

Original Article

Methicillin and vancomycin resistance in coagulase-negative *Staphylococci* isolated from the nostrils of hospitalized patients

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Abstract

Introduction: Nasal colonization by coagulase-negative *Staphylococci* (CoNS) play an important role in nosocomial infections. This study aims to determine antibiotics susceptibility pattern and molecular screening of methicillin- and vancomycin-resistant nasal CoNS among hospitalized patients.

Methodology: Nasal swabs were collected from 202 inpatients at Prince Hamzah Hospital, Jordan. Swabs were processed according to standard microbiological procedures to isolate *Staphylococci*. Antibiotic susceptibility testing was performed using disk diffusion, E-test, microdilution, and Vitek 2. Molecular analysis was performed using PCR for the detection of *mecA*, *vanA*, and *vanB* genes.

Results: Nasal *Staphylococci* was isolated in 64/202 (31.7%) samples. Thirty isolates (14.8%) were CoNS, including *S. haemolyticus* (n = 17, 8.4%), *S. sciuri* (n = 6, 3%), *S. epidermidis* (n = 2, 1%), *S. warneri* (n = 2, 1%), *S. hominis* (n = 2, 1%), and *S. lentus* (n = 1, 0.5%). Twenty-two (10.9%) isolates were MR-CoNS harboring *mecA* gene. CoNS and MR-CoNS isolates were highly resistant to benzylpenicillin, erythromycin, fosfomicin, and imipenem. All isolates were sensitive to vancomycin by E-test and microdilution test and were negative for *vanA* and *vanB* genes. Nasal CoNS colonization was associated with an increased number of family members living with the participant (P = 0.04) and with admission to the orthopedic department (P = 0.03), while MR-CoNS colonization was associated with smoking (P = 0.03).

Conclusions: Nasal colonization by unusual CoNS species and *mecA*-positive MR-CoNS are common among hospitalized patients. Absence of *vanA* and *vanB* genes suggests little contribution of nasal CoNS to vancomycin resistance transmission.

Key words: methicillin; vancomycin; coagulase-negative *Staphylococcus*; *mecA*; *vanA*; *vanB*.

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Introduction

Staphylococci is a common cause of human diseases [1,2]. Traditionally, *Staphylococci* is divided into two major groups based on their ability to coagulate plasma. The coagulase-positive *Staphylococci* (CoPS) includes *Staphylococcus aureus* (*S. aureus*) [2] and the coagulase-negative *Staphylococci* (CoNS) includes over 30 species, of which *Staphylococcus epidermidis* (*S. epidermidis*) and *Staphylococcus haemolyticus* (*S. haemolyticus*) are the most important [3]. The CoNS are common commensals on the skin and mucous membranes, although some species can cause a wide variety of severe opportunistic and nosocomial infections [3]. Infections caused by Methicillin-, vancomycin-, and linezolid-resistant CoNS have been reported worldwide and represent an imminent challenge in hospitals [4-6]. The rates of methicillin and vancomycin resistance in CoNS are generally higher than in CoPS [7]. Methicillin resistance is mediated by the *mecA* gene while vancomycin resistance is mediated

by the *vanA* and, to a lesser extent, by *vanB* genes [5-8].

Nasal colonization is an important and common reservoir of CoNS associated with an increased risk of nosocomial infections [9]. Furthermore, resistance genes among CoNS are transferred horizontally to other *Staphylococci* including *S. aureus* and thus facilitate the spread of antibiotic resistance [3,8]. Multiple studies have indicated a high prevalence of nasal colonization by CoNS and methicillin-resistant CoNS (MR-CoNS) among community individuals, hospitalized patients, and health care workers [10-14]. The epidemiology and molecular characterization of MR-CoNS among hospitalized patients in the Middle East, including Jordan, has not been investigated before [15,16]. More importantly, nasal colonization with vancomycin-resistant CoNS has not been reported before [5,6]. All nasal CoNS isolates from different populations worldwide were sensitive to vancomycin by different phenotypic tests; however, molecular analysis of

vancomycin resistance genes among CoNS has not been investigated before [3,9,14,17-23]. Therefore, the aim of this study is to investigate the molecular epidemiology of nasal methicillin- and vancomycin-resistant CoNS among hospitalized patients.

Methodology

Study participants

In this cross sectional study, 202 inpatients at Prince Hamzah Hospital, Amman, Jordan, were recruited voluntarily from January 2016 to August 2017 to obtain nasal swabs. Each participant signed a consent form and had demographic data, clinical information, and data about potential risk factors for nasal *Staphylococci* colonization collected [24]. This study was approved by the Institutional Review Board (IRB) of the Hashemite University and Prince Hamzah Hospital.

Microbiological identifications

Nasal swabs were cultured overnight on nutrient agar (Mast, Bootle, UK), sheep blood agar (Mast, Bootle, UK), and mannitol salt agar (Biolab, Budapest, Hungary) at 37°C. Positive growth was observed for colony morphology, Gram-stain (GCC diagnostics, Sandycroft, UK), and biochemical tests. Coagulase-positive versus coagulase-negative *Staphylococci* were assessed using a slide and tube coagulase test with rabbit plasma [24,25]. Species identification was carried out using the automatic Vitek 2 compact system for Gram-positive identification (GP card, REF 21342) according to manufacturer instructions. Isolates were stored at -80°C for further testing of antibiotic susceptibility and molecular analysis. The following strains were used as quality controls: ATCC® 33591 (MRSA), ATCC 12228 (*Staphylococcus epidermidis*), ATCC® 700221 (*vanA*-positive *Enterococcus faecium*), ATCC® 51299 (*vanB*-positive *Enterococcus faecalis*) [24-26].

Antibiotics susceptibility tests

Antibiotic susceptibility testing was performed using the standard disk diffusion method using oxacillin 1 µg and cefoxitin 30 µg (Bioanalyse, Ankara, Turkey) according to CLSI guidelines [26]. Vancomycin minimum inhibitory concentration (MIC) was determined using the E-test (BioMérieux, Marcy l'Etoile, France) and the broth microdilution method [26]. Furthermore, antibiotic susceptibility pattern was determined using a Gram-positive susceptibility card (AST P592, REF 22287) analyzed by the Vitek 2 compact system according to the manufacturer's recommendation (BioMérieux, Marcy l'Etoile, France).

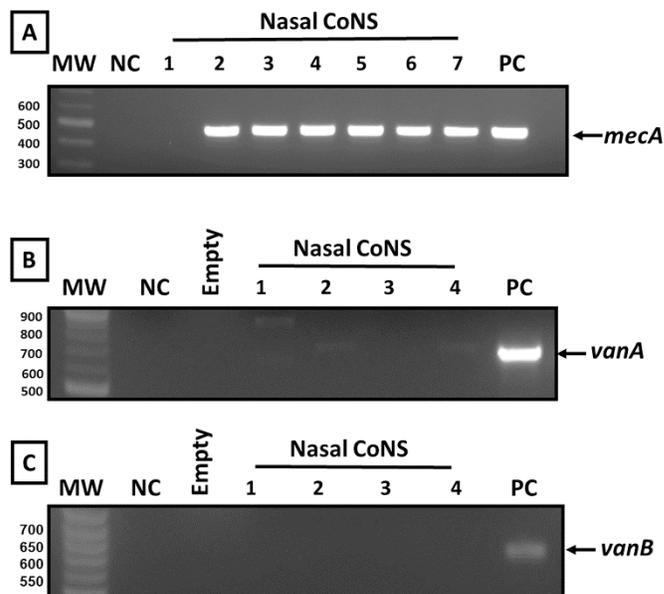
Molecular characterization

Bacterial DNA was extracted from culture samples using a DNA extraction kit (Norgen Biotek Corp, Ontario, Canada) followed by PCR reaction using specific primers for *mecA*, *vanA* and *vanB* as previously described [24,25]. PCR products were electrophoresed in a 1.5% agarose gel stained with ethidium bromide and visualized using UV transillumination (Figure 1) [24,25]. Positive and negative control strains were included in each run.

Statistical analysis

Statistical analysis was performed using SPSS version 20. Association between nasal CoNS colonization and risk factors was assessed using the Chi-square test by measuring differences in frequencies and it was reported as odds ratio (OR), 95% confidence intervals (95% CI), and P values. P values < 0.05 were considered statistically significant.

Figure 1. Analysis of PCR products by gel electrophoresis stained with ethidium bromide for *mecA*, *vanA*, and *vanB* genes in nasal CoNS samples.



(A) Showing a band at around 500 base pairs (bp) for *mecA* gene. Nasal CoNS sample 1 was negative for *mecA* while samples 2-7 were positive for *mecA*, PC: positive control (*mecA*-positive ATCC® 33591). (B) Showing a band at around 732 bp for *vanA* gene. Nasal CoNS samples 1-4 were negative for *vanA*, PC: positive control (*vanA*-positive *Enterococcus faecium* ATCC® 700221). (C) Showing a band at around 635 bp for *vanB* gene. Nasal CoNS samples 1-4 were negative for *vanB*, PC: positive control (*vanB*-positive *Enterococcus faecalis* ATCC® 51299). MW: molecular weight, NC: negative control, Empty: empty control.

Table 1. Distribution of nasal coagulase-negative Staphylococcal isolates of participants.

Nasal CoNS n = 30					
<i>S. hominis</i>	<i>S. epidermidis</i>	<i>S. lentus</i>	<i>S. warneri</i>	<i>S. sciuri</i>	<i>S. haemolyticus</i>
2	2	1	2	6	17

Results

Nasal colonization by CoNS

Study participants were recruited from different hospital departments over one and a half years. The mean age of participants was 50.17 ± 18.18 years and 59.4% were females. Sixty-four of 202 nasal swab samples tested positive for *Staphylococci* (31.7%). Thirty out of 202 were CoNS (14.8%). *S. haemolyticus* was the most common isolate (n = 17, 8.4%), followed by *S. sciuri* (n = 6, 3%), *S. epidermidis* (n = 2, 1%), *S. warneri* (n = 2, 1%), *S. hominis* (n = 2, 1%), and *S. lentus* (n = 1, 0.5%) (Table 1). Methicillin screening with an oxacillin disk diffusion test identified 24 isolates as resistant and 1 isolate as intermediately resistant, while the cefoxitin disk diffusion test identified 19 samples as resistant and 3 samples as intermediately resistant. Analysis with the Vitek 2 susceptibility card identified 25 samples as methicillin resistance. Screening of all isolates for the presence of the *mecA* gene identified 22 (10.9%) isolates as *mecA*-positive (Figure 1 and Table 2). Accordingly, 73.3% of nasal CoNS isolates were methicillin-resistant harboring the *mecA* gene. All isolates were sensitive to vancomycin by E-test and microdilution test, while one isolate was resistant to vancomycin by Vitek 2 (MIC > 32 µg/mL). *VanA* and *vanB* were not detectable in this isolate. Furthermore, screening of all CoNS isolates was negative for both *vanA* and *vanB* (Figure 1 and Table 3).

Nasal CoNS correlation with potential risk factors

There was a positive association between nasal colonization with CoNS and increased number of family members living with the participant (P = 0.04) (Table 4). Colonization with CoNS was significantly higher in the orthopedic department (P = 0.03) (Table 4). Association between CoNS and other potential risk factors was negative (Table 4). Furthermore, there was

a significant association between nasal colonization with MR-CoNS and smoking (P = 0.03). Association between MR-CoNS nasal colonization and other potential risk factors was negative (data not shown).

Nasal CoNS antibiotics susceptibility pattern

The antibiotic susceptibility pattern of nasal CoNS isolates indicated high resistance rates for benzylpenicillin (91.7%), erythromycin (91.7%), fosfomycin (79.2%), and imipenem (62.5%) (Figure 2). Moderate resistance was observed for clindamycin (50.0%) (Figure 2). A low resistance rate was observed for ciprofloxacin (37.5%), fusidic acid (29.2%), trimethoprim/sulfamethoxazole (29.2%), gentamycin (25%), tetracycline (16.7%), rifampicin (8.3%), levofloxacin (8.3%), and tobramycin (4.2%) (Figure 2). The susceptibility profile of each nasal CoNS isolate using disk diffusion test, Vitek 2 system, and vancomycin MIC by microdilution and E-test (Table 5) indicated discrepancies in phenotypic testing of some isolates. The antibiotic susceptibility pattern of MR-CoNS indicated higher resistance rates to fosfomycin 90%, benzylpenicillin 95.2%, imipenem 66.6%, ciprofloxacin 45.9%, fusidic acid 33.3%, and

Figure 2. Antibiotic susceptibility pattern of nasal CoNS (n = 24).

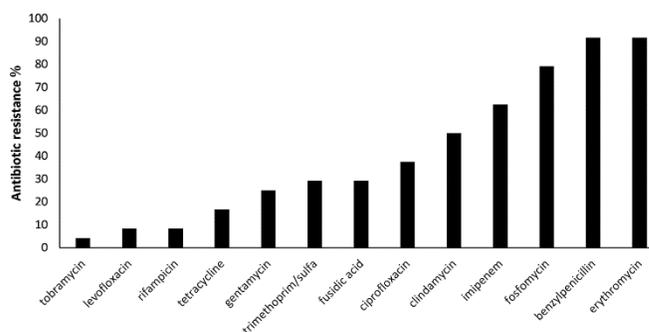


Table 2. Phenotypic and molecular screening of methicillin resistance among nasal CoNS (n = 30).

Phenotypic screening						Molecular screening			
Oxacillin disk			Cefoxitin disk			Vitek 2		<i>mecA</i>	
R	I	S	R	I	S	Positive	Negative	Positive	Negative
24	1	5	19	3	8	25	5	22	8

Phenotypic screening was performed using disk diffusion test with oxacillin and cefoxitin and automatic Vitek 2 analysis. Molecular screening was performed using PCR analysis of *mecA* gene. R: resistant, I: intermediate, S: sensitive.

Table 3. Phenotypic and molecular screening of vancomycin resistance among nasal CoNS (n = 30).

Phenotypic screening						Molecular screening					
Vancomycin E-test			Vancomycin micro dilution			Vitek 2		vanA gene		vanB gene	
R	I	S	R	I	S	Positive	Negative	Positive	Negative	Positive	Negative
0	0	30	0	0	30	1	29	0	30	0	30

Phenotypic screening was performed using vancomycin E-test, micro dilution test, and automatic Vitek 2 analysis. Molecular screening was performed using PCR analysis of *vanA* and *vanB* genes. R: resistant, I: intermediate, S: sensitive.

gentamycin 28.6% and a similar resistance pattern for erythromycin 90.5%, clindamycin 47.6%, and trimethoprim/sulfamethoxazole 28.6% (data not shown).

Discussion

CoNS have increasingly been recognized as a cause of clinically significant nosocomial infections including bacteremia, endocarditis, foreign body-related infections and infections in preterm newborns [3].

Table 4. Risk factors for CoNS nasal colonization.

		Number	Nasal CoNS n = 30	
			Chi-square test	
			OR (95% CI)	P value
Age (years)	< 31	3		0.82
	31 – 50	14		
	51 – 70	10		
	> 71	3		
Gender	Male	11	0.8 (0.3 – 1.8)	0.69
	Female	19		
Department	Medicine	5		0.03
	Surgery	6		
	Orthopedic	10		
	Gynecology	9		
Number of family members living with participant	≤ 3	6		0.04
	4-6	12		
	> 6	12		
Smoking	Yes	14	1.4 (0.6 - 3.2)	0.41
	No	16		
History of Smoking	Yes	1	0.8 (0.1 – 6.8)	1.00
	No	29		
Previous hospitalization	Yes	21	1.1 (0.4 – 2.6)	0.83
	No	9		
Hospitalization period	0 days	1		0.73
	1 – 5 days	9		
	6 - 10 days	3		
	> 10 days	4		
Previous surgeries	Yes	16	0.7 (0.3 – 1.7)	0.55
	No	14		
Chronic diseases	Yes	10	0.5 (0.2 – 1.1)	0.11
	No	20		
Skin infections	Yes	2	0.9 (0.2 – 4.4)	1.0
	No	28		
Medications	Yes	15	0.7 (0.3 – 1.5)	0.42
	No	15		
Antibiotic use in the past 2 weeks	Yes	13	0.7 (0.3 - 1.7)	0.56
	No	17		
URTI symptoms	Yes	2	0.7 (0.1 – 3.4)	1.0
	No	28		
Fever within last 48 hours	Yes	4	0.9 (0.3 – 3.1)	0.12
	No	26		

Association between potential risk factors and CoNS nasal colonization among study participants (n = 30). OR: Odds Ratio, 95% CI: 95% Confidence Intervals.

Treatment of CoNS-related infections is challenging because there is a higher chance of being a methicillin-resistant strain or having a reduced susceptibility to glycopeptides [6]. Compared to MRSA (methicillin-resistant *Staphylococcus aureus*), much less is known about the epidemiology of CoNS in health care facilities [3]. Multiple studies have shown the high prevalence of methicillin resistance among nasal CoNS [10-14], identified its role in the dissemination of nosocomial infections [9], and proposed nasal CoNS as an important reservoir for genes mediating methicillin resistance [8]. The prevalence of vancomycin resistance genes among nasal CoNS and its role in the dissemination of vancomycin resistance have not been investigated.

A lower rate of CoNS nasal colonization was observed in this study compared to other studies [18,20,21,23]. No similar studies among hospitalized patients were conducted in the Middle East. *S. haemolyticus* was the most common CoNS isolate in this study followed by *S. sciuri*. A recent comprehensive review, as well as many other studies, indicated *S. epidermidis* was the most common CoNS nasal isolate [3,9,17,20,27]. *S. haemolyticus* was considered an unusual isolate of nasal CoNS among health care workers [16]. Similarly, *S. sciuri* is less common and has been rarely isolated from the nasal cavity [28]. In agreement with this study, one study showed higher colonization with *S. haemolyticus* compared to *S. epidermidis* in hospitalized patients

Table 5. Antibiotic susceptibility profile of each Nasal CoNS isolate.

No	CoNS species	Disk diffusion		Vitek		Antibiotics susceptibility															Vancomycin MIC (µg/mL)	
		Oxacillin	Cefoxitin	Vancomycin	Oxacillin	Vancomycin	Benzylpenicillin	Erythromycin	Clindamycin	Fosfomycin	Imipenem	Ciprofloxacin	Tetracycline	Fusidic acid	Gentamycin	Trimetho/sulfa	Rifampicin	Levofloxacin	Tobramycin	Teicoplanin		
1	<i>S. haemolyticus</i>	S	R	S	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	≤ 0.5
2	<i>S. haemolyticus</i>	R	R	S	R	S	R	R	S	R	R	R	R	R	S	S	S	S	S	S	S	≤ 0.5
3	<i>S. haemolyticus</i>	R	R	S	R	S	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	≤ 0.5
4	<i>S. haemolyticus</i>	R	R	S	R	S	R	R	R	R	R	R	S	S	R	S	S	S	S	S	S	1
5	<i>S. haemolyticus</i>	R	R	S	R	S	R	R	R	R	R	R	S	R	S	R	S	S	S	S	S	1
6	<i>S. sciuri</i>	R	S	S	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	≤ 0.5
7	<i>S. sciuri</i>	R	S	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	≤ 0.5
8	<i>S. sciuri</i>	R	S	S	R	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	≤ 0.5
9	<i>S. haemolyticus</i>	R	R	S	R	S	R	R	R	R	R	R	S	S	R	S	R	S	S	S	S	1
10	<i>S. epidermidis</i>	R	R	S	R	S	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	≤ 0.5
11	<i>S. haemolyticus</i>	R	R	S	R	S	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	≤ 0.5
12	<i>S. lentus</i>	S	S	S	S	S	S	R	R	R	S	S	R	S	S	R	S	S	S	S	S	≤ 0.5
13	<i>S. haemolyticus</i>	R	R	S	R	S	R	R	R	R	R	R	S	R	R	R	S	S	S	S	S	1
14	<i>S. hominis</i>	R	I	S	R	S	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	≤ 0.5
15	<i>S. sciuri</i>	R	S	S	S	S	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	≤ 0.5
16	<i>S. warneri</i>	S	I	S	R	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	≤ 0.5
17	<i>S. warneri</i>	R	R	S	R	S	R	R	S	R	R	S	S	S	S	S	S	S	S	S	S	≤ 0.5
18	<i>S. haemolyticus</i>	R	R	S	R	S	R	R	S	R	R	S	R	S	S	S	S	S	S	S	S	≤ 0.5
19	<i>S. hominis</i>	R	R	S	R	R	R	R	R	S	S	S	S	R	S	R	R	S	S	R	S	≤ 0.5
20	<i>S. haemolyticus</i>	R	R	S	R	S	R	R	R	R	S	S	S	R	S	R	S	R	S	S	S	1
21	<i>S. haemolyticus</i>	R	R	S	R	S	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	≤ 0.5
22	<i>S. haemolyticus</i>	R	R	S	R	S	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	≤ 0.5
23	<i>S. haemolyticus</i>	R	R	S	S	S	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	≤ 0.5
24	<i>S. haemolyticus</i>	R	R	S	R	S	R	R	R	R	S	S	S	S	R	R	R	R	R	S	S	1
25	<i>S. haemolyticus</i>	R	S	S	R	S	R	R	S	R	R	R	S	R	R	R	S	S	S	S	S	2
26	<i>S. epidermidis</i>	S	S	S	S	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	1
27	<i>S. haemolyticus</i>	S	I	S	R	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	≤ 0.5
28	<i>S. sciuri</i>	R	S	S	S	S	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	≤ 0.5
29	<i>S. sciuri</i>	R	R	S	R	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	≤ 0.5
30	<i>S. haemolyticus</i>	I	R	S	R	S	R	R	S	R	R	R	S	R	R	R	S	S	S	S	S	≤ 0.5

Antibiotics susceptibility profile of each nasal CoNS isolate (n = 30) using disk diffusion method, Vitek 2, and MIC by micro dilution and E-test. R: resistant, I: intermediate, S: sensitive, na: not available.

compared to community controls [19], and one study showed higher colonization with *S. sciuri* in admitted patients [28]. This suggests the possibility that hospitalization might increase the prevalence of unusual CoNS isolates including *S. haemolyticus* and *S. sciuri*. Reduced susceptibility to vancomycin is more common in *S. haemolyticus* compared to *S. epidermidis* [4].

In this study, nasal MR-CoNS accounted for 73.3% of total isolates, which is higher than most other studies that reported nasal MR-CoNS at 9% [23], 25% [18], 58.1% [29], and 62.8% [21]. This is mostly due to the higher frequency of *S. haemolyticus* and *S. sciuri* observed in this study. A higher prevalence of methicillin resistance among *S. haemolyticus* and *S. sciuri* can be noted compared to *S. epidermidis* [3,28].

The *mecA* gene is commonly reported from the nasal isolates of non-hospitalized patients and from clinic isolates [3,8]. The *mecA* gene was detected in 22/30 (73.3%) CoNS nasal isolates, indicating *mecA* as the primary gene responsible for colonization with methicillin resistance in hospitalized patients, which could contribute to the dissemination of methicillin resistance in a hospital setting. Most studies did not investigate the occurrence of the *mecA* gene in nasal MR-CoNS among hospitalized patients [17-19, 21,23,27]. Other studies focused on *mecA* polymorphism [20], SCCmec typing [9], and spa typing [29].

In the current study, nasal colonization with CoNS was significantly higher in orthopedic department patients in agreement with another study which reported 73% colonization with CoNS in an orthopedics department [18]. MR-CoNS colonization rate was ward-specific at 33.8% in orthopedic surgery patients [29]. Here we report for the first time that nasal CoNS colonization was associated with increased number of family members living with the participant, while MR-CoNS colonization was associated with smoking. Other studies on patients' nasal colonization with CoNS have not investigated the role of risk factors [9,17-21,23]. Smoking was identified as an independent risk factor for MRSA colonization [30]. Cigarette smoking enhances immune and antibiotic resistance of *Staphylococci* [31], and electronic cigarettes decrease the host immune response, promote inflammation, and increase the virulence of *S. aureus* colonizing bacteria [32]. MRSA colonization was associated with an increased number of children in the family and crowded conditions [33]. Some factors enhancing *S. aureus* colonization, including smoking and increased family member number, could also enhance colonization with

CoNS. In this study, nasal CoNS and MR-CoNS did not correlate with age, gender, previous hospitalization, previous surgery, chronic illness, and antibiotic use in agreement with other studies in school students [14], health care workers [15], and remote communities [22]. Nasal colonization correlated with lower birth weight, parenteral nutrition, previous hospitalization, and a younger age at admission in admitted neonates. Also, MR-CoNS correlated with the empiric use of antibiotics [34].

The results of the Vitek 2 automatic system in the detection of methicillin and vancomycin resistance in CoNS were sometimes misleading in comparison to molecular detection of *mecA*, *vanA*, and *vanB* genes. Similar observations were reported by other studies [35,36]. Manual vancomycin MIC determination by microdilution or an agar dilution test is currently the reference method [26,37], while molecular analysis remains the gold standard [5-8]. Accordingly, all isolates were considered sensitive to vancomycin based on MIC and genotyping results despite Vitek 2 results which indicated one isolate was resistant. The antibiotic resistance pattern of CoNS and MR-CoNS showed slightly higher resistance rates to most antibiotics including benzylpenicillin, erythromycin, fosfomicin, ciprofloxacin, fusidic acid, gentamycin, and imipenem compared to other studies [9,19,23,27]. Most MR-CoNS isolates (76.2%) were multi-drug resistant (resistance to 3 antibiotics of 3 different classes).

Methicillin- and vancomycin-resistant CoNS were isolated from different clinical infections, and the rate is generally higher than CoPS-related infections [5-7]. Similarly, nasal colonization by MR-CoNS is common and proposed to be the source of methicillin resistance genes in CoPS [3,8]. Few studies have documented nasal isolation of hetero-resistant (h-VISA) *S. aureus* [38] and vancomycin-intermediate *S. aureus* (VISA) [39,40], while nasal CoNS with decreased susceptibility to vancomycin was not reported before [5,6]. In this study, we systematically screened all nasal CoNS isolates for vancomycin resistance using multiple phenotypic methods and molecular detection of *vanA* and *vanB*. All isolates were negative for vancomycin resistance using MIC and molecular testing which would suggest a poor contribution of nasal CoNS in vertical dissemination of vancomycin resistance to other species including *S. aureus*. Similarly, other studies indicated that all nasal CoNS isolates were sensitive to vancomycin by different phenotypic tests without molecular screening of vancomycin resistance genes, mostly due to the absence of vancomycin-resistant isolates [3,9,14,17-23]. Currently, the

mechanism of vancomycin resistance in *Staphylococci* is not fully understood. Vancomycin-resistant *S. aureus* (VRSA) strains carry transposon Tn1546, acquired from vancomycin-resistant *Enterococcus faecalis*, but the resistance mechanisms in VISA isolates and in CoNS are less well defined [4].

Conclusions

Nasal colonization by CoNS among hospitalized patients was generally low and predominated by unusual isolates including *S. haemolyticus* and *S. sciuri*. Methicillin and other antibiotic resistance was very high among these isolates. *MecA* was detected in 73.3% of CoNS nasal isolates which could contribute to the dissemination of methicillin-resistant infections among hospitalized patients. All nasal CoNS isolates were sensitive to vancomycin by multiple susceptibility tests except one isolate which was resistant to vancomycin by Vitek 2. Molecular analysis confirmed that all isolates were negative for known vancomycin resistance genes, including the *vanA* and *vanB* genes. These findings will decrease the possibility that nasal CoNS might contribute to the dissemination of vancomycin resistance and suggests other mechanisms might apply. Increased numbers of family members living with individuals and admission to the orthopedic department increased the risk for nasal CoNS colonization, while smoking increased the risk for MR-CoNS nasal colonization.

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References

- Tang YW, Stratton CW (2010) *Staphylococcus aureus*: An old pathogen with new weapons. Clin Lab Med 30: 179-208.
- Savini V, Passeri C, Mancini G, Iuliani O, Marrollo R, Argentieri AV, Fazii P, D'Antonio D, Carretto E (2013) Coagulase-positive staphylococci: my pet's two faces. Res Microbiol 164: 371-374.
- Becker K, Heilmann C, Peters G (2014) Coagulase-negative staphylococci. Clin Microbiol Rev 27: 870-926.
- Srinivasan A, Dick JD, Perl TM (2002) Vancomycin resistance in staphylococci. Clin Microbiol Rev. 15: 430-438.
- Cercenado E (2010) Update of antimicrobial resistance in Gram-positive microorganisms. Med Clin 135 Suppl 3: 10-15.
- Biavasco F, Vignaroli C, Varaldo PE (2000) Glycopeptide resistance in coagulase-negative staphylococci. Eur J Clin Microbiol Infect Dis 19: 403-417.
- Morgenstern M, Erichsen C, Hackl S, Mily J, Militz M, Friederichs J, Hungerer S, Bühren V, Moriarty TF, Post V, Richards RG, Kates SL (2016) Antibiotic resistance of commensal *Staphylococcus aureus* and coagulase-negative staphylococci in an international cohort of surgeons: A prospective point-prevalence study. PLoS One 11: e0148437.
- Otto M (2013) Coagulase-negative staphylococci as reservoirs of genes facilitating MRSA infection: staphylococcal commensal species such as *Staphylococcus epidermidis* are being recognized as important sources of genes promoting MRSA colonization and virulence. Bioessays 35: 4-11.
- Faria NA, Conceição T, Miragaia M, Bartels MD, de Lencastre H, Westh H (2014) Nasal carriage of methicillin resistant staphylococci. Microb Drug Resist 20: 108-117.
- Baragundi MC, Solabannavar SS, Gokale SK, Sonth SB (2012) Methicillin and multidrug resistant coagulase negative staphylococcal nasal carriage in medical students. J Commun Dis 44: 231-237.
- Ternes YM, Lamaro-Cardoso J, André MC, Pessoa VP Jr, Vieira MA, Minamisava R, Andrade AL, Kipnis A (2013) Molecular epidemiology of coagulase-negative *Staphylococcus* carriage in neonates admitted to an intensive care unit in Brazil. BMC Infect Dis 13: 572.
- Drago L, Cappelletti L, Lamartina C, Berjano P, Mattina R, De Vecchi E (2015) Colonization by methicillin resistant staphylococci of nares and skin in healthcare workers: a pilot study in spinal surgeries. Injury 46 Suppl 8: 77-80.
- Murugesan S, Perumal N, Mahalingam SP, Dilliappan SK, Krishnan P (2015) Analysis of antibiotic resistance genes and its associated SCCmec types among nasal carriage of methicillin resistant coagulase negative staphylococci from community settings, Chennai, Southern India. J Clin Diagn Res 9: DC01-5.
- Iravani Mohammad Abadi M, Moniri R, Khorshidi A, Piroozmand A, Mousavi SG, Dastehgoli K, Mirzaei Ghazikalayeh H (2015) Molecular characteristics of nasal carriage methicillin-resistant coagulase negative staphylococci in school students. Jundishapur J Microbiol 8: e18591.
- Al-Bakri AG, Al-Hadithi H, Kasabri V, Othman G, Kriegeskorte A, Becker K (2013) The epidemiology and molecular characterization of methicillin-resistant staphylococci sampled from a healthy Jordanian population. Epidemiol Infect 141: 2384-2391.
- Salimi E, Pakbaz Z, Pourmand MR, Avakh Majelan P, Dehbashi S (2016) Nasal carriage of uncommon coagulase-negative staphylococci in nurses and physicians of Tehran university hospitals. Acta Med Iran 54: 330-333.
- Hamilton-Miller JM, Iliffe A (1984) Characterization of coagulase-negative staphylococci from patients with a malignancy compared with those from other sources. Zentralbl Bakteriol Mikrobiol Hyg A 258: 368-373.
- Sanzén L, Walder M (1988) Antibiotic resistance of coagulase-negative staphylococci in an orthopaedic department. J Hosp Infect 12: 103-108.
- Thurn JR, Crossley KB, Gerdtz A, Baken L (1992) Dynamics of coagulase-negative staphylococcal colonization in patients and employees in a surgical intensive care unit. J Hosp Infect 20: 247-255.

20. Aires De Sousa M, Santos Sanches I, Ferro ML, De Lencastre H (2000) Epidemiological study of staphylococcal colonization and cross-infection in two West African Hospitals. *Microb Drug Resist* 6: 133-141.
21. Altöparlak U, Kadanali A, Celebi S (2004) Slime factor positivity in coagulase negative staphylococci isolated from nasal samples of haemodialysis patients. *Int J Clin Pract* 58: 1112-1114.
22. Lebeaux D, Barbier F, Angebault C, Benmahdi L, Ruppé E, Felix B, Gaillard K, Djossou F, Epelboin L, Dupont C, Renard M, Peroz G, Vandenesch F, Wolff M, Andremont A, Ruimy R (2012) Evolution of nasal carriage of methicillin-resistant coagulase-negative staphylococci in a remote population. *Antimicrob Agents Chemother* 56: 315-323.
23. Stefánsdóttir A, Johansson A, Lidgren L, Wagner P, W-Dahl A (2013) Bacterial colonization and resistance patterns in 133 patients undergoing a primary hip- or knee replacement in Southern Sweden. *Acta Orthop* 84: 87-91.
24. Al-Tamimi M, Himsawi N, Abu-Raideh J, Abu jazar D, Al-jawaldeh H, Al Haj Mahmoud S, Hijjawi N, Hawamdeh H (2018) Nasal colonization by methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* among medical students. *J Infect Dev Ctries* 12: 326-335 doi: 10.3855/jidc.9908.
25. Al-Tamimi M, Himsawi N, Abu-Raideh J, Abu jazar D, Al-jawaldeh H (2018) Isolation of fully vancomycin-resistant *Streptococcus thoraltensis* from the nasal cavity of a healthy young adult. *Microb Drug Resist* 25: 421-426, <https://doi.org/10.1089/mdr.2018.0092>.
26. Clinical and Laboratory Standards Institute (CLSI) (2018) Performance standards for antimicrobial susceptibility testing, 28th informational supplement. CLSI document M100-S28 (ISBN 1-56238-839-8).
27. Crossley KB, Ross J (1985) Colonization of hospitalized patients by *Staphylococcus aureus*, *Staphylococcus epidermidis* and enterococci. *J Hosp Infect* 6: 179-186.
28. Cirkovic I, Trajkovic J, Hauschild T, Andersen PS, Shittu A, Larsen AR (2017) Nasal and pharyngeal carriage of methicillin-resistant *Staphylococcus sciuri* among hospitalised patients and healthcare workers in a Serbian university hospital. *PLoS One* 12: e0185181.
29. Trouillet-Assant S, Rasigade JP, Lustig S, Lhoste Y, Valour F, Guerin C, Aubrun F, Tigaud S, Laurent F (2013) Ward-specific rates of nasal cocolonization with methicillin-susceptible and -resistant *Staphylococcus* spp. and potential impact on molecular methicillin-resistant *Staphylococcus aureus* screening tests. *J Clin Microbiol* 51: 2418-2420.
30. Casey JA, Cosgrove SE, Stewart WF, Pollak J, Schwartz BS (2013) A population-based study of the epidemiology and clinical features of methicillin-resistant *Staphylococcus aureus* infection in Pennsylvania, 2001-2010. *Epidemiol Infect* 141: 1166-1179.
31. McEachern EK, Hwang JH, Sladewski KM, Nicatia S, Dewitz C, Mathew DP, Nizet V, Crotty Alexander LE (2015) Analysis of the effects of cigarette smoke on staphylococcal virulence phenotypes. *Infect Immun* 83: 2443-2452.
32. Hwang JH, Lyes M, Sladewski K, Enany S, McEachern E, Mathew DP, Das S, Moshensky A, Bapat S, Pride DT, Ongkeko WM, Crotty Alexander LE (2016) Electronic cigarette inhalation alters innate immunity and airway cytokines while increasing the virulence of colonizing bacteria. *J Mol Med* 94: 667-679
33. Chen CJ, Hsu KH, Lin TY, Hwang KP, Chen PY, Huang YC (2011) Factors associated with nasal colonization of methicillin-resistant *Staphylococcus aureus* among healthy children in Taiwan. *J Clin Microbiol* 49: 131-137.
34. Campeotto F, Garnier F, Kalach N, Soulaines P, Dupont C, Raymond J (2004) A routine prospective survey process to detect nosocomial bacterial colonization in a neonatal unit: risk factors for acquisition. *Arch Pediatr* 11: 1314-1318.
35. Raney PM, Williams PP, McGowan JE, Tenover FC (2002) Validation of Vitek version 7.01 software for testing staphylococci against vancomycin. *Diagn Microbiol Infect Dis* 43: 135-140.
36. John MA, Burden J, Stuart JI, Reyes RC, Lannigan R, Milburn S, Diagre D, Wilson B, Hussain Z (2009) Comparison of three phenotypic techniques for detection of methicillin resistance in *Staphylococcus* spp. reveals a species-dependent performance. *J Antimicrob Chemother* 63: 493-496.
37. European Committee on Antimicrobial Susceptibility Testing (2018) Version 8.1. Breakpoint tables for interpretation of MICs and zone diameters. Available: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.1_Breakpoint_Tables.pdf Accessed: 20 June 2018.
38. Varona-Barquín A, Iglesias-Losada JJ, Ezpeleta G, Eraso E, Quindós G (2017) Vancomycin heteroresistant community associated methicillin-resistant *Staphylococcus aureus* ST72-SCCmecIVa strain colonizing the nostrils of a five-year-old Spanish girl. *Enferm Infecc Microbiol Clin* 35: 148-152.
39. Banerjee T, Anupurba S (2012) Colonization with vancomycin-intermediate *Staphylococcus aureus* strains containing the vanA resistance gene in a tertiary-care center in north India. *J Clin Microbiol* 50: 1730-1732.
40. Carvalho SP, Almeida JB, Andrade YMFS, Silva LSCD, Oliveira AC, Nascimento FS, Campos GB, Oliveira MV, Timenetsky J, Marques LM (2017) Community-acquired methicillin-resistant *Staphylococcus aureus* carrying SCCmec type IV and V isolated from healthy children attending public daycares in northeastern Brazil. *Braz J Infect Dis* 21: 464-467.

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