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Hospital surface contamination in wards occupied by patients infected with MRSA or MSSA in a Brazilian university hospital

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ABSTRACT

The hospital environment, especially surfaces, represents a secondary reservoir for pathogens such as *Staphylococcus aureus*. The present study was carried out at the Hospital de Clínicas da Universidade Federal de Uberlândia (HC-UFU) in Brazil, from January to August 2004, with the aim of qualitatively and quantitatively assessing the presence of *S. aureus* on surfaces touched by hands (bed rail, bedside table and door handle), on the floor and in the air, in wards where the patients were infected or not infected with this organism. Twenty-six wards with at least one infected patient and 26 wards with uninfected patients were investigated. Surfaces and air were sampled during bed making, by means of sterile 10cm^2 adhesive tapes and 90mm-diameter exposed culture plates, respectively. Cultures were carried out on Egg Yolk Salt Agar, containing 7.5% NaCl and 1% egg yolk, and colonies identified by lecithinase and coagulase tests and Gram stain. About 50% of the wards were *S. aureus* contaminated, without significant differences between the groups (46.1% in the infected patient wards *versus* 53.8% in controls); the correlation between counts of the pathogen on the surfaces and in the air was 66.7%. In spite of the fact that these bacteria were frequently present in the assessed wards, the microbial density was low (less than 1 CFU/cm^2). The association between environmental contamination and the epidemiology of *S. aureus* nosocomial infections is complex and thus further investigations are needed to reach a better understanding of this relationship.

Keywords: environmental contamination; nosocomial infections; *Staphylococcus aureus*.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria are more and more prevalent in the hospital environment and represent a challenge to infection control practices in most countries (Lemmen et al., 2004). The importance of the environment as a secondary reservoir for multi-resistant microorganisms, such as MRSA, is currently being emphasized because of their apparent ability to survive on dry surfaces (Lemmen et al., 2004). The main mechanism of transmission of infections within hospital is by direct contact, in particular with the hands of health professionals (Boyce, 2004), which may both contaminate or be contaminated by hospital surfaces (Rutala & Weber, 2002). Surfaces can be divided into two categories: those where little hand contact occurs (e.g. floors), and those where the hand contact is frequent (door handle, bed rail) (Sehulster et al., 2004). Surface microbiological monitoring for *S. aureus* presence is normally qualitative and does not reveal the density of microbial contamination, which is closely related to infection (Oie et al., 2002).

The present study was elaborated to evaluate qualitatively and quantitatively environmental surface contamination in wards of patients infected or not with methicillin-resistant (MRSA) and/or methicillin-susceptible (MSSA) *S. aureus*, as well as to test the air as a source of surface contamination.

MATERIAL AND METHODS

The study was carried out at the Hospital de Clínicas da Universidade Federal de Uberlândia (HC - UFU), Minas Gerais State, Brazil, which has 570 beds and offers tertiary level care. Between January and August, 2004, 52 wards were investigated for environmental contamination of: bedside table, bed rail, door handle, floor and air. The evaluation was performed in wards of Surgical Units I and II and the Medical Unit.

The evaluated (two bed) wards were divided into two groups: those which hosted at least one patient infected with *S. aureus* (26) on the first day of Vancomycin treatment, and control wards which had no *S. aureus* infected patients (26). For each infected case ward investigated, a control ward in the same unit was also evaluated.

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Analysis

Surface and air sampling was performed in the morning, during ward cleaning. Surface quantitative collections were performed with sterile 10cm^2 adhesive tapes (Pimaco, Rio de Janeiro, RJ, Brazil) and swabs used for qualitative analysis of each surface. The swabs were transported in Tryptic Soy Broth (TSB - Oxoid, Basingstoke, Hampshire, England) tubes and then processed in the laboratory. Air collection was simultaneously performed by exposing 9cm diameter agar plates, as proposed by Pasquarella et al. (2000).

Air and surface contamination was evaluated for *S. aureus* presence on Egg Yolk Salt Agar plates (Finegold et al., 1978). *S. aureus* samples were identified by the opaque halo around the colony, due to the presence of lecithinase, Gram staining and, additionally, by the tube coagulase test (Coagu-plasma, Laborclin, Pinhais, PR, Brazil). Antimicrobial resistance of sampled colonies was evaluated *in vitro* by the agar diffusion technique (NCCLS, 2003).

Statistical Analysis

Results were statistically analyzed by univariate comparison using the χ^2 test, Fishers, exact test and the Student *t* test to differentiate between means, besides the Mann-Whitney test for non-parametric data. Potential risk factors were evaluated by dichotomization, i. e. present vs. absent in a 2 x 2 contingency table.

RESULTS

The two groups (infected and uninfected) did not differ in gender and age. Fifty-two wards were evaluated for *S. aureus* presence on surfaces (floor, bed rail, bedside table, door handle) and in the air. Half of the analyzed wards were contaminated with *S. aureus* in at least one qualitatively sampled environmental site (air and / or surface). Considering only surface contamination, 21 (40.4%) of the wards were positive, among cases (34.6%) and controls (46.1%). *S. aureus* was recovered from 35 (16.8%) of the 208 analyzed surfaces, corresponding to 65.4% and 69.2% in wards of patients with and without staphylococcal infection, respectively (Table 1).

Data referring to quantitative investigation (Table 1) also does not show significant differences between surfaces corresponding to the two groups. On the other hand, floor contamination was outstanding in comparison with other investigated surfaces ($P < 0.05$) (Table 1).

S. aureus counts in the air were also not significantly different in the two evaluated groups, with 9.5 CFU/plate (cases) and 4.4 CFU/plate (controls) (P=0.25). A clear relationship between air and surface contamination in the wards can be seen in Table 2. About two-thirds of wards with contaminated air showed surface contamination. Only a third of those with no detected aerial contamination had the same result.

Staphylococcal infections with microbiological confirmation had MRSA as the etiologic agent, excepting three (12.5%), and in total they mainly corresponded to surgical site infection (47.6%) and blood flow infection (23.8%).

Results of surface contamination per sample with methicillin-susceptible (MSSA) and resistant (MRSA) *S. aureus* are shown in Table 3. There were no differences between the frequencies of these microorganisms on case and control ward surfaces, with a lower proportion of MRSA samples (33.3%) than MSSA (66.7%).

Table 1 - Qualitative and quantitative analysis of *S. aureus* surface contamination in wards of infected and uninfected patients at the Hospital de Clínicas, Universidade Federal de Uberlândia - MG

Surfaces	Qualitative[a]			Quantitative[a]		
	Cases n=26 (%)	Controls n=26 (%)	*P*	Cases n=26 $\bar{X}$[b] (range)	Controls n=26 $\bar{X}$ (range)	*P*
Floor	8 (30.8)	10 (38.5)	0.79	7.88 (0-32)	6.66 (0-30)	0.70
Bed rail	4 (15.4)	4 (15.4)	0.99	0.76 (0-5)	1.28 (0-13)	0.94
Bedside table	4 (15.4)	3 (11.5)	1.00	1.40 (0-15)	1.30 (0-13)	0.70
Door handle	1 (3.8)	1 (3.8)	1.00	0.18 (0-3)	0.58 (0-8)	1.00
Total	17 (65.4)	18 (69.2)	0.76			

[a] *P* there was no significant differences between surfaces corresponding to both analysis (P <0.05);
[b] Data as mean (range) in CFU (colony-forming unit) / 10 cm^2

Table 2 - Relationship between *S. aureus* air and surface contamination in wards of infected and uninfected patients at the Hospital de Clínicas, Universidade Federal de Uberlândia - MG

Surfaces[a]	Air			
	Cases		Controls	
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
Positive	6 (66.7)	3 (17.7)	3 (60.0)	7 (38.9)
Negative	3 (33.3)	14 (82.3)	2 (40.0)	11 (61.1)
Total	9 (100.0)	17 (100.0)	5 (100.0)	18 (100.0)

[a] Surfaces evaluated: door handle, bed rail, bedside table and floor.

Table 3 - Surface contamination with methicillin-sensitive (MSSA) / methicillin-resistant (MRSA) *S. aureus* in wards of infected and uninfected patients at the Hospital de Clínicas, Universidade Federal Uberlândia - MG

Microorganism	Floor		Others[a]		Total	
	Cases	Controls	Cases	Controls	Cases	Controls
	n[b]=26(%)	n=26(%)	n=78(%)	n=77[c] (%)	n=104(%)	n=103[c](%)
MRSA	4 (15.4)	3 (11.5)	1 (1.3)	3 (3.9)	5 (4.8)	6 (5.8)
MSSA	4 (15.4)	7 (26.9)	8 (10.2)	4 (5.2)	12 (11.5)	11 (10.7)
MRSA and MSSA	0	0	0	1 (1.3)	0	1 (0.9)
Total	8 (30.8)	10 (38.4)	9 (11.5)	8 (10.4)	17 (16.3)	18 (17.4)

[a] bedside table, bed rail, door handle; [b] Sampled surfaces; n=77[c] - one sample from a bed rail was lost

DISCUSSION

About 40% of investigated ward surfaces in HC-UFU were contaminated with *S. aureus*, irrespective of whether they had infected patients or not, which reflects the importance of this microorganism within the hospital.

Currently, the MRSA phenotype is responsible for 50% of *S. aureus* infections in critical patients in the USA (Karchmer et al., 2002). In Brazil, this situation is even more severe. Although the proportion is similar, MRSA infection is present in the hospital as a whole (Moraes et al., 2000); in HC-UFU, this phenotype is responsible for 44% of hospital staphylococcal infections (Sadoyama & Gontijo Filho, 2000). In our investigation, among patients listed in the case wards group, 87.5% were infected with MRSA.

The main transmission route for nosocomial infections, particularly those associated with multidrug resistant microorganisms, is contact with health professionals' hands (Boyce, 2004). According to Obee et al. (2007), hand contact surfaces with a high aerobic bacterial count have been found to carry a great number of *S. aureus*. Thus, for hospital surfaces with which hands are likely to make contact, such as door handles and bed rails, the current recommendation is regular cleaning and disinfecting, according to the Association for Professionals in Infection Control and Epidemiology (APIC) (Sehulster et al., 2004). The effectiveness of environmental cleaning is an important factor in strategies to prevent the nosocomial transmission of MRSA (Loveday at al., 2006).

In our qualitative surface evaluation, this pathogen

was present in 40.4% of the wards, without significant differences between cases (34.6%) and controls (46.1%). This result was lower than that observed by Boyce et al. (1997) (71%) in infected patients' rooms, and compatible with the report by Blythe et al. (1998) (46%). Our overall data for specific surfaces of case wards were similar to those observed by Boyce et al. (1997), respectively, for bed rail and table (15.4% vs. 18.0%), door handle (3.8% vs. 3.0%), and floor (30.8% vs. 50.0%), and did not differ significantly from the corresponding controls.

One of the risk factors for infection is the microbial density at the entrance into the patient space (Oie et al., 2002). Shiomori et al. (2002), analyzing qualitative surface contamination between *S. aureus* infected and colonized patients, found: 6.1 CFU/ 10cm^2 (infected) and 2.3 CFU/ 10cm2 (colonized) on the floor and 3.4 CFU/ 10cm^2 (infected) and 1.2 CFU/ 10cm^2 (colonized) on the bed tables. Our quantitative data also showed no differences between case and control wards, suggesting, as in the qualitative analysis, the epidemiological importance of in-patient colonization by *S. aureus* (Shiomori et al., 2002). The door-handle contamination in HC-UFU (7.7%) was lower than reported by Oie et al. (2002) (27.0%). In none of our samples were more than 8 CFU/ 10cm^2 found, and the average of the positive handles was 0.4 CFU/ 10cm^2. It is important to emphasize that, besides a smaller sampled area (10cm^2 rather than the total area of the handle) compared to the cited study, ward doors in HC-UFU usually remain open, unlike the situation when there is a controlled environment.

There is evidence that epidemic MRSA samples are more virulent than non-epidemic, having a higher viability in the environment (Karchmer et al., 2002). Others have reported an absence of differences in virulence between MRSA and MSSA samples (Duckworth & Jordens, 1990). Our results were obtained in endemic conditions and revealed MRSA prevalence among infected (87.5%) and colonized (62.5%) patients but, on ward surfaces, a greater incidence of MSSA (66.7%) than MRSA (33.3%) was found and, when the analysis was restricted to surfaces likely to contaminate hands, the MSSA frequency was even higher (88.9%). These data fit those reported by Oie et al. (2002), when they evaluated *S. aureus* door-handle contamination.

The Index of Microbial Air Contamination (IMA), proposed by Pasquarella et al. (2000) for the assessment of total mesophiles in the air, was used in our study of aerial *S. aureus*, and the values obtained for this microorganism were higher in the air in the case wards (9.5 CFU/plate) than in the controls (4.4 CFU/plate). Pasquarella et al. (2000) report a relationship between air and surface contamination, and Shiomori et al. (2002), using an air volumetric sampling technique, showed that the amount of MRSA on the floor varies with that of the air in wards of patients infected with this microorganism. In our study, this relationship was 66.6% and 60.0%, respectively, in case and control environments. It is important to emphasize that IMA was not intended to evaluate microorganisms in the turbulent conditions usually present in HC-UFU wards, due to the air flow resulting from open windows and doors.

Hygiene standards, proposed by Dancer (2004) for hospital surfaces, include *S. aureus* monitoring (lower than 1 CFU/cm^2) for frequently touched surfaces. Our data on hospital practice meet this requirement and reflect the cleaning routine of wards, which includes the use of alcoholic solution (70%) on surfaces close to patients.

Although the present results point to an extensive (40%) contamination with *S. aureus* in the environment, without significant differences between wards of infected and uninfected patients, the density of microorganisms, important in establishing infection, was low (< 1 CFU/cm^2), reflecting cleaning routines and suitable disinfection in the hospital. The importance of the environment, including surfaces, as a reservoir of nosocomial infections, is still the subject of controversy. Further investigation of this theme is needed and must be encouraged.

RESUMO

Contaminação de superfícies hospitalares em enfermarias ocupadas por pacientes infectados por MRSA ou MSSA em um hospital universitário brasileiro

O ambiente hospitalar, particularmente superfícies, representa um reservatório secundário de patógenos como *Staphylococcus aureus*. O presente estudo foi realizado no Hospital de Clínicas da Universidade Federal de Uberlândia (HC-UFU), no período de Janeiro a Agosto de 2004, com o objetivo de avaliar a contaminação qualitativa e quantitativa de superfícies tocadas pelas mãos (grade, mesa de cabeceira e maçaneta da porta), de piso e de ar atmosférico, por *S. aureus* em enfermarias de pacientes infectados ou não por este microrganismo. Foram investigados dois grupos: pacientes infectados (26) e não infectados (26). Superfícies e ar foram amostrados durante a arrumação das camas, por meio de fitas adesivas estéreis com área de 10cm^2 e placas de exposição de 90mm de diâmetro, respectivamente. As culturas foram realizadas em "Egg Yolk Salt Agar", contendo 7,5% de NaCl e 1% de gema de ovo e a identificação pelos testes de lecitinase e coagulase. Aproximadamente 50% das enfermarias estavam contaminadas por *S. aureus*, sem diferenças entre os dois grupos (46,1% nos infectados versus 53,8% nos controles); a correlação entre as contagens deste patógeno nas superfícies e no ar foi de 66,7%. Embora sua presença nas enfermarias avaliadas tenha sido extensa, a densidade microbiana foi baixa (menos de 1 UFC/cm^2). A associação entre contaminação ambiental e epidemiologia de infecções hospitalares é complexa, exigindo mais investigações para uma melhor compreensão desta relação.

Palavras-chave: contaminação ambiental; infecções hospitalares; *Staphylococcus aureus*.

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