30 years old and have a nosocomial character, with the acquisition of MRSA related to recent hospitalization or prolonged and recurrent exposure to antibiotics.

Recent studies have documented that the epidemiology of MRSA has changed, as the isolation of MRSA is no longer contained to hospital settings or patients with predisposing risk factors [4,5], Asymptomatic CA-MRSA colonization has been noticed in healthy children attending the emergency departments and outpatient clinics of children's hospitals. These are community-acquired MRSA infections (CA-MRSA) [6].

Nasal or skin colonization of *S. aureus*, including MRSA, is quite common among children, and constitutes a major risk factor for subsequent invasive *staphylococcal* infection. The ecological niches of *S. aureus* strains are the anterior nares [7].

Given the relationship between nasal carriage and risk of infection, knowledge of the prevalence of nasal MRSA carriage in a community provides sense of probability of acquiring an MRSA infection in that community [8].

In the current study, which was the first in Morocco, we aimed to address the extent of this issue by evaluating the nasal carriage prevalence and antimicrobial resistance profiles of *S. aureus* and CA-MRSA in healthy children.

Materials and Methods

Setting and population

This prospective observational study was conducted from June 2017 to June 2017 at the pediatric consultation of Mohamed VI University Hospital.

A total of 300 patients having 15 years or under were in subject to the study, excluding those having taken an antibiotic during the previous 3 months, those with hospitalization record in the previous 6 months, with a chronic disease (diabetes, hemodialysis, asthma, ...), and those having immunodeficiency (primitive, acquired, or iatrogenic) or indwelling catheters.

Statistical analysis

A convenient sample technique was used for sample selection. Results were compiled, tabulated and all data were subjected to SPSS, version17.0 software statistical package for analysis. Association was done using Chi-square test. A p-value of <0.05 was considered significant.

Questionnaire and ethical considerations

Written questionnaires concerning demographics and medical history were completed by the children's parents. An informed consent is approved by parents.

Procedure of sampling

Procedure of nasal sampling

- Take sterile dry cotton swab (soaked in 0.5 ml of sterile physiological saline).
- Insert the swab into the patient's anterior nostril (1-2 cm) and collect nasal secretions by performing 2 to 3 full rotations of the swab.
- Repeat the same procedure in the other nostril of the patient without changing the swab.

Procedure of skin sampling

A skin sample was taken from children with dermatological lesions

- The gallbladder, bubble or pustule was opened using a sterile needle
- A swab was made
- Then the swab was placed in its carrying case.

The samples were sent to the microbiology laboratory in less than 4 hours.

Microbiological methods

The bacteriological study and the study of antibiotic susceptibility were carried out in the microbiology department of Med VI University Hospital.

The inoculation of the collected swabs was done on Mannitol salt agar medium. The plates were incubated aerobically at 37°C for 24 hours and they were examined for growth. The Staphylococcus aureus which was isolated from samples was identified by standard methods based on morphological criteria (Gram-positive Cocci in clusters), culture (pigmented colonies), biochemical (mannitol fermentation, catalase, production, coagulase, biochemical tests). The identification and antibiogram were performed in liquid medium by the BD Phoenix® system. The confirmation of Meticillin resistance was made by a cefoxitin disk on agar medium (Müller Hinton medium). MRSA was confirmed by the disc diffusion method by using cefoxitin (30 mcg) and also by its growth on Oxacillin Resistant Screen Agar. The results were interpreted according to the recommendations of the Committee on antibiogram of the French Microbiology Society CA-SFM EUCAST [9]. The following antibiotics were tested: penicillin G, cefoxitin, erythromycin, lincomycin, pristinamycin, cotrimoxazole, fusidic acid, fosfomycin, rifampicin, kanamycin, gentamicin. tobramycin, ciprofloxacin, vancomycin, teicoplanin, tetracycline, linezolid and mupirocin.

Results

A total of 300 children consulting at different pediatric specialties were enrolled in this study (**Table 1**).

Table 1 Frequency distribution of S. aureus nasal carriagebased on different pediatric specialties.

Specialty	No. of subjects (%)	No.of subjects (%) with S. aureus		
Pediatric emergencies	115 (38.3%)	15 (13%)		
pediatric dermatology	105 (35%)	19 (18%)		
Pediatrics	57 (19%)	9 (16%)		
Infant surgery	19 (6.3%)	4 (21%)		
Neonatology	4 (1.3%)	2 (50%)		

Their age is ranging from 1 to 180 months, with a mean age of 60.45months.

55.7% (n=167) were boys. The majority of consultants (93%) did not have a particular pathological history. Infectious

pathology was the most frequent reason for consultation with 60% of cases. Fever, respiratory discomfort and cough dominated the clinical symptomatology, with the percentages being (60%), (20%) and (14%) respectively. Six patients were carriers of cutaneous lesions.

Forty nine strains of *Staphylococcus aureus* were isolated, giving a prevalence of nasal carriage of *S. aureus* of 16.33% (49/300), (95% confidence interval [CI]:12.1-20.5).

The prevalence of MRSA was 0.66% (2/300), this figure corresponds to 4% (2/49) of *S. aureus* isolates. Skin and nasal samples were positive in children with dermatological lesions.

The mean age in children with *S. aureus* nasal carriage was 75.14 months (p=0.05). A male predominance was noted (sex ratio M/F=1.33).

The prevalence of SA nasal carriage was higher in the summer (p=0.068) and most patients identified having SA nasal carriage were from urban areas (76%) and 24% from rural areas (p=0.214). The community life represented essentially by the number of close contacts under the same roof with the patient, it was noted a high carriage in living patients with five or more people (0.024).

Among the 300 children included in the study the statistically significant risk factor for nasal colonization of *S. aureus* was number of members of joint families (**Table 2**).

Variable	n (%)	S. aureus nasal carriage n (%)		p-value	CA-MRSA nasal carriage n (%)	
		Positive	Negative	Positive	Negative	
Age group (years)	·	I		1		
0-3	84 (28)	19 (6.33)	65 (21.66)	0.05	1 (0.33)	83 (27.66)
>3-6	60 (20)	5 (1.66)	55 (18.33)		1 (0.33)	59 (19.66)
>6-10	84 (28)	13 (4.33)	71 (23.66)		0	84 (28)
>10-15	72 (24)	12 (4)	60 (20)		0	72 (24)
Sex	I			- <u> </u>		
Male	167 (55.66)	28 (9.33)	139 (46.33)	0.352	2 (0.66)	165 (55)
Female	133 (44.33)	21 (7)	112 (37.33)		0	133 (44.33)
Season	I					
Summer	164 (54.66)	32 (10.66)	132 (44)	0.068	1 (0.33)	163 (54.33)
Winter	136 (45.33)	17 (5.66)	119 (39.66)		1 (0.33)	135 (45)
Owelling	I	1		- <u> </u>		
Urban	237 (79)	37 (12.33)	200 (66.66)	0.214	0	237 (79)
Rural	63 (21)	12 (4)	51 (17)		2 (0.66)	61 (20.33)
lumber of membe	ers of joint families			I		
<5	160 (53.33)	11 (3.66)	149 (49.66)	0.024	1 (0.33)	159 (53)
≥5	140 (46.66)	38 (12.66)	102 (34)		1 (0.33)	139 (46.33)

Table 2 Frequency repartition of S. aureus and CA-MRSA nasal carriage based on demographic variables.

The two children with CA-MRSA, were male, and they were 42 months and 26 months respectively.

They had no contact with a person with a current skin lesion. Both patients came from rural areas. The close contacts living with them under the same roof were 5 people for the first case and 4 people for the second.

One of our patients was seen in pediatric emergencies for progressive symptoms and signs of motor weakness develop rapidly of two limbs. The Guillain Barré Syndrome was retained after a complete assessment. The 2nd patient was seen at the pediatric dermatology consultation where he was being followed for atopic dermatitis.

The study of the antibiotic susceptibility of the 49 strains of *S. aureus* showed that apart penicillin G, *S. aureus* strains were sensitive to other antibiotics. No acquired resistance was found in methicillin-sensitive *S. aureus* strains.

Isolated MRSA strains remained sensitive for other families of antibiotics including erythromycin, linezolid, pristinamycin, rifampicin, fluoro-quinolones and glycopeptides. These strains

expressed a 100% resistance to cotrimoxazole and fusidic acid and a 50% to kanamycin **(Table 3)**.

Table 3 Sensitivity of CA-MRSA Strains to Antibiotics.

Antibiotic	Susceptibility (%)
Penicillin G	0
Cefoxitin	0
Gentamycin	100
Kanmycin	50
Tobramycin	100
Daptomycin	100
Teicoplanin	100
Vancomycin	100
Erythromycin	100
Pristinamycin	100
Linezolid	100
Levofloxacin	100
Rifampicin	100
Tetracyclin	100
Tigecyclin	100
Cotrimoxazole	0
Fucidic acid	0

The bacteriological data had demonstrated that the resistance of the two MRSA-C strains is mediated by the *mec A* gene.

Discussion

S. aureus is a bacterium that has the ability to asymptomatically colonize human mucosa for varying periods of time [10,11]. Its carriage is frequent in the pediatric population [12]. Nasal carriage of MRSA has been verified as a significant risk factor for MRSA infection. The first cases of MRSA infection were reported more than 30 years ago and had a nosocomial character, the acquisition of MRSA being related to recent hospitalization or prolonged and recurrent exposure to antibiotics.

After nearly three decades of being solely confined to hospitals and long-term care facilities, MRSA has emerged in various geographically distinct communities outside of health care settings, without known health care-associated risk factors [13].

In 2000, the CDC created a case definition for a CA-MRSA infection: any MRSA infection diagnosed for an outpatient or within 48 h of hospitalization if the patient lacks the following health care-associated MRSA risk factors: hemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling

catheter or a percutaneous device at the time of culture, or previous isolation of MRSA from the patient [14,15].

This study from Morocco reports the nasal carriage of *S. aureus* and MRSA_C among healthy children in the community in the age group of 1 month to 15 years.

Our study showed that 16.33% of healthy children attending pediatric consultation of Mohamed VI University Hospital Center, were colonized with *S. aureus* in the anterior nares.

Our findings are consistent with previous reports: In a study from Andhra Pradesh, India, a carriage rate of 16% for *S. aureus* was documented [16]. Studies from Taiwan, US, Iran have documented prevalence of nasal carriage of *S. aureus* among children ranging from 16%, 23% and 28% respectively. [13,17,18].

In contrast, Chaterjee et al. [19] studied 489 school children aged 5-15 years by PCR and found nasal colonization of *S. aureus* in 256 (52.5%) of children, which is much higher compared to our study and other reports [19]. Other studies from tunis and Tanzania have documented a carriage rate of 47%, 40% respectively [20,21].

The comparatively higher prevalence rate may be attributed to the characteristics of the study population, although other factors may have played a contributory role.

Studies have shown that colonization with *S. aureus* varies with age [22-24] with a peak at 2-3 years [22]. Similarly, in our study the predominant age groups were 0 to 3 years and 6 to 10 years. These differences were not statistically significant.

In our study, the statistically significant risk factor for nasal colonization of *S. aureus* was living with more than 5 persons. The study conducted by Shetty et al. (India) [5] has shown that the prevalence of SA carriers is higher in patients living with 10 people compared to patients with close contacts \leq 4; this could be due to poor hygiene and overcrowding. Another study done by Chen et al. (Taiwan) reported that living with more children is an important factor for colonization with MRSA [25].

The low prevalence of MRSA (0.66%) in the current study concurs with findings of resource-limited countries: in the studies done in Ghana by Eibach et al. [37], in Malaysia by Azmawati et al. [26], in Ahvaz, Iran by Nikfar et al. [27], and in Szolnok, Hungary by Krisztina et al. [28], the rates are respectively 2%, 1.6%, 1.3% and 0.8% but these findings are in contrast with previous reports from other countries, which documented height prevalence of MRSA colonization among healthy children in the community ranging from 13.2% to 22% [29-31].

In one study from the US, the nasal colonization rate of MRSA among healthy children increased from 0.8% in 2001 to 9.2% in 2004 [25,32].

The high MRSA carriage in healthy children is of great importance because MRSA colonization may subsequently cause infections [5], has been reported to be associated with outbreaks in a pediatric hospital resulting in the death of children [33], also asymptomatic colonization can persist for months to years [34] and the potential for spread of the

pathogen to settings such as hospitals can cause outbreaks among vulnerable populations.

The resistance of *Staphylococcus aureus* to penicillin, through the acquisition of a penicillin-degrading penicillinase plasmid penicillinase, was initially restricted to the hospital setting. Currently, this resistance has spread quickly in community affects more than 90% of strains of *S. aureus.*

Methicillin resistance is related to the acquisition of another penicillin binding protein, PLP2a. This PLP2a transpeptidase has a low affinity for β -lactams. The production of this PLP2a is encoded by the *mec A* chromosomal gene [35]. Strains of *S. aureus* possessing the *mec A* gene are therefore resistant to the entire family of β -lactams, especially meticillin or oxacillin. The *mec A* gene is included in a mobile genetic element: the *staphylococcal* cassette [36]. The strains of MRSA isolated in our study carried *mec A* gene.

Staphylococcus aureus has a strong adaptive power. It is a formidable pathogen that has developed resistance to each new antibiotic introduced for half a century. The plasticity of its genome gives it the ability to adapt to all environmental conditions, including the acquisition of antibiotic resistance genes other than beta lactamines and to develop regulatory mechanisms to adapt to increasing concentrations of antibiotics [36]. This explains the resistance of MRSA strains to the many families of antibiotics with resistance rates of up to 90-95% for some [36].

In our study, the *S. aureus* isolates exhibited resistance to Penicillin G resistance (97%), The two strains isolated of CA-MRSA were resistant to Cotrimoxazole (100%), Fusidic acid (100%) and Kanamycin (50%). They were susceptible to Glycopeptid, Linézolid and Fluoroquinolones.

Eibach [37] found a high resistance to Penicillin (95.1%), Tetracyclines (53.7%) and Erythromycin (11.4%). Isolated strains were susceptible to Vancomycin and Linézolide.

In the study of Azmawati et al. [26] the majority of strains isolated were sensitive to oxacillin, 94.7% were resistant to penicillin and 50% were sensitive to erythromycin, 95.8% and 72, 9% were the susceptibility rates for doxycycline and ciprofloxacin, respectively. Levels of resistance to voncomycin and clindamycin were low.

Recommendations

There is insufficient evidence to support the routine use of decolonization treatment for nasal carriage of CA-MRSA. CA-MRSA management should focus on preventing the spread of infection with emphasis on good hygiene. Good hygiene should emphasize proper hand washing, keeping draining wounds covered, and disinfecting the environment. Topical decolonization achieves short-term decolonization. Decolonizing pre-surgical patients may decrease the rate of postoperative wound infections. Topical decolonization strategies involve the use of antiseptic wipes and/or daily nasal antibiotics. If CA-MRSA decolonization treatment regimens are used, it may be useful to treat close cohorts [38].

For our 2 cases of CA-MRSA, we tried to re-contact the 2 identified nasal carriers of CA-MRSA, a survey was conducted to know the circumstances and experience of both patients, raise awareness and propose an effective treatment to limit the contamination of the environment.

Conclusion

S. aureus is a pathogen whose high adaptability allows survival through the successive acquisition of antibiotic resistance genes, growth-regulating mechanisms in the presence of antibiotics.

Children of consultation in Marrakech, Morocco have a high rate of colonization with *S. auerus*. Nasal colonization of CA-MRSA exists but is still low among children lacking traditional risk factors for MRSA infection. This study has demonstrated the baseline colonization rate, and continued surveillance of this population is necessary to assess the ongoing risk CA-MRSA poses to this community. Presence of resistance to commonly used antibiotics is a big concern. Elimination of nasal carriage would theoretically reduce the infection rates in populations in which it has been identified as a risk factor.

The vigilance of clinicians and microbiologists is required to signal the emergence of new epidemiological phenomena.

New studies will be needed to better investigate the other *S. aureus* porting sites, to better appreciate the colonization dynamics of this bacterium over time and to better specify the risk factors favoring carriage.

Antibiotic surveillance program and infection control strategy should be instituted to monitor the development of antibiotic resistance nationally through clear and feasible implementation strategies to prevent community spread of these community-acquired MRSA.

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