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Commensal *Staphylococcus* isolates from the nasal cavity of community older adults in Valencia (Spain) and their resistance to methicillin and other antibiotics

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Methicillin-resistant *Staphylococcus aureus* strains (MRSA) have been gradually disseminated worldwide, causing nosocomial and community-acquired infections, and healthy carriers of commensal MRSA constitute a reservoir of the pathogen. Other *Staphylococcus* species (coagulase-negative, CoNS) colonize animals and humans and include also methicillin-resistant strains (MRCoNS). Here we have determined the prevalence of *S. aureus* and CoNS species in the nasal cavity of community healthy older adults (*n*= 27, average age: 63.7 years) and their resistance to methicillin and other antibiotics. A total of 35 *Staphylococcus* isolates were obtained. All individuals (100%) were carriers of at least one *Staphylococcus* strain; 15% of subjects were *S. aureus* carriers, and eight subjects (30%) carried two strains. Prevalence of resistance to methicillin was 25% and 35% for *S. aureus* and CoNS isolates, respectively. Most isolates were resistant to penicillin G (90%) and clarithromycin (45%). Other resistances were less frequent (rifampicin, tetracycline, fosfomycin, ciprofloxacin), and no resistant isolates to chloramphenicol or vancomycin were found. Multiresistant isolates to three or four chemotherapeutic agents were detected (20% of isolates). These results suggest that the nasal cavity of healthy adults may represent an ecological niche for the transfer of resistant determinants between staphylococcal species, and point out that epidemiological surveillance of commensal MRSA carriers should extended also to MRCoNS carriers.

Key words: Staphylococcus, methicillin resistance, nasal carriers, older adults.

Aislamiento de estafilococos comensales de la cavidad nasal de adultos mayores en Valencia (España) y su resistencia a meticilina y otros antibióticos. Las cepas de *Staphylococcus aureus* resistentes a meticilina (SARM) están ampliamente diseminadas, causando infecciones hospitalarias y comunitarias, y los portadores sanos de SARM constituyen un reservorio del patógeno. Otras especies de estafilococos coagulasa negativos (CoNS) colonizan animales y humanos, e incluyen cepas resistentes a meticilina (CoNSRM). En este trabajo hemos determinado la prevalencia de *S. aureus* y CoNS en la cavidad nasal de adultos mayores sanos (*n*= 27, edad media: 63.7 años) y su resistencia a meticilina y otros antibióticos. Se obtuvieron 35 aislados de *Staphylococcus*. Todos los individuos (100%) portaban al menos una cepa de *Staphylococcus*; el 15% eran portadores de *S. aureus*, y en ocho sujetos (30%) se aislaron dos cepas. La resistencia a meticilina fue del 25% y del 35% para los aislados de *S. aureus* y CoNS, respectivamente. La mayoría de aislados fueron resistentes a penicilina G (90%) y claritromicina (45%). Otras resistencias fueron menos frecuentes (rifampicina, tetraciclina, fosfomicina, ciprofloxacino) y no se encontraron resistencias a cloranfenicol ni vancomicina. Se detectaron aislados multirresistentes a tres o cuatro quimioterápicos (20% de aislados). Estos resultados sugieren que la cavidad nasal puede constituir un nicho para la transferencia de resistencias entre estafilococos, y que la vigilancia epidemiológica debe incluir tanto a los portadores de SARM como de CoNSMR.

Palabras clave: Staphylococcus aureus, resistencia a meticilina, portadores nasales, adultos mayors.

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Staphylococcus aureus is a Gram-positive bacterial species that often colonizes the skin, skin glands and mucous membranes of healthy individuals (20-30% of human population are nasal carriers of *S. aureus*) (Gorwitz et al., 2008; Krismer et al., 2017; Weidenmayer et al., 2012). Besides, *S. aureus* may act as a human pathogen that causes a plethora of infectious diseases both in hospital and community environments, in addition to food poisoning (Fisher et al., 2018; Sievert et al., 2013; Tong et al., 2015). Hospital-acquired infections by *S. aureus* particularly affect to immunocompromised patients that are prone to nosocomial infections due to their impaired immune system and the increasing use of clinical devices, such as ventilator-associated infections in intensive care units. In Spain, *S. aureus* is, after *Escherichia coli*, the most frequent etiologic agent causing both nosocomial infections (10.32 % of total hospital-acquired infections) and community-acquired infections (8.80% of total community infections) (EPINE-EPPS, 2017). The role of healthy carriers of *S. aureus* in the epidemiology of infections is well known, as they constitute a reservoir of the pathogen, and most infections are caused by the host own commensal *S. aureus* strains (Miller and Kaplan, 2009; Wertheim et al., 2004).

The clinical relevance of *S. aureus* infections is enhanced by the remarkable ability of this species to develop antimicrobial resistance, being resistance to methicillin (first described in 1961) the most important clinical concern, as methicillin-resistant *S. aureus* (MRSA) infections are not susceptible to treatment with beta-lactam antibiotics (Dulon et al., 2011; Otto, 2012). The prevalence of MRSA among clinical isolates of *S. aureus* has been roughly constant (25-30%) during last years in Spain (Cercenado, 2016). Methicillin resistance results from the acquisition of the *mec*A gene carried on a large genetic mobile element (staphylococcal cassette chromosome *mec*, SCC*mec*), which may differ among MRSA strains (Hiramatsu et al., 2014; Lakhundi and Zhang, 2018). MRSA strains have been gradually disseminated worldwide over the last decades, causing serious nosocomial infections, which in some settings may have a higher frequency that methicillin-susceptible *S. aureus* (MSSA) infections (Dulon et al., 2011; Otto, 2012). More recently, community-acquired (CA-MRSA) infections are also emerging worldwide, including Spain, probably by spreading from hospital environment, although transmissibility mechanisms of MRSA are not well known; new CA-MRSA strains have been emerging continuously and some clones are able to cause infections in individuals without predisposing risk factors (Lakhundi and Zhang, 2018; Otto, 2012; Vindel et al., 2014). MRSA strains are also present in companion and livestock animals that may act as MRSA reservoirs, since transmission of MRSA among humans and these animals may occur (Couto et al., 2015; Lakhundi and Zhang, 2018; Pomba et al., 2017).

In addition to *S. aureus*, other coagulase positive species, as the *S. intermedius* group (mainly *S. pseudointermedius*) are commensal species of companion animals and the most frequent staphylococcal pathogens in small animal practice, thus suggesting an

endogenous origin of the infection (Bond and Loeffler, 2012; Kadlec and Schwarz, 2012; Ruscher et al., 2009). Interestingly, *S. intermedius* isolates have been found in human exposed to companion animals (Pomba et al., 2017). Acquisition of SCC*mec* by S. intermedius strains accounted for the important emergence of methicillin-resistant *S. intermedius* isolates reported in Europe and North America (Bond and Loeffler, 2012; Kadlec and Schwarz, 2012).

Coagulase-negative *Staphylococcus* (CoNS) species constitute a heterogeneous group, now considered as nosocomial pathogens (with *S. epidermidis* and *S. haemolyticus* as the most significant species), which includes members that can colonize the skin and mucous membranes of animals and humans, causing community-acquired infections worldwide, including Spain (Becker et al., 2014; Cercenado, 2016, EPINE-EPPS, 2017). Resistance to methicillin in CoNS is conferred by the SCC*mec*, although non-*mec*A SCC elements have been reported in some species (Barbier et al., 2010); the described overall frequency of MRCoNS in healthcare-associated isolates is roughly 60%, similar to the prevalence of MRCoNS in clinical isolates in Spain (50-60%) (Barbier et al., 2010; Cercenado et al., 2016; Cuevas et al., 2004). Studies with healthcare-associated strains suggested that MRCoNS may serve as a source of SCC*mec* for *S. aureus*, and besides MR-CoNS are probably disseminated into the community, including individuals with no health care-associated risks (Barbier et al., 2010; Hanssen et al., 2004)

S. aureus is able to acquire genetic determinants, by interspecies gene transfer, conferring resistance to clinically relevant antibiotics (in addition to methicillin). Multi-drug resistance phenotype makes some *S. aureus* strains one of most intractable pathogenic bacteria in hospital environment (Hiramatsu et al., 2014; Lakhundi et al, 2018). Other *Staphylococcus* species, such *S. pseudointermedius* and CoNS may also develop resistances to antibiotics other than methicillin (Becker et al., 2014; Bond and Loeffler, 2012).

Despite the stabilization of the prevalence of MRSA during last years in Spain, MRSA remains as one of the most important nosocomial pathogen, and MRSA infections have emerged in the community (EPINE-EPPS, 2017; Pujol and Limón, 2013, Vindel et al., 2014). However, the information on dissemination of MRSA strains in the community in Spain is still scant, particularly in healthy individuals with no obvious risk factors for colonization. Two independent studies reported 14% and 22 % of nasal carriers of *S. aureus* among high school students, with differing prevalence of MRSA among isolates (40% and 6%) (Falomir et al., 2018; Teira et al., 2013). Besides, there is also a lack of information on MRSA dissemination in the elderly population; a recent study performed in residential care homes for older people reported that 21% of subjects were colonized by *S. aureus* (including 3.8% by MRSA) (Galán-Sánchez et al., 2019).

Similarly, there is a lack of data about nasal colonization by *Staphylococcus* species other than *S. aureus* and co-carriage of more than one commensal strain; Falomir et al. (2018) reported preliminary data showing that about 5% of young adults are co-carriers of two mannitol-positive *Staphylococcus* species. In addition, antibiotic resistance patterns of commensal *S. aureus*, and particularly non-aureus *Staphylococcus* species, are largely unknown, in contrast to the information available on antibiotic resistances in clinical isolates. Therefore, the aim of this study was to determine in community healthy older adults (outside hospital and community healthcare homes) the prevalence of carriers harboring commensal *Staphylococcus* species in their nasal cavity, and to determine the antibiotic resistance of the isolates, in order gain valuable information to support our hypothesis concerning to (i) their potential role as reservoirs for infection and dissemination of antibiotic resistance in the community and (ii) the potential role of nasal cavity co-colonized by *Staphylococcus* species as an ecological niche for the transfer of resistant determinants between staphylococcal species.

METHODS

Population under study

The study was performed in unselected older adults, all of them students attending (during the academic year 2017-18) the second course of the Health Sciences itinerary, included in "la Nau Gran", the teaching program for the elderly of the "Universitat de València" (Spain). A total of 27 students (19 woman and 8 men) participated in the study, with ages ranging from 58 to 84 years old (average 63.7 years: 64.2 for women and 62.5 for men). Participation in this study was on strict volunteer basis, written consent was given in all cases, and results obtained were kept anonymous. In addition to the age, another data potentially related with *S. aureus* colonization were obtained, such whether individuals are in contact with companion animals (dogs and cats) (seven adults) and whether individuals have been long-term smokers (seven adults, but none current smoker). Odds ratio was calculated to find possible association between colonization by *Staphylococcus* and exposure to these factors.

Isolation and identification of Staphylococcus *species*

Nasal swabs, aseptically taken from each subject, were immediately streaked on selective mannitol salt agar (Chapman mannitol plates) (Conda) and incubated for 24/48h at 37ºC for colony development, as previously described (Falomir et al., 2018). All colony types isolated, both mannitol-fermenting and mannitol-non fermenting colonies, were routinely cultured and maintained on Trypticase Soy Agar (Liofilchem). Identification of *Staphylococcus* isolates was performed according to standard procedures (Falomir et al., 2018): coagulase test with plasma rabbit (BioMerieux),

agglutination test using the Staph Plus Latex Kit (DiaMondial), catalase assay and biochemical tests using the BBL Crystal Gram-Positive (GP) Identification (ID) System (Becton Dickinson).

Determination of resistance to chemotherapeutic agents

Susceptibility to antibiotics was determined by disk diffusion methods on Moeller-Hinton agar (Liofilchem), according to standard microbiological procedures (Falomir et al., 2014, 2018). Discs of nine common antibacterial chemotherapeutic agents in clinical use against gram-positive bacteria were used (Liofilchem): oxacillin 1 µg (to test methicillin resistance), tetracycline 30 µg, clarithromycin 15 µg, rifampicin 30 µg, fosfomycin 200 µg, chloramphenicol 30 µg, ciprofloxacin 5 µg, vancomycin 30 µg, and penicillin G 10 IU. Resistance to the chemotherapeutic agents was deduced from the obtained growth inhibition halos according to the known diameters for clinical resistance/susceptibility provided by Liofilchem Diagnostici (Italy).

RESULTS

A total of 35 isolates were obtained from 27 nasal swabs (10 mannitol-fermenting and 25 mannitol non-fermenting). All 10 mannitol fermenting isolates were identified at the species level: four isolates were coagulase positive and further characterized as *S. aureus* by agglutination and biochemical tests; the other six isolates were coagulase negative and identified as *S. warneri* (3 isolates), *S. pasteuri, S. kloosii* and *S. xylosus*. The 25 mannitol negative isolates were confirmed as *Staphyloccus* spp. (gram-positive cocci, catalase positive), but no further identified at the species level (Table 1). Therefore, 15% (4 out of 27) of the subjects under study were nasal carriers of *S. aureus*, with similar percentages between woman (15.7%, 3 out of 19) and men (12.5%, one out of eight).

Table 1. Staphylococcus isolates from nasal cavities

Phenotype	Species	Number of isolates
Manitol (+) Coagulase (+)	*S. aureus*	4
Manitol (+) Coagulase (-)	*S. warneri*	3
	S. kloosii	1
	S. xylosus	1
	S. pasteuri	1
Manitol (-) Coagulase (-)	*Staphylococcus* spp.	25

All 27 individuals assayed were carriers of at least one commensal *Staphylococcus* isolate in their nares, and 8 out of 27 (30%) carried two isolates, with an average of 1.37 isolates per nasal cavity (Table 2). Among men, 50% (4 out of 8) were carriers of two isolates (with an average of 1.5 isolates per sample), whereas among

women this percentage decreased (21%, 4 out of 19), with an average of 1.2 isolates per nasal swab. Only one individual was simultaneously colonized by *S. aureus* and a coagulase-negative *Staphylococcus* spp., whereas the other seven individuals carried two coagulase-negative *Staphylococcus* spp. (see below).

Table 2. Number of *Staphylococcus* isolates per individual

Number of isolates	Number of individuals (%)
1	19 (70.4)
2	8 (29.6)
1.37 (average)	27 (100)

Resistance to methicillin was detected in one (out of four) coagulase positive *S. aureus* isolates (25%), whereas 35% of the coagulase-negative isolates (11 out of 31) were methicillin resistant (Table 3).

Table 3. Methicillin (oxacillin) resistance in *Staphylococcus* isolates

Phenotype	Total number of isolates	Resistant isolates (%)
Coagulase (+) (*S. aureus*)	4	1 (25)
Coagulase (-) (Other *Staphylococcus* spp.)	31	11 (35)
	35 (Total)	12 (34)

Resistance to other chemotherapeutic agents was determined only in 20 selected isolates: all mannitol-fermenting isolates (4 coagulase positive and 6 coagulase negative isolates) and ten mannitol-negative (coagulase negative) isolates. These 20 isolates included all eight pair of isolates obtained from the same subject. Among *S. aureus*, only one isolate was found to be resistant to clarithromycin (25%), and all isolates were resistant to penicillin G (100%), whereas no resistances to the other six antibacterial agents were detected (Table 4). In coagulase-negative isolates (n= 16), resistance to penicillin G was the most frequently found (87.5%), followed by resistance to clarithromycin (50%), fosfomycin (12.5%) and tetracycline, rifampicin and ciprofloxacin (6.25%); no resistances to chloramphenicol and vancomycin were found among coagulase negative isolates (Table 4). Considering all 20 isolates tested, prevalence of resistances ranged from 90% (for penicillin G) and 45% (for clarithromycin) to 10 % (fosfomycin), 5% (tetracycline, ciprofloxacin and rifampicin) and 0% (chloramphenicol and vancomycin) (Table 4).

Table 4. Antibiotic resistances (other than methicillin) in *Staphylococcus aureus* and selected coagulase negative *Staphylococcus* isolates

Antibiotic	*S. aureus* isolates Total/resistant (%)	Coagulase (-) isolates Total/resistant (%)	All isolates Total/resistant (%)
Tetracycline	4/0 (0)	16/1 (6.25)	20/1 (5)
Rifampicin	4/0 (0)	16/1 (6.25)	20/1 (5)
Chloramphenicol	4/0 (0)	16/0 (0)	20/0 (0)
Fosfomycin	4/0 (0)	16/2 (12.5)	20/2 (10)
Clarithromycin	4/1 (25)	16/8 (50)	20/9 (45)
Ciprofloxacin	4/0 (0)	16/1 (6.25)	20/1 (5)
Vancomycin	4/0 (0)	0/16 (0)	20/0 (0)
Penicillin G	4/4 (100)	6/14 (87.5)	18/20 (90)

Resistance to more than one chemotherapeutic agent was also found among isolates, as shown in tables 5-7. In addition to penicillin G, one coagulase positive (*S. aureus*) isolate was also resistant to methicillin (oxacillin) and clarithromycin (Tables 5 and 6). Among coagulase-negative isolates, only two (out of 16) were susceptible to all agents tested; one isolate was resistant only to penicillin G, ten isolates showed resistance to two antibiotics (penicillin G and either rifampicin, clarithromycin or oxacillin), whereas one and two isolates were resistant to three and four antimicrobial agents, respectively, as detailed in tables 5 and 7. Interestingly, one of these latter isolates was resistant to methicillin. Considering all 20 isolates, multiresistance to three or four agents was found in 4 isolates (20%), whereas 10% showed no resistances, 20% showed one resistance (to penicillin G in all cases), and 50% showed resistance to two chemotherapeutic agents (Table 5).

Table 5. Multiresistances in *Stahylococcus aureus* and selected coagulase negative *Staphylococcus* isolates

Number of antibiotic resistances	Number of resistant isolates (%)		
	Coagulase (+) *n*= 4	Coagulase (-) *n*=16	Total *n*= 20
0	0	2 (12.5)	2 (10)
1	3 (75)	1 (6.25)	4 (20)
2	0	10 (62.5)	10 50)
3	1 (25)	1 (6.25)	2 (10)
4	0	2 8 (12.5)	2 (10)

Table 6. Antibiotic resistances in *S. aureus* isolates

Antibiotics	Number of resistant isolates (%)
Penicillin G	3 (75)
Penicillin G, Clarithromycin, Oxacillin	1 (25)

Table 7. Resistances in selected coagulase negative *Staphylococcus* isolates (*n*= 16)

Antibiotic	Number of resistant isolates (%)
Penicillin G	1 (6.25)
Penicillin G, Rifampicin	1 (6.25)
Penicillin G, Clarithromycin	5 (31.25)
Penicillin G, Oxacillin	4 (31.25)
Penicillin G, Clarithromycin, Fosfomycin	1 (6.25)
Penicillin G, Clarithromycin, fosfomycin, Tetracycline	1 (6.25)
Penicillin G, Clarithromycin, Ciprofloxacin, Oxacillin	1 (6.25)
None	2 (12.5)

Antibiotic resistances in *Staphylococcus* isolates colonizing the same subject are shown in table 8. It should be noted (i) that two of the subjects (numbers 7 and 8 in Table 8) were colonized by the coagulase negative *Staphylococcus* isolates multiresistant to four antimicrobial agents, (ii) that subject 8 was also colonized by a coagulase-positive (*S. aureus*) strain, and (iii) that subject 7 was colonized by a MRCoNS. In addition, another four subjects were co-carriers of methicillin (oxacillin)-resistant coagulase-negative *Staphylococcus* spp.

Table 8. Antibiotic resistances in *Staphylococcus* isolates carried by the same subject

Individual	Isolate	Antibiotic resistances
1	*S. pasteuri*	Penicillin G
	Coagulase (-) spp.	Penicillin G, Clarithromycin
2	*S. kloosii*	Penicillin G, Oxacillin
	Coagulase (-) spp.	Penicillin G, Rifampicin
3	*S. warneri*	Penicillin G, Oxacillin
	Coagulase (-) spp.	Penicillin G, Clarithromycin
4	Coagulase (-) spp.	None
	Coagulase (-) spp.	Penicillin G, Clarithromycin
5	*S. warneri*	Penicillin G, Oxacillin
	Coagulase (-) spp.	Penicillin G, Clatrithromycin, Fosfomycin
6	*S. xylosus*	Penicillin G, Oxacillin
	Coagulase (-) spp.	Penicillin G, Clarithromycin
7	*S. warneri*	Penicillin G, Clarithromycin
	Coagulase (-) spp.	Penicillin G, Oxacillin, Clarithromycin, Cipofloxacin
8	*S. aureus*	Penicillin G
	Coagulase (-) spp.	Penicillin G, Clarithromycin, Fosfomycin, Tetracycline

DISCUSSION AND CONCLUSIONS

It is well known that MRSA strains have been disseminated worldwide and are the etiologic agent of both hospital and community-acquired infections. Besides, in most cases the staphylococcal infective process is caused by host own endogenous bacterial strains (Hiramatsu et al., 2014; Lakhundi and Zhang, 2018; Otto, 2012). In addition, many non-aureus *Staphylococcus* species (CoNS and *S. intermedius*) are able

to colonize both humans and animals, and include a large proportion of methicillin resistant strains, that can be transmitted between these hosts (Barbier et al., 2010; Becker et al., 2014). Consequently, horizontal gene transfer mechanisms between species colonizing the same subject might contribute to acquisition of methicillin resistance (as well as to other antibiotics) by methicillin susceptible *S. aureus* strains. Therefore, it is epidemiologically relevant to obtain information about the prevalence of community healthy carriers of *S. aureus* and/or non-aureus *Staphylococcus* that may serve as reservoirs for infection and dissemination of antibiotic resistances. As we have previously studied the prevalence of nasal carriers of MRSA within healthy young adults in Valencia (Spain) with no healthcare-associated risks (Falomir et al., 2014; Falomir et al., 2018), in this work we have dealt with the study of the prevalence of methicillin resistant *Staphylococcus* species in community older adults in Valencia.

The prevalence of nasal colonization by *Staphylococcus* species was 100% (no nasal swabs were free of staphylococci). The prevalence of *S. aureus* carriers in older adults (15%) was not much different compared to that previously found in young adults in the same place, Valencia (22%), and in other urban community in Northern Spain (13.7%) (Falomir et al., 2014; Falomir et al., 2018; Teira et al., 2013), suggesting that aging itself does not represent a risk factor for colonization despite the small sample size in our study. Similar results have been reported in a cross-sectional epidemiological study conducted in older population in community residential care homes in Spain, as 21% subjects were *S. aureus* carriers (Galán-Sánchez et al. 2019). Odds ratios indicated no association between nasal colonization by *S. aureus* with other conditions: sex (1.31 for men, and 0.76 for women), long-term (no current) smokers (1.2), or contact with companion animals (0.94).

Interestingly, a high proportion of individuals (30%) were carriers of two *Staphylococcus* strains, particularly in the case of men (50%), as compared to women (21%). Despite the odds ratios suggest a moderate/weak association between colonization with two strains and male sex (3.75), long-term smokers (2.25) and contact with companion animals (2.25), the actual significance of these associations should be relativized due to the small sample size.

Resistance to methicillin was detected in 25% *S. aureus* isolates, which is between the percentage of MRSA isolates described in two studies with young adults in Spain (6% and 40%, respectively) and similar to the percentage of MRSA isolates (18%) found in older population in community residential care homes in Spain (Falomir et al., 2018; Galán-Sánchez et al., 2019; Teira et al., 2013). In other countries a lower MRSA percentage has been reported (1.5-3%) in isolates from young students, as well as in individuals with no healthcare-associated risk factors in European countries (0-2.1%), including Spain; however, in other studies performed in a wide range of geographic sites the reported percentage of commensal MRSA strains in healthy children and adults

varied widely (6-43%) (Gardella et al., 2011; Teira et al., 2013; van Bijnen et al., 2015; Wang et al., 2017). In Spain, a study reported a rate of 2.9% of MRSA in community-associated isolates (Vindel et al., 2014). Taken together all these data suggest that most probably the frequency of MRSA is overestimated in our study (one out of 4 isolates). However, our data on the percentage of MRCoNS seems to be more reliable (35%, 11 out of 35 isolates). The percentages of methicillin resistance obtained in our study are not much lower than those described for clinical *S. aureus* and CoNS in Spain during last years (28% and 51%, respectively) (Cercenado, 2016). These results point out that both MRSA and MRCoNS are significantly spreading in the community, despite the minor selective pressure for selection of resistance outside hospital environment.

As expected, most *Staphylococcus* isolates were resistant to the widely used penicillin G, and among the other antibiotics tested, resistance to macrolides (clarithromycin) was the most frequent in both *S. aureus* (25%) and CoNS (50%), similar to the reported resistance to macrolides (erythromycin) in clinical *Staphylococcus* isolates in Spain (Cercenado, 2016; Vindel et al., 2014). Similarly, no resistances to vancomycin were found in our study as previously reported in clinical isolates. Interestingly, a distinct of pattern of resistance to ciprofloxacin was found in our work (5% of resistant *Staphylococcus* isolates) as compared to that reported in clinical isolates (33% and 43% of resistances in *S. aureus* and CoNS, respectively) (Cercenado, 2016), and quite similar to that found in community-associated-*S. aureus* isolates (3.1%) (Vindel et al., 2014). On the other hand, a significant percentage of multiresistant isolates to three or four (out of the nine chemotherapeutic agents assayed) was detected (20%, four out of 20). Most multiresistant strains were isolated from adults carrying two nasal *Staphylococcus* strains, although isolates from the same individual (Table 8) do not share antibiotic resistances other than those most frequently found (penicillin G and clarithromycin). These results indicate that probably multiresistant strains spreading in the community, at least in part, are not related to clinical isolates (as suggested by the resistances to ciprofloxacin, mentioned above). Furthermore, our data suggest that interspecies exchange of resistance determinants by horizontal gene transfer mechanisms is probably limited in the absence of a strong selective pressure for resistance selection in the community. It should be noted that nasal microbiota is complex and highly variable, and plays a role in controlling *S. aureus* colonization (Krismer et al., 2017). Therefore, as a complex bacterial niche, nasal cavity may be considered an ecosystem where multiple genetic events intra- and interspecies may occur, such as the transfer of resistance determinants among strains, as described in gut microbiota (Baquero, 2012). In these context, studies on multiresistant nasal isolates of Staphylocccus species in young and older adults are needed to gain information about this issue. In fact, the major limitation of our study is the low number of subjects analyzed, as well as the lack of characterization of the antibiotic resistances at the molecular level. Therefore, to confirm

our hypothesis a higher number of subjects, belonging to different population subsets (within hospital and community environments) should be analyzed, as well the molecular basis accounting for the antibiotic resistances in commensal *Staphylococcus* isolates.

As a conclusion, our study shows that older adults are carriers of both *S. aureus* and CoNS, with a significant proportion of antibiotic resistant and multiresistant isolates (to methicillin and other antibiotics), and these carriers may have an impact in public health at various levels. First, they can serve as reservoirs for MRSA and MRCoNS infections in the community, particularly in immunocompromised older individuals (Show et al., 2012). This is of interest considering the empiric antibiotic treatment of these infections, and indicates that beta-lactam antibiotics may be not the first choice. Second, *S. aureus* carriers may also serve: (i) as a source of food contamination, contributing to food poisoning outbreaks, taken into account that 80% of total *S. aureus* isolates carry staphylococcal enterotoxin genes and that food handlers colonized by enterotoxin-producing *S. aureus* are regarded as the main source of food contamination causing these outbreaks (Fisher et al., 2018), (ii) to the entry of antibiotic resistant bacteria into the food chain (Argudín et al., 2010; Doulgeraki et al., 2016). A third concern is the role of MRSA and MRCoS in spreading resistances within the community, that makes necessary to implement continued surveillance not only restricted to commensal MRSA dissemination, but extended also to MRCoNS, particularly in individuals in contact with livestock animals and/or with companion animals (such as veterinarians, farm workers, etc.) in order to improve prevention and control of infections by resistant *Staphylococcus* species, mainly MRSA.

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