

Staphylococcus sp. colonization in workers from areas of hospital support: epidemiological and microbiological aspects

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ABSTRACT

The objectives were to determine the prevalence of workers colonized by *Staphylococcus* sp., to identify colonization predictors and, to assess their susceptibility to antimicrobials. A questionnaire was applied and saliva was collected in 130 workers from support areas of an oncology institution. Microbiological analysis was conducted following standard procedures. The prevalence of workers colonized by *Staphylococcus* sp. was 37.7%, and the isolated majority was *Staphylococcus coagulase-negative*. Resistance to methicillin was detected in 35.1% of *Staphylococcus coagulase-negative*, being 12 *epidermidis* and one *S. haemolyticus*. From those. 92.3% had the *mecA*. gene. All *S. aureus* were sensitive to methicillin. Working shift and sector were identified as predictors for colonization. The colonization in workers from support areas assisting oncology patients indicates that workers should be target of occupational health policies, and it should include pre- and post-colonization measures in investigations/discussions of future studies aiming at worker and patient safety.

Descriptors: Staphylococcus; Methicillin Resistance; Hospital Services; Occupational Health; Nursing Care.

INTRODUCTION

Health workers are exposed to many occupational risks because of their activities in health services. In special, biological risk due to

attention to patients with many infectious pathologies that requires care, including innumerable procedures risking the worker's health, as well as the contact with circulating microbiota in this environment, increasingly more virulent and resistant to antimicrobials⁽¹⁾.

This scenario explains higher vulnerability of these workers to colonization by pathogenic microorganisms, as the bacteria from the *Staphylococcus* sp. genus, which are clinically and epidemiologically relevant in the context of Infections Related to Health Assistance (IRHA). In the literature, it was found the subsequent colonization and infection of health workers by these microorganisms, as well as its transmission from the colonized worker to patients and community contacts; and most studies about this theme addressed workers directly assisting patient⁽²⁻³⁾.

Despite of the indirect contact with patients, workers from support areas (WSA), as the ones from the Center of Material and Sterilization (CMS), Sectors of Clothing Reprocessing (SCR), of Hygiene and Cleaning (SHC) and Nutrition and Diet (SND), they are exposed to many risks, including of colonization by *Staphylococcus* sp.. It occurs due to their working routine when they handle utensils used by patients, and there is contact with the microbiota of health services environments. On the other hand, once colonized, these workers represent risk to patients, because of the direct contact with utensils that can pass microorganisms to patients.

It is important to highlight the WSA from specialized health services for cancer patients, because of the impairment of these patients' immunological system, one of the fundamental factors to develop infection, besides innumerable hospitalizations, diagnostic and therapeutic procedures that prolong their permanence in hospital environments⁽⁴⁾.

Although the anterior nostril is the chosen location for *Staphylococcus* sp. colonization screening, the oral cavity represents an important reservoir when we consider the easy and constant dispersion of droplets produced when speaking, coughing, sneezing and, when touching hands in this cavity. As consequence, the asymptomatic carrier represents an important source of microorganisms transference to susceptible individuals⁽⁵⁾.

In this context and, considering the lack of studies addressing the status of WSA colonization⁽²⁻³⁾, who has the fundamental role in the flux and in the quality of health assistance; this study aimed to determine the prevalence of WSA colonized by *Staphylococcus* sp.; to assess the profile susceptible to antimicrobials from microorganisms isolated in the oral cavity and, to identify the predictors for the colonization of these workers.

METHODS

A descriptive cross-sectional study conducted in a large health institution in the Center-West region of Brazil, that is reference for cancer treatment and, it is integrated to the Unified Healthcare System (SUS).

Those WSA who worked at CMS, SCR, SHC and SND when data collection occurred, participated in the study. We excluded professionals who were using antimicrobial or who used until seven days prior to data

collection, those who were on vacation or on sick leave. Thus, the study population was composed by 130 WSA, corresponding to 89.6% (130/145) of total workers.

The data collection was from May 2009 to November 2010, during three work shifts (morning, afternoon and night), according with the routine of investigated sectors. The data collection was conducted individually through the application of a questionnaire addressing sociodemographic (gender, age, education) and work related characteristics (working sector and shift, time of service in the institution, weekly working hours, to have or not another employment). After, we conducted the collection of specimens (saliva).

A saliva sample without gustatory stimulation (0.7 to 1.0 mL) was collected from each participant in sterile disposable polypropylene vials, kept in Styrofoam boxes and sent to the Laboratory of Medical Bacteriology of the Institute of Tropical Pathology and Public Health from Universidade Federal de Goiás (IPTSP/UFG), where they were processed in a maximum period of 48 hours after collection.

The specimens were homogenized through vortex and aliquots of 20 μ L planted in mannitol salt agar, tryptic soy agar (TSA) and tryptic soy broth (TSB) supplemented with 4% of NaCl and 6mcg/mL oxacillin, which were incubated at 35°C for 48 hours⁽⁶⁾.

Colonies characterized by *Staphylococcus* sp. were submitted to Gram staining, and those with microscopic morphology of Gram-positive cocci were cultivated on nutrient agar and incubated at 35°C for 24 hours, to later conduct identification tests: production of catalase, free coagulase and, deoxyribonuclease. The isolated ones were also characterized regarding production of lecithinase enzyme⁽⁷⁾.

To assess susceptibility profile to antimicrobials, the isolated ones were submitted to disk-diffusion test, following the one preconized by the Clinical and Laboratory Standard Institute (CLSI). Resistance to methicillin was detected through the use of cefoxitin and oxacillin as phenotypic markers (screening) and confirmed by the E-test[®]. It was also conducted a research for phenotype of inducible resistance to macrolide, lincosamides, and type B streptogramins (MLS_B) through the induction test by disk-approximation, with the use of clindamycin and erythromycin disks⁽⁶⁾.

The *mecA* gene in methicillin resistant *Staphylococcus* sp. was verified through the Polymerase Chain Reaction (PCR) technique, and species of coagulase-negative staphylococci (CoNS) were identified by Internal Transcribed spacer-PCR (ITS-PCR), with modifications⁽⁷⁾.

The data were entered in databases on Excel and exported to the statistical package SPSS (Statistical Package for the Social Sciences), version 18.0. The prevalence was calculated with a confidence interval of 95%. Differences in proportions were compared using the Chi-Square test (χ^2) or the Fisher's Exact test, when appropriated. Values of $p < 0.05$ were considered statistically significant.

This study is linked to the project "Isolated microorganisms in saliva of health professionals of an oncology hospital in the Center-West region of Brazil". It was evaluated and approved by the Ethics in Research Committee, under the protocol nº 040/08. All participants gave their consent by signing the Free and Informed Consent Term, meeting the Brazilian norms for research with human beings.

RESULTS

One-hundred and thirty WSA participated in the study. From those 101 (77.7%) were female. Regarding age and education, there was a predominance of workers older than 30 years (67.0% / n= 87) who completed high school (56.1%/ n = 73). From 130 WSA, 49 (37.7%) were colonized by *Staphylococcus* sp., 26 (53.0%) by SCN, 18 (36.8%) by *S. aureus* and five (10.2%) by both (Table 1).

Table 1: Characterization of workers colonized by *Staphylococcus* sp. (n=49) according to investigated variables. Goiânia, GO, Brazil, 2010.

Variables	<i>Staphylococcus</i> sp.			
	n /Total	(%)	χ^2	P
Gender			0.81	0.37
Female	36/101	35.6		
Male	13/29	44.8		
Age			1.1	0.3
Up to 30 years	13/41	31.7		
< 30 years	36/87	41.4		
Not informed	02	-		
Education			0.83	0.95
≤ Middle school	17/47	36.2%		
High school	28/73	38.4%		
≥ College education	04/10	40.0%		
Working sector			12.8	0.005
Sector of Nutrition and Diet – SND	18/31	58.1		
Sector of Clothing Reprocessing – SCR	08/16	50.0		
Sector of Hygiene and Cleaning – SHC	21/65	32.3		
Center of Material and Sterilization – CMS	02/18	11.1		
Working shift			7.4	0.006
Daytime	37/112	33.0		
Night	12/18	66.7		
Service time (years)			1.4	0.5
< 2 years	09/27	33.3		
2 to 4 years	13/27	48.1		
> 4 years	25/68	36.8		
Not informed	08	—		
Working hours (weekly hours)			2.0	0.15
< 40 hours	06/24	25.0		
≥ 40 hours	43/106	40.6		
Work in another institution			0.37	0.55
No	42/112	37.5		
Yes	06/13	46.2		
Not informed	05	—		

The variables working sector and working shift were identified as predictors for *Staphylococcus* sp. colonization in WSA ($p < 0.05$) (Table 1). The SND and SCR presented higher prevalence of colonized WSA, 58.1% and 50.0%, respectively. The same way, the WSA from night shifts were proportionally more affected by *Staphylococcus* sp. colonization (66.7%).

A total of 59 *Staphylococcus* sp. were isolated, 37 (62.7%) SCN and 22 (37.3%) *S. aureus*. The

susceptibility profile of *Staphylococcus* sp. to antimicrobials are presented on Table 2.

Table 2. Susceptibility profile to *Staphylococcus* sp. antimicrobials isolated in saliva of workers from support areas (n=59) in an oncology institution in the Center-West region. Goiânia, GO, Brasil, 2010.

Antimicrobial	Resistant		Intermediate		Sensitive	
	n	%	n	%	n	%
Clindamycin	-	-	01	1.7	58	98.3
Erytromycin	18	30.5	03	5.1	38	64.4
Ciprofloxacin	02	3.4	-	-	57	96.6
Gentamicin	02	3.4	01	1.7	56	95.0
Sulfamethoxazole/trimethoprim	02	3.4	-	-	57	96.6
Tetracycline	13	22.0	-	-	46	78.0
Rifampicin	-	-	-	-	59	100.0
Mupirocin	02	3.4	-	-	57	96.6
Cefoxitin	09	15.2	-	-	50	84.7
Oxacillin	23	39.0	01	1.7	35	59.3

According to the confirmatory test of resistance to oxacillin (E-Test[®]), methicillin resistant *S. aureus* (MRSA) was not identified. However, 35.1% (13/37) the SCN isolated presented themselves resistant to antimicrobial, being 12 identified as *S. epidermidis* and one as *S. haemolyticus*. The presence of *mecA* gene was detected in 92.3% of ECN resistant to methicillin (Table 3).

Table 3. Phenotypic and genotypic characteristics of isolated *Staphylococcus coagulase-negative* resistant to methicillin (n=13) of workers from support areas in an oncology institution in the Center-West region. Goiânia, GO, Brazil, 2010.

Participant	Code ECN	Susceptibility profile		CIM ^c (µg/ml)	Gene <i>mecA</i> ^d	Species ^e
		Cefoxitin ^a	Oxacillin ^b			
011DEH	011 MN	R	R	1.5	+	<i>S. epidermidis</i>
021DEH	021aTSA	R	R	1.0	+	<i>S. epidermidis</i>
121SHL	121a MN	S	R	0.75	-	<i>S. haemolyticus</i>
139 SHL	139 MN	R	R	1.5	+	<i>S. epidermidis</i>
159 SHL	159 TSB	R	R	1.5	+	<i>S. epidermidis</i>
177 SHL	177a TSA	R	R	1.5	+	<i>S. epidermidis</i>
178 SHL	178 TSA	R	R	6.0	+	<i>S. epidermidis</i>
186 SND	186 MN	R	R	1.5	+	<i>S. epidermidis</i>
189 SND	189 TSA	S	R	0.5	+	<i>S. epidermidis</i>
192 SND	192 TSB	S	R	1.0	+	<i>S. epidermidis</i>
194 SND	194a MN	R	R	1.5	+	<i>S. epidermidis</i>
198 SND	198 TSA	R	R	2.0	+	<i>S. epidermidis</i>
200 SND	200a MN	S	R	1.5	+	<i>S. epidermidis</i>

R = resistant.

S = sensitive.

^a Halo growth inhibition \geq 17mm (antibiogram).

^b Halo of growth inhibition \geq 24mm (antibiogram).

^c E-test for oxacillin– CIM \geq 0,5 µg/ml.

^d By PCR.

^e By ITS-PCR.

From the 13 WSA colonized by oxacillin resistant ECN, 12 (92.3%) were women, nine (69.2%) were 30 years or older, eight (61.5%) worked for more than four years in the institution and 11 (84.6%) worked 40

hours/week or more. According to the working sector, six (19.4%) worked at SND, five (7.7%) at SHC and two (11.1%) at CMS.

From 22 identified *S. aureus* isolated, 36.3% (n=8) were fibrinolysin and 100,0% lecithinase productors. After the D-test, 9.1% of *S. aureus* (n=2) presented the resistance phenotype MLS_B of inducible type. All *S. aureus* and isolated SCN presented themselves sensitive to vancomycin, linezolid and quinupristin / dalfopristin.

DISCUSSION

The oral cavity colonization of WSA by *S. aureus* and SCN, including *S. epidermidis* and methicillin resistant *S. haemolyticus*, was identified in a hospital specialized in attending patients at high risk to develop an infection^(4,8). This is the first study that investigated colonization in the oral cavity by *Staphylococcus* sp. of WSA working in assistance for oncologic patients.

Oral cavity colonization of workers from direct assistance sectors from the same health institution⁽⁹⁾ and from a Brazilian public hospital⁽¹⁰⁾ by methicillin resistant SCN, as well as the presence of MRSA⁽¹¹⁾ and *Enterobacteriaceae*⁽¹²⁾ in WSA, was found in other studies, reinforcing this site as a reservoir for these infectious agents.

The absence of MRSA within the WSA isolated in this study, as well as within assistencial workers in the same institution⁽⁹⁾, can be the success reflected from measures implanted in the institution regarding antimicrobial rational use, an essential factor in the fight against bacterial resistance. At the same time, it points to SCN emergency, which until few decades, was considered only contaminating, but it has been increasingly recognized as important cause of IRAS, and as a reservoir of genes that shows resistance to antimicrobials⁽¹³⁾.

As a risk to worker's health, as well as in cases with biological material, the colonization by multi resistant bacteria, for example the methicillin resistant SCN and MRSA, should be addressed by working policies to guarantee occupational safety to workers in health services⁽²⁾, including those from support areas that are also exposed to risk, as shown in this study.

The working sector can be a risk factor for *Staphylococcus* sp. colonization, due to the biological risk inherent from activities developed in these sectors, that is, contact with materials (CMS), clothing (SCR), utensils (SND) and residuals (SHC), and workers have direct contact with patients or with environments of health services.

In this study, the prevalence of WSA colonized by *Staphylococcus* sp. was higher in the SND and SCR. A possible justification for this finding is that in these sectors, the visible presence of body fluids, as blood, does not occur at the same frequency as observed in CMS and SHC. Consequently, it can lead to non-adherence of SND and SCR workers to biosafety measures, exposing themselves to microorganisms that although invisible to our eyes, are present in utensils surfaces, and clothing used by patients.

The SND is responsible for the production, processing, packaging, storage, transport and, distribution

of food. A study in health institutions identified that 16.2% (13/80) of collected samples from hands of SND workers were positive for *S. aureus*⁽¹⁴⁾. Additionally, enteral feedings from a health institution presented worrying levels of contamination by *S. aureus* (7.0×10^2 UFC)⁽¹⁵⁾.

The SCR is responsible for the reprocessing and distribution of clothing for patient assistance, beyond the previous steps, of collection, transportation and packaging of dirty laundry, and storage of clean laundry. A study that assessed the presence of bacteria in surgical clothing pointed that 79% of those were contaminated by some type of gram-positive cocci, as the *S. aureus*⁽¹⁴⁾. To propitiate occupational quality and safety in this service has been one of the major challenges, once cloths can storage virulent microorganisms resistant to antimicrobials, from patients' secretions and body fluids⁽¹⁾.

The night working shift in special, with distinct work hours, added to physical, biological and psychic poor conditions could result in higher risk of occupational exposure, especially to biological risk. Working hours correlates with health problems, especially cardiovascular, psychosocial and sleep disorders⁽¹⁶⁾. All these changes can result in lower rigor of workers to adhere to standard precautions, accentuating their exposition to diverse pathogenic agents.

In this study, the percentage of WSA colonization by SCN and its methicillin resistance profile was noted. The resistance to this antimicrobial among isolated SCN of workers from assistencial sectors were highlighted in a study conducted in the same health institution⁽⁹⁾ and in a Brazilian public hospital⁽¹⁰⁾. This bacteria group classifies as emergent opportunists, especially in immunocompromised and institutionalized patients, making them more vulnerable to develop severe infections^(13,17).

Higher resistance prevalence to antimicrobial among SCN was seen in isolated from health service workers in relation to isolated of individuals from the community⁽¹⁸⁾, warning for the contact with insalubrious environments in health services, that can change the colonization profile of these individuals, as well as the resistance profiles of colonizing microorganisms⁽³⁾.

It is important to emphasize that resistance of these bacteria to methicillin confers a crossed resistance to all beta-lactams antimicrobial, therefore, considered multi resistant. In addition, isolated resistant to methicillin are also frequently resistant to non-beta-lactams antimicrobials, that are used in the treatment of staphylococcal infections, restraining the therapeutic options^(6,17).

In the present study, the WSA colonization by *S. aureus* was also noted, 100% of these lecithinase producers. The phosphatidylinositol-specific phospholipase C, also known as lecithinase, is one of extra-cellular enzymes produced by *Staphylococcus* sp., especially the *S. aureus*. This enzyme is found in the cytoplasmic membrane of eggs and human cells, and it is capable to catalyze the phosphatidylinositol, which function is to determine the link between proteins to human cells. It is known that the virulence factor can be related to development of acute respiratory syndromes in adults, the intravascular disseminated coagulation, demyelination of vases and meninges, capable of causing important diseases, as the bacterial meningitis^(5,7).

Some isolated of *S. aureus* can produce fibrinolisinases that degrades fibrin clots and allow the bacterial

dissemination to adjacent tissues^(5,7). The production of this virulent factor by *S. aureus* was also seen in this study.

S. aureus as phenotype of inducible resistance to the MLS_B group was found in this study. Such phenotype is determined by the *erm* (*erythromycin ribosome methylation*) gene, responsible for changing the connection site of macrolides in the bacterial ribosome, also conferring crossed resistance to lincosamides and streptogramin B⁽⁶⁻⁷⁾. It is important to note that erythromycin and clindamycin are therapeutic options to treat infections in the skin and in soft parts caused by methicillin resistant *Staphylococcus* sp.

CONCLUSION

Workers from support areas who provide services to patients at high risk of infections were identified as colonized by the oral cavity by SCN resistant to methicillin and *S. aureus* producers of lecithinase and fibrinolisinases, as well as, isolated resistant to the MLS_B group, being sector and working shift identified as predictors to colonization.

Facing this findings, it is recommended for WSA to also be targeted by occupational health policies, once colonization can contribute for subsequent infection. Such policies should include pre- and post-colonization measures. Educational activities, control regarding adherence of biosafety measures, screenings (including the oral cavity), treatment (with and without medicines) and, the accompaniment of colonized, although controversial, are strategies to be discussed and investigated in future studies.

In this sense, it is important to highlight nursing participation in Control Services for Hospital Infections, aiming the implantation of evidence-based measures to contribute for the worker's safety and to minimize the dissemination of multi resistant bacteria in health services, at the workers home and in the community.

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