

Research Paper

Methicillin-resistant *Staphylococcus* sp. colonizing health care workers of a cancer hospital

Dayane de Melo Costa¹, André Kipnis², Lara Stefânia Netto de Oliveira Leão-Vasconcelos², Larissa Oliveira Rocha-Vilefort³, Sheila Araújo Telles⁴, Maria Cláudia Dantas Porfírio Borges André², Anaclara Ferreira Veiga Tipple⁴, Ana Beatriz Mori Lima³, Nádia Ferreira Gonçalves Ribeiro³, Mayara Regina Pereira¹, Marinésia Aparecida Prado-Palos⁴

¹Núcleo de Estudos e Pesquisa de Enfermagem em Prevenção e Controle de Infecções Relacionadas à Assistência à Saúde, Faculdade de Enfermagem, Universidade Federal de Goiás, Goiânia, GO, Brazil..

²Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brazil.

³Secretaria Municipal de Saúde de Goiânia, Goiânia, GO, Brazil.

⁴Faculdade de Enfermagem, Universidade Federal de Goiás, Goiânia, GO, Brazil.

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Abstract

The aim of the study was to analyze epidemiological and microbiological aspects of oral colonization by methicillin-resistant *Staphylococcus* of health care workers in a cancer hospital. Interview and saliva sampling were performed with 149 health care workers. Antimicrobial resistance was determined by disk diffusion and minimum inhibitory concentration. Polymerase Chain Reaction, Internal Transcribed Spacer-Polymerase Chain Reaction and Pulsed Field Gel Electrophoresis were performed for genotypic characterization of methicillin-resistant *Staphylococcus*. Risk factors were determined by logistic regression. Methicillin-resistant *Staphylococcus* colonization prevalence was 19.5%, denture wearing ($p = 0.03$), habit of nail biting ($p = 0.04$) and preparation and administration of antimicrobial ($p = 0.04$) were risk factors identified. All methicillin-resistant *Staphylococcus* were *S. epidermidis*, 94.4% of them had *mecA* gene. Closely related and indistinguishable methicillin-resistant *S. epidermidis* were detected. These results highlight that HCWs which have contact with patient at high risk for developing infections were identified as colonized by MRSE in the oral cavity, reinforcing this cavity as a reservoir of these bacteria and the risk to themselves and patients safety, because these microorganisms may be spread by coughing and talking.

Key words: health personnel, occupational health, *Staphylococcus*, methicillin resistance, oncology service, hospital.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is recognized as an important pathogen in the context of health care-associated infections (HAIs). Over recent years, coagulase-negative *Staphylococcus* (CoNS), has also distinguished itself in HAIs, concurrently with advances in medical practice including increased use of im-

planted devices, and an increased number of immunocompromised patients (Casey *et al.*, 2007).

Health care workers (HCWs) colonized with methicillin-resistant *Staphylococcus* (MRS) may serve as reservoirs and potential spreaders of these bacteria in health care and community settings (Eveillard *et al.*, 2004; Albrich and Harbarth, 2008; Liakopoulos *et al.*, 2008; Lis *et al.*, 2009; Nubel *et al.*, 2013). In addition, the HCWs themselves may

suffer to this condition, getting infection due to colonization (Albrich and Harbarth, 2008; Haamann *et al.*, 2011). In most studies, nasal colonization is investigated (Albrich and Harbarth, 2008). However, the oral cavity (saliva) has been identified as a potential reservoir for these microorganisms (Ohara-Nemoto *et al.*, 2008; Rosa *et al.*, 2009; Prado-Palos *et al.*, 2010), which may be spread by coughing and talking. Further, upper respiratory tract infections are among the most frequent in HCWs colonized by MRSA (Albrich and Harbarth, 2008; Haamann *et al.*, 2011).

Due to severity of infections by *Staphylococcus* and its multidrug resistance, HCWs colonized by these bacteria who treat of patients considered at high risk for infections (such as cancer patients) require particular attention. These patients are especially susceptible to infections due to, among other factors, a suppressed immune system caused by underlying malignancy and its treatment (Kamboj and Sepkowitz, 2009).

In this context, we aimed to evaluate epidemiological and microbiological aspects of the oral colonization by MRS of HCWs in a cancer hospital.

Material and Methods

Design, local and population

This cross-sectional study was carried out from May 2009 to November 2010 in a hospital with 212 beds, referral for cancer treatment in Central Brazil, located in Goiânia, Goiás. Study population was composed by HCWs who worked in the wards (WD) (WD1A, WD1B, WD1C, WD2A, WD3A e WDBC), surgical center (SC), service of hospital infection control (SHIC), dressing sector (DS), endoscopy sector (EDS), emergency sector (ES), chemotherapy sector (adult and child) (QTS), rehabilitation and physiotherapy sector (RFS), radiotherapy sector (RTS), intensive care unit (ICU) and bone marrow transplantation (BMT), and administrative secretaries of the ICU. Those who exercised administrative function, except in the ICU, and those who were using antimicrobial agents at the time or within the last seven days before data collection were excluded.

The study was approved by the Ethical Committee of the Associação de Combate ao Câncer em Goiás (ACCG) (Protocol: CEP-ACCG/040/08), in agreement with the Declaration of Helsinki. Interview and saliva sampling were performed after signing of the Informed Consent Term by the participants.

Data and saliva sampling

Data were collected through interviews, and saliva collection (1.0 mL), without stimulation (Nauntoffe *et al.*, 2005) in sterilized polyethylene bottles, which were transferred to the laboratory, stored to 2 to 8 °C and processed within 24 h of collection. Data and saliva sampling were

collected by researchers and trained undergraduate nursing students from the Universidade Federal de Goiás.

Microbiological procedures

The saliva collected (20 µL) were streaked on Trypticase Soy Agar (TSA), Trypticase Soy Broth (TSB) with 4% NaCl and 6 µg/mL of oxacillin, and Mannitol Salt Agar, incubated at 35 °C for 48 h. Characteristic colonies of *Staphylococcus* were submitted to Gram's staining and standard identification tests (Koneman *et al.*, 2008). *S. aureus* and CoNS were tested for antimicrobial susceptibility by disk diffusion (Clinical and Laboratory Standards Institute, 2007; 2009) to: cefoxitin (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), dalbapristin-quinupristin (15 µg), erythromycin (15 µg), gentamicin (10 µg), linezolid (30 µg), mupirocin (5 µg), oxacillin (1 µg), rifampicin (5 µg), sulfamethoxazole-trimethoprim (25 µg) and tetracycline (30 µg). The *S. aureus* and CoNS that were resistant to oxacillin and/or cefoxitin on the disk diffusion test were tested in the minimum inhibitory concentration (MIC) to oxacillin by *Etest*®. *S. aureus* with MIC ≥ 4 µg/mL and CoNS with MIC ≥ 0.5 µg/mL were considered meticillin-resistant (Clinical and Laboratory Standards Institute, 2009). ATCC 25923 *S. aureus* was used as quality control.

Molecular typing of MRS

The *mecA* gene in MRS was verified through the Polymerase Chain Reaction (PCR) (Murakami *et al.*, 1991). The species of MR-CoNS were identified by Internal Transcribed Spacer-PCR (ITS-PCR), with minor modifications (Couto *et al.*, 2001). Pulsed Field Gel Electrophoresis (PFGE) with *SmaI* was performed as described previously (Chung *et al.*, 2000).

The PFGE were interpreted according to the Tenover *et al.* (1995) criteria and by computed analysis through the program BioNumerics (version 5.0; Applied Maths, Ghent, Belgium). The dendrogram was constructed based on the position and presence of bands and unweighted pair group method for arithmetic averages and using Dice coefficient of similarity with optimization and tolerance parameters of 0.7% and 1.3%, respectively. The pulsotype (PT) was defined as a single electrophoretic profile identified with one or two capital letters. Cluster (CL) was defined as a group of profiles (n > 2) with a coefficient ≥ 80% of similarity and identified with Arabic numeral. The subtypes within the same cluster were identified with the cluster number added as a subscript Arabic numeral.

Statistical analysis

Statistical evaluations were performed using Statistical Package for the Social Sciences, version 18.0 (SPSS Inc., Chicago, IL). Prevalence of colonization by *Staphylococcus* spp. and MRS were calculated to a confidence interval of 95% (95% CI). Univariate analysis was performed to

estimate the odds ratio of colonization by MRS associated with the prediction variables investigated: age, sex, level of education, occupation, work shift, work contract, length of professional activity, working at another health care setting, preparation and administration of antimicrobial, use of personal protective equipment, frequency of hand hygiene, use of ring, watch/bracelet, long earrings and necklace, nails size, habit of nail-biting, eating outside the feeding area, use of mouthwash, denture wearing, self-medication (antimicrobial) and frequency of upper respiratory tract infection. Those that had p values < 0.10 were submitted to multivariate analysis using the logistic regression model. Proportion differences were compared using the chi-square test (χ^2) or Fisher's exact test, when appropriate. P values < 0.05 were considered statistically significant.

Results

Participated of the study 53.6% (149/278) of the total population, the most were female (129/149; 86.6%), nursing technician (112/149; 75.2%) and worked at WD (42/149; 28.2%) (Table 1).

The prevalence of HCWs colonized by CoNS was 25.5% (38/149), by *S. aureus* (21/149) was 14.1% and by both was 6.7% (10/149). MR-CoNS were isolated from 19.5% (29/149) (95% CI: 13.7-26.4) of them. MRSA was not detected.

Among the variables investigated, denture wearing, habit of nail biting, as well as preparation and administration of antimicrobial were independently associated with colonization by MRS (Table 2).

Sixty-one (66.3%) CoNS and 31 (33.7%) *S. aureus* were isolated. According to the MIC to oxacillin, 59.0% (36/61) of CoNS were methicillin-resistant. The resistance to other antimicrobials tested was more prevalent among MR-CoNS than those methicillin-sensitive (Figure 1). All MR-CoNS isolates were identified as *S. epidermidis*, and 94.4% of them were *mecA* gene positive.

The prevalence of antimicrobial susceptibility profile of *S. aureus* isolates was: 83.9% to erythromycin, 87.1% to tetracycline, 90.3% to clindamycin, 90.3% to rifampicin, 93.6% to gentamicin, 96.8% to mupirocin and trimethoprim-sulfamethoxazole, and all isolates (100%) were sensitive to ciprofloxacin, dalfopristin/quinupristin and linezolid.

The dendrogram constructed (Figure 2) from the restriction patterns of chromosomal DNA generated by PFGE showed: 35 pulsotypes (A to II), six clusters (1-6) and 21 subtypes. The two biggest clusters, 1 and 4, were composed by methicillin-resistant *S. epidermidis* (MRSE) isolates from five and seven HCWs, respectively, of distinct sectors. Two MRSE of cluster 4 (subtype 4₀), isolated from HCWs of the SC (289 TSA) and ES (045 MN), were indistinguishable.

Table 1 - Demographic and occupational characteristics of health care workers (n = 149) at a referral cancer treatment hospital. Goiânia/GO, Brazil.

| Demographic and occupational characteristics | n | % |
|---|-----|------|
| Sex | | |
| Female | 129 | 86.6 |
| Male | 20 | 13.4 |
| Age (years) | | |
| ≤ 30 | 53 | 35.6 |
| 31-40 | 51 | 34.2 |
| > 40 | 45 | 30.2 |
| Occupation | | |
| Nursing Technician | 112 | 75.2 |
| Nurse | 10 | 6.7 |
| Physician | 10 | 6.7 |
| Radiotherapy Technician | 6 | 4.0 |
| Surgical Instrumentator | 3 | 2.0 |
| Physiotherapist | 2 | 1.3 |
| Nursing Assistant | 2 | 1.3 |
| Secretary of ICU | 2 | 1.3 |
| Assistant of Plaster | 1 | 0.7 |
| Radiotherapy Physicist | 1 | 0.7 |
| Working sector | | |
| Wards (WD) | 42 | 28.2 |
| Chemotherapy Sector (adult and child) (QTS) | 29 | 19.5 |
| Intensive Care Unit (ICU) | 16 | 10.7 |
| Dressing Sector (DS) | 12 | 8.1 |
| Surgical Center (SC) | 11 | 7.4 |
| Radiotherapy Sector (RTS) | 12 | 8.1 |
| Bone Marrow Transplantation (BMT) | 9 | 6.0 |
| Emergency Sector (ES) | 8 | 5.4 |
| Service of Hospital Infection Control (SHIC) | 4 | 2.7 |
| Endoscopy Sector (EDS) | 4 | 2.7 |
| Rehabilitation and Physiotherapy Sector (RFS) | 2 | 1.3 |

Discussion

In this study, *S. aureus* and CoNS, including MRSE, were isolated from the oral cavity of HCWs from a referral cancer treatment hospital. The presence of MRSE (Rosa *et al.*, 2009) and MRSA (Prado-Palos *et al.*, 2010) was identified in the oral cavity of HCWs from public hospitals in Brazil, reinforcing this cavity as a reservoir of these bacteria and the risk to HCWs' health. Additionally, there is the potential exchange of *Staphylococcus* spp. between this cavity and the nasal cavity (Ohara-Nemoto *et al.*, 2008).

Poor hygiene of dentures may influence the presence of MRSE in the saliva of the denture-wearing population. Moreover, association between the isolation of *Candida* from the oral cavity and hands/fingers prosthesis users was

Table 2 - Univariate and multivariate analyzes of risk factors for oral colonization by methicillin-resistant *Staphylococcus* spp. (MRS) among health care workers at a referral cancer treatment hospital. Goiânia/GO, Brazil.

| Variables | Colonized by MRS/n† | (%) | OR Non-adjusted (95% CI)* | p | OR Adjusted§ (95% CI)* | p |
|---|---------------------|--------|---------------------------|-------|------------------------|------|
| Preparation and administration of antimicrobial ‡ | | | | | | |
| No | 3/34 | (8.8) | 1.0 | | 1.0 | |
| Yes | 22/89 | (24.7) | 3.39 (0.94-12.1) | 0.07 | 5.5 (1.0-29.1) | 0.04 |
| Habit of nail-biting | | | | | | |
| No | 23/136 | (16.9) | 1.0 | | 1.0 | |
| Yes | 6/13 | (46.2) | 4.2 (1.29-13.69) | 0.01 | 3.5 (1.0-2.3) | 0.04 |
| Denture wearing | | | | | | |
| No | 20/118 | (16.9) | 1.0 | | 1.0 | |
| Yes | 9/21 | (42.9) | 3.67 (1.36-9.88) | 0.007 | 3.1 (1.1-8.7) | 0.03 |

*OR: odds ratio, 95% CI: 95% confidence interval.

†Included in the denominator valid values.

‡Included in the denominator only participants of the nursing staff.

§Adjusted by age, sex, work contract (hour per week), working at another health care setting, use of mouthwash, denture wearing, nail biting habit and self-medication (antimicrobial) in the multivariate analysis, because they showed $p < 0.1$ in the univariate analysis.

Adjusted by age, sex, work contract (hour per week), working at another health care setting, use of mouthwash, denture wearing, nail biting habit and self-medication (antimicrobial) and preparation and administration of antimicrobial in the multivariate analysis, because they showed $p < 0.1$ in the univariate analysis.

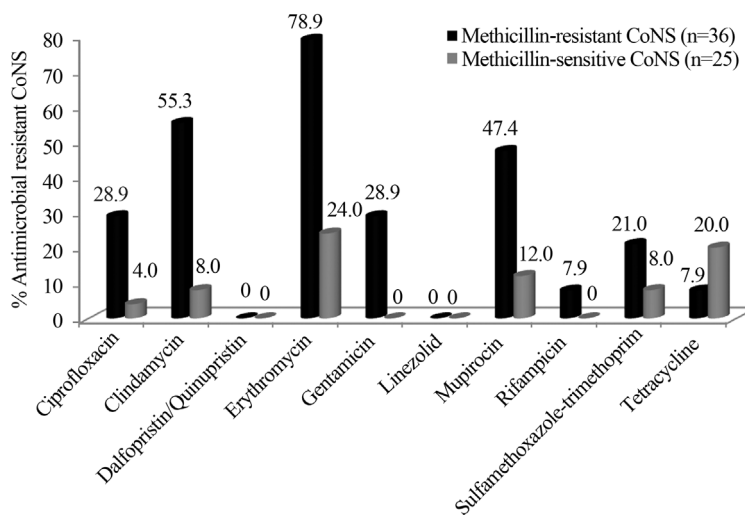


Figure 1 - Antimicrobial susceptibility profile of methicillin-resistant and methicillin-sensitive coagulase-negative *Staphylococcus* isolated from oral cavity of health care workers at a referral cancer treatment hospital. Goiânia/GO, Brazil.

reported (Darwazeh *et al.*, 2001). Therefore, adequate denture and hand washing, especially to HCWs, are emphasized as simple and safe measures to prevent colonization by these microorganisms.

It is known that subungual areas harbor high concentrations of bacteria, predominantly CoNS (McGinley *et al.*, 1988). Thus, the habit of nail-biting contributes to the transport of microorganisms to the oral cavity and is characterized as a bad hygiene habit and a risk behavior, particularly to HCWs. This also highlights the importance of keeping

nails natural, clean, and short, with no artificial fingernails (World Health Organization, 2009).

We suggest a possible explanation for the association between preparation and administration of antimicrobial agents and colonization by methicillin-resistant isolates may be related to inadequate attitudes of HCWs during this procedure, when handling them without gloves, improper disposal of materials/waste used, and no hand washing, providing the selective pressure of bacteria on the hands and environment of drug compounding. However, no evi-

dence was found about this issue, indicating the needed investigation about this relationship.

MRSE was more resistant to other antibiotics tested when compared to methicillin-susceptibility CoNS (Fig-

ure 1), emphasizing that methicillin-resistant isolates are frequently resistant to antimicrobial non-beta lactams indicated for the treatment of staphylococcal infections. For example, 47.4% of MRSE were resistant to mupirocin which

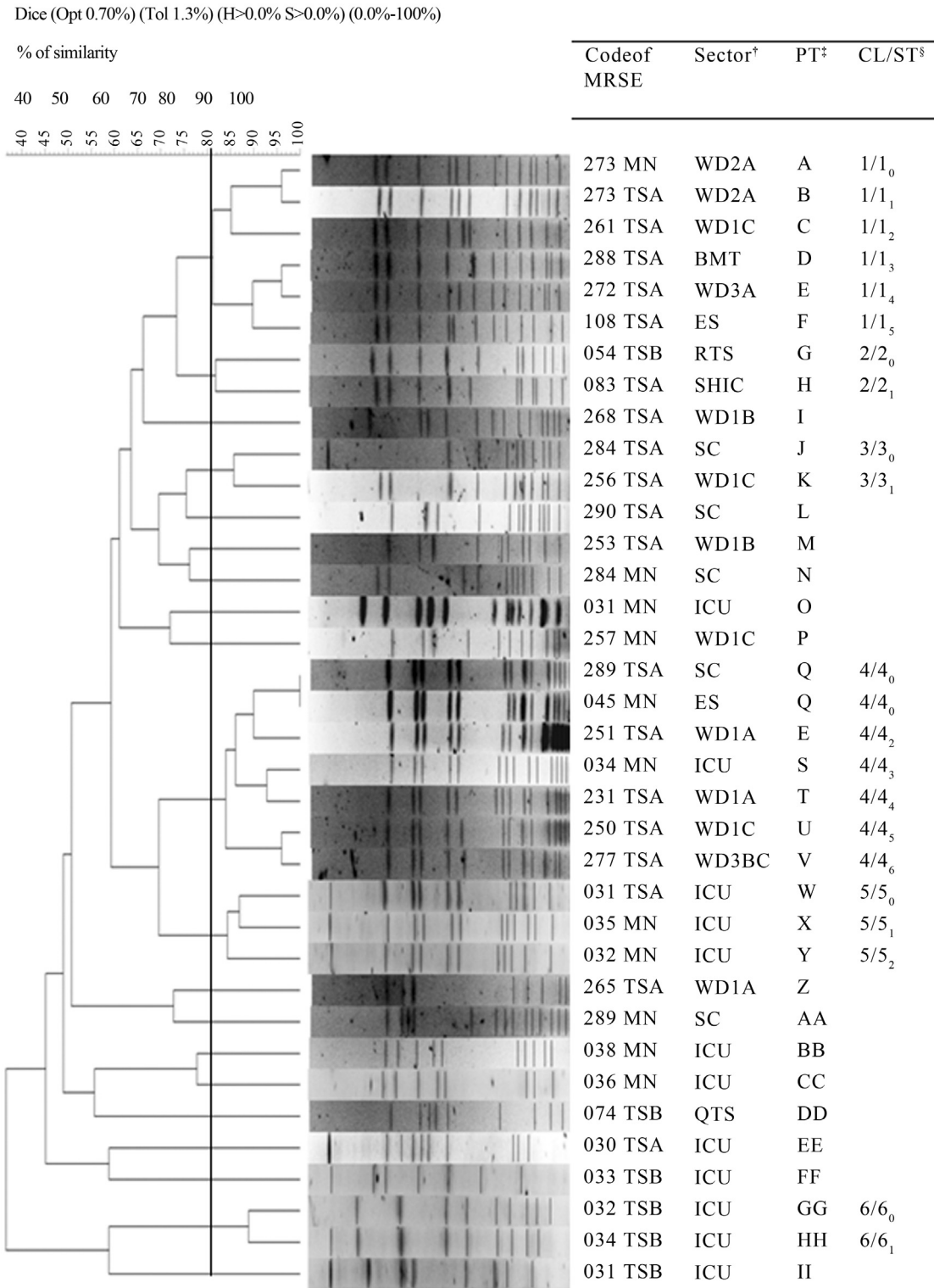


Figure 2 - Dendrogram of MRSE isolated from oral cavity of health care workers at a referral cancer treatment hospital, Goiânia/GO, Brazil. †WD1A, WD1B, WD1C, WD2A, WD3A e WD3C - Wards; SC - Surgical Center; SHIC - Service of Hospital Infection Control; QTS-Chemotherapy Sector (adult and child); ES-Emergency Sector; RTS - Radiotherapy Sector; ICU - Intensive Care Unit; BMT - Bone Marrow Transplantation. ‡PT-Pulsotype. §CL/ST - Cluster/Subtype.

is recommended for nasal decolonization by MRSA. It is interesting to emphasize that *S. epidermidis* might be a possible reservoir of the *mupA* gene, which confers resistance to mupirocin, for MRSA (Hurdle *et al.*, 2005). However, all *S. aureus*, CoNS and MRSE were sensitive to dalbapristin/quinupristin and linezolid, indicating that these antimicrobials are effective to treat infections by them.

Closely related isolates (Clusters 1-6) were identified among HCWs in different sectors and MRSE isolated from two HCWs, one in SC and one in ES, were genetically indistinguishable (Cluster 4/subtype 4₀). The close relation between isolates from HCWs may be due to nosocomial exposure of the workers by a dominant group of MRSE.

Significant reduction in the incidence of MRSA in a trauma ICU was observed after identification and decolonization of colonized HCWs (Ben-David *et al.*, 2008), emphasizing that unrecognized MRSA-colonized HCWs may serve as significant reservoirs, and thus could impair other infection control measures (Ben-David *et al.*, 2008; Nubel *et al.*, 2013). We agree with this view and extend it to HCWs colonized by MR-CoNS. We also believe that knowledge of MRS colonization may contribute to changing HCWs attitudes in relation to prevention and control measures (Eveillard *et al.*, 2004). With regard to occupational safety, colonization or infection of HCWs with MRSA should be considered an occupational hazard and injury according to local legislation (Albrich and Harbarth, 2008; Dulon *et al.*, 2013), and we emphasize that colonization by MR-CoNS also represents a risk to HCWs' health.

In conclusion, HCWs which have contact with patient at high risk for developing infections were identified as colonized by MRSE in the oral cavity, highlighting a risk to themselves and patients safety, and other individuals in the hospital and community/home. Furthermore, it reinforces the role of the oral cavity (saliva) as a reservoir of these bacteria and the need to re-think policies for occupational health combined with the control of multidrug-resistant organisms and, consequently, to contribute to the successful control of HAIs.

This study has limitations regarding the method to data collection, this was based only on subjectivity of the report of the participants and the size of studied population, this was relatively small, so the generalization of the risk factors for colonization by MRSE, to other health care settings may be limited. Therefore, further studies on this theme are needed.

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