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Nasal isolates of commensal *Staphylococcus aureus* and non-*aureus* species from healthy young adults in Valencia (Spain) and their resistance to chemotherapeutic agents

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Abstract

Objective: Methicillin-resistant *S. aureus* (MRSA) have been gradually disseminated causing serious nosocomial infections worldwide, and recently MRSA infections have emerged in healthy individuals. Besides, non-*aureus staphylococci* can colonize humans and animals, and show a large proportion of methicillin resistant strains that can be transmitted between these hosts. The aim of this study was to determine the incidence of commensal *Staphylococcus* species in the nasal cavity of healthy young adults, and their resistance to methicillin and other antibiotics, as potential reservoirs for spreading disease and antibiotic resistance into the community.

Methods: Nasal carriage of *S. aureus* and other manitol-fermenting non-*aureus* species was studied in 445 and 89 healthy volunteer university students (University of Valencia, Spain), respectively. *Staphylococcus* isolation, identification, and resistance tests to eight chemotherapeutic agents (ciprofloxacin, clindamycin, co-trimoxazole, erythromycin, gentamicin, mupirocin, oxacillin and vancomycin) were performed according to standard microbiological procedures.

Results: The nasal carriage rate was 22% for *S. aureus* (99 isolates from 445 students) and 13.5% for other manitol-fermenting non-*aureus* species (12 out of 89 students). Non-*aureus* isolates were identified as *S. intermedius* (7 isolates), *S. saprophyticus* (4 isolates), *S. caprae* (2 isolates), *S. kloosii* (1), S.warneri (1), and *S. capitis* (1). Interestingly, four students out of 89 (4,5%) were carriers of two *Staphylococccus* species. Methicillin-resistant isolates were 6% (*S. aureus*) and 25% (non-*aureus* species). Resistances to mupirocin and particularly to erythromycin were detected; no resistances to co-trimoxazole, ciprofloxacin, gentamicin and

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vancomycin were found.

Conclusion: There is a need to implement continued surveillance of MRSA dissemination within the community in order to improve MRSA prevention and control. We suggest that this surveillance should be not limited to *S. aureus* but extended to other *Staphylococcus* species to avoid the possibility of resistance transfer among non-*S. aureus* (most belonging to species normally found as commensals in animals) and *S. aureus* isolates.

Introduction

Staphylococcus aureus is a commensal bacterial species often found in skin and mucous membranes of healthy individuals; a significant percentage (20-30%) of human population are nasal carriers of *S. aureus* [1]. These healthy carriers constitute a reservoir of the pathogen, as most bacterial infections are caused by the patients' own commensal microbiota. Colonization significantly increases the risk of infection in immune compromised patients which are frequently infected with the same strain they carry as a commensal. Therefore, hospitalized patients are often exposed to nosocomial infections by *S. aureus*, which is able to cause a plethora of diseases [2-4]. The clinical impact of *S. aureus* infections is enhanced by its potential to acquire antimicrobial resistance, with the development of resistance to methicillin as the most relevant concern. Over the last decades, Methicillin-resistant *S. aureus* (MRSA) have been gradually disseminated causing serious nosocomial infections worldwide, although it was considered as an uncommon community pathogen. However, more recently MRSA infections have emerged in healthy individuals with no obvious risk factors for its acquisition [2-6]. At present, community acquired MRSA is emerging worldwide, probably by disseminating from hospital environment, although not much is known about its transmissibility. Transmission of MRSA between companion animals and humans has been also suggested, as well as between specific livestock-associated MRSA and livestock veterinarians and farms workers [7-12]. The situation is more complex, as coagulase-positive *staphylococci* (CoPS) other than *S. aureus*, such as members of the *S. intermedius* group (mainly *S. pseudointermedius*), show resistance to methicillin and are commensal species of companion animals that occasionally are found in human exposed to those animals [8-10,13-15]. Besides, the heterogeneous group of coagulase-negative *staphylococci* (CoNS), regarded as less pathogenic *staphylococci*, are now considered also as major nosocomial pathogens, which colonize humans and animals, show a large proportion of methicillin resistant strains, and a few studies suggest transmission between these hosts [8,10,15].

In Spain, MRSA still continues to be an important nosocomial pathogen, although resistance to methicillin in clinical isolates appears to be stabilized both in *S. aureus* (25-30%) and CoNS (50-60%) [16]. However, the information concerning MRSA dissemination within the community, particularly in healthy individuals with no obvious risk factors for colonization, is still scant [17]. Similarly, there is a lack of information concerning colonization of healthy individuals by non-*aureus staphylococci*. Besides, in contrast to clinical isolates, antibiotic resistances of commensal isolates of *S. aureus*, and particularly of non-*aureus* isolates are largely unknown. Therefore, the aim of this study was to determine the incidence of commensal *Staphylococcus* species in the nasal cavity of healthy young adults (university students of the University of Valencia, Spain), and their resistance to methicillin and other antibiotics, in order to obtain relevant epidemiological data concerning the rate of healthy carriers of MRSA and other *Staphylococcus* species, as potential reservoirs for developing infections and spreading antibiotic resistance into the community.

Methods

Nasal swabs were streaked on selective mannitol salt agar (Chapman mannitol) and incubated 24/48 h at 37°C for colony isolation. Manitol-fermenting isolates were routinely cultured and maintained on Tryticase Soy Agar (TSA). *Staphylococcus* species were identified by standard procedures: coagulase test using plasma rabbit (BioMerieux), agglutination test using the Staph plus Latex Kit (DiaMondiaL), and biochemical tests using the BBL Crystal Gram-Positive (GP) Identification (ID) system (Becton Dickinson). All isolates were identified with a confidence > 0.95. Antibiotic susceptibility was determined by disk diffusion methods according to standard microbiological procedures [18]. Disks of eight antibacterial chemotherapeutic agents commonly in clinical use against gram-positive bacteria were used (Liofilchem): ciprofloxacin 5µg, clindamycin 2µg, co-trimoxazole (trimethoprim/sulfamethoxazole) 25µg, erythromycin 15µg, gentamicin 10µg, mupirocin 200µg, oxacillin 1µg and vancomycin 30µg. The study of nasal *S. aureus* carriers is routinely included in the laboratory practice of the students of the Food Hygiene subject in the Nutrition and Human Dietetics degree (University of Valencia) (official teaching guide code 33954, available on line at the university website); participation in the study reported in this work was on strict volunteer basis (written consents were given) and all the personal information was kept anonymous.

Results

A total of 99 isolates were identified as *S. aureus*, showing that 22% of healthy students (99 out of 445) were carriers of *S. aureus* as commensal bacteria in their nasal cavity. Six of these *S. aureus* isolates (6%) were found to be oxacillin (methicillin)-resistant.

S. aureus isolates (n: 35, including five MRSA) from selected students (n: 89) were tested for resistance to other seven antibiotics (Table 1). Interestingly, none out of 35 isolates was resistant to ciprofloxacin, vancomycin, gentamicin, co-trimoxazole nor clindamycin. Two isolates showed resistance to mupirocin, whereas about 54% (19 isolates) were resistant to erythromycin. Only two isolates were resistant to two agents (oxacillin and erythromycin), whereas 11 isolates (31%) showed no resistances to the antibiotics tested.

In our study, manitol-fermenting *Staphylococcus* isolates other than *S. aureus* were also identified in the above cited selected samples (n: 89). A total of 16 isolates were obtained from 12 carriers (13,5%, 12 out of 89 students). Six different species were identified: *S. intermedius* (7 isolates), *S. saprophyticus* (4 isolates), *S. caprae* (2 isolates), *S. kloosii* (1), *S. warneri* (1), and *S. capitis* (1). To gain more information about antibiotic resistances in these isolates, susceptibility tests to all eight antibiotics was assayed. Results (Table 1) showed that none out of 16 isolates was resistant to ciprofloxacin, vancomycin, gentamicin, nor co-trimoxazole; four isolates (*S. caprae*, *S. kloosii*, *S. warneri* and *S. intermedius*) showed resistance to methicillin (25%), whereas resistance to erythromycin was observed in nine isolates (55%: five *S. intermedius*, three *S. saprophyticus*, and one

S. caprae); three isolates (*S. intermedius*) showed resistance to mupirocin (19%), and only one isolate (*S. intermedius*) was resistant to clindamycin (6%); five isolates (31%) were resistant to two agents: erythromycin and either oxacillin (one *S. caprae* isolate), clindamycin (one *S. intermedius* isolate) or mupirocin (three *S. intermedius* isolates). Four isolates (25%) were susceptible to all antibiotic tested (*S. caprae*, *S. capitis*, *S. intermedius* and *S. saprophyticus*).

Interestingly, four students (out of 89) were carriers of two *Staphylocccus* species in their nasal cavity (Table 2): *S. saprophyticus* and *S. intermedius* (one student), *S. aureus* and *S. capitis* (one student), *S. aureus* and *S. intermedius* (one student), and *S. aureus* and *S. caprae* (one students); in all cases isolates from the same individual did not share antibiotic resistance (students 1 and 4), other than erythromycin resistance (student 3) (Table 2).

Discussion

Our results confirm that a significant percentage (22%) of healthy young adults are nasal carriers of *S. aureus*, and show that 6% of the *S. aureus* isolates are resistant to methicillin, despite none of the volunteer students participating in the study had been exposed to risk factors for *S. aureus* colonization or antibiotic treatments for at least two months prior to their participation in the study. This percentage of resistance to methicillin is lower than that described for clinical isolates of *S. aureus* in Spain (around 28%) [16], and higher than the percentage of MRSA isolates from healthy students found in other countries (1.5-3%) [19,20] as well as to the percentage of MRSA found in individuals with no healthcare associated risks in European countries (0-2.1%) [17], whereas MRSA prevalence in community in Delhi area was found to be higher: 18.1% [21].

Similarly, in our study resistance of *S. aureus* isolates to other antibiotics (clindamycin, gentamicin, and particularly ciprofloxacin) was also lower in isolates from healthy individuals (0%) than that described for clinical isolates in Spain (15%, 8% and 33%, respectively) [16]. Only resistance to erythromycin was increased in commensal isolates (about 54%) as compared to clinical isolates (28%). These observations point out that probably the relationship between *S. aureus* nasal isolates in the community and *S. aureus* from hospital environment is not obvious. Also methicillin resistance in non-*aureus Staphylococcus* isolates (25%) was lower than that reported in clinical isolates (51%), as well as resistances to clindamycin (6% versus 51%), gentamicin (0% versus 36%), co-trimoxazole (0% versus 23%) and ciprofloxacin (0% versus 43%), whereas resistance to erythromycin was similar in both commensal and clinical isolates [16]. Interestingly, resistance to mupirocin, the antibiotic commonly used to treat nasal carriers of *S. aureus*, was found both in *S. aureus* (6%) and non-*aureus* isolates (20%).

These results suggest that the ability of *S. aureus* to develop resistance to methicillin (and other antibiotics) and to spread resistance within the community is limited, probably due to the absence of selective pressure to develop/select resistance outside hospital environment. In addition, as a significant percentage of individuals (13.5%) were nasal carriers of manitol-fermenting non-*aureus Staphylococcus* species, the possibility of resistance transfer among non-*S. aureus* and *S. aureus* isolates colonizing the same individual should be considered (although in the few cases found in our study such a resistance transfer did not occur, as no common resistances were detected in these isolates). Since most non-*aureus* isolates belong to species normally found as commensals in animals (particularly *S. intermedius* in companion animals), genetic transfer of resistance determinants between animal (veterinary) and human isolates may represent a factor contributing to the spreading of methicillin resistances into the community. Therefore, due to the emergence of community acquired MRSA, there is a need to implement continued surveillance of MRSA dissemination within the community, as well as in livestock and companion animals, in order to improve MRSA prevention and control, and we suggest that this surveillance should be not limited to *S. aureus* but extended to other *Staphylococcus* species.

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Tables

Table 1: Resistance to methicillin and other antibiotics in *S. aureus* and non-*aureus Staphylococcus* isolates from nasal cavity of selected young adults (n: 89).

	S. aureus isolates	Non-*aureus* isolates
Antibiotic	(n: 35)	(n: 16)
Oxacillin	5	4
Erythromycin	19	9
Mupirocin	2	3
Clindamycin	0	1
Gentamicin	0	0
Co-trimoxazole	0	0
Ciprofloxacin	0	0
Vancomycin	0	0
None	11	4
Erythromycin/Oxacillin*	2	1
Erythromycin/Clindamycin*	0	1
Erithromycin/Mupirocin*	0	3

*Isolates resistant to two antimicrobial agents are also included in the data of single resistances.

Table 2: Antibiotic resistances in *Staphylococcus* isolates from the same nasal cavities

Nasal swab	Isolates	Resistances
Student 1	*S. saprophyticus*	Erythromycin
	S. intermedius	Oxacillin
Student 2	*S. aureus*	none
	S. capitis	none
Student 3	*S. aureus*	Erythromycin
	S. intermedius	Erythromycin/mupirocin
Student 4	*S. aureus*	Oxacillin
	S. caprae	none

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