

Prevalence of *Staphylococcus aureus* Introduced into Intensive Care Units of a University Hospital

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Staphylococcus aureus is one of the principal human pathogens that colonize healthy individuals in the community in general, and it is responsible for severe infections in hospitalized patients. Due to an increase in the prevalence of strains of methicillin-resistant *S. aureus* (MRSA), combating these microorganisms has become increasingly difficult. A descriptive study was carried out on 231 patients in intensive care at the Oswaldo Cruz University Hospital (HUOC) in Recife, Brazil between January and April 2003 to determine the prevalence of *S. aureus* and MRSA and to evaluate risk factors for colonization by these bacteria when introduced into Intensive Care Units (ICUs). Body secretions were collected from the nostrils, axillary and perineal regions, and from broken skin lesions, of all patients during the first 48 hours following admission to the ICU. Samples were inoculated into blood agar and mannitol-salt-agar culture medium and identified by Gram staining, and by coagulase, DNase and agglutination (Slidex Staph Test®) tests. Growth in Mueller-Hinton agar with 4% sodium chloride and 6mg/L oxacillin was used to identify MRSA. In addition, the latex agglutination test was performed to identify penicillin-binding protein, PBP 2A. The prevalence of *S. aureus* and MRSA was 87/231 (37.7%) and 30/231 (12.98%), respectively. There was no association between any risk factor studied (age, sex, origin of the patient – whether hospital or community, previous hospitalization, use of current or previous antibiotic therapy, corticotherapy and/or immunotherapy, reason for hospitalization and place of hospitalization) and the presence of *S. aureus*. However, a significant association was established between previous hospitalization and the presence of MRSA (RR:1.85; CI:1.00-3.41; p=0.041). The nostrils were the principal site of colonization by both *S. aureus* (80.4%) and MRSA (26.4%), followed by the perineal area, with rates of 27.6% and 12.6%, respectively. If only the nostrils had been investigated, the study would have failed to diagnose 17 patients (19.5%) as carriers of the pathogen into the ICU, thus contributing towards cross-dissemination.

Key Words: *Staphylococcus aureus*, methicillin-resistant, intensive care unit.

When present in a host, *Staphylococcus aureus* may induce clinically manifested diseases, or the host may remain completely asymptomatic. It can be present in a host and not cause apparent lesions; this condition is known as colonization [1-4].

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Staphylococcus aureus constitutes part of the transient skin flora of up to one-third of the general population, the principal reservoir sites being the nasal vestibule (35%) and the perineal region (20%), as well as the umbilical, axillary and interdigital regions (5%-10%), from where dissemination may occur, provoking disease and permitting transmission to other individuals [5].

Transmission may be carried out by direct contact [6]. In hospitals, health workers [7,8] caring for infected patients or handling objects that have been colonized may contaminate their hands and subsequently transmit the organism to other patients [8,9].

According to the Canadian Pediatric Society, environmental transmission and transmission via air passages are uncommon, except under certain circumstances, such as in burn units and in ICUs, where transmission may occur in cases of tracheostomized patients with pneumonia caused by *S. aureus* [10].

Carriers of *S. aureus* play a key role in epidemiology and in the pathogenesis of the infection, and they are the greatest risk factor, both for the development of hospital infections and for infections acquired in the community [8,11,12]. The majority of staphylococcus infections originate endogenously [13].

In the pre-antibiotic era, around 70% of patients developed metastatic infections, and bacteremia caused by *S. aureus* was responsible for more than 80% of deaths. With the introduction of benzylpenicillin (penicillin G) in the mid-1940s, there was a significant improvement in the prognosis of patients with staphylococcus infections. However, in 1942, the continued use of this antibiotic had already caused the development of strains resistant to benzylpenicillin, due to their ability to synthesize beta-lactamase [14-16].

The increased frequency of strains of benzylpenicillin-resistant *S. aureus* led to efforts to create a penicillin-derivative that would be resistant to hydrolysis by beta-lactamase. Methicillin was synthesized in 1959; by substituting the phenol group of benzylpenicillin for the methoxy group, this new compound was resistant to the action of the staphylococcal beta-lactamase [15]. However, methicillin-resistant *S. aureus* (MRSA) subsequently appeared; these were resistant due to the production of penicillin-binding proteins (PBPs), [3,15,16].

Penicillin-binding proteins are transpeptidases and carboxypeptidases responsible for the synthesis of the bacterial cell wall; they can be targeted by all the beta-lactamic antibiotics [1]. Staphylococcus produces four PBPs, referred to as PBP1-4 [15]; the MRSA strains express sub-type PBP2a or 2', acquired from other species of staphylococcus and coded by the *mecA* gene. PBP2a shows low affinity, not only to methicillin, but to practically all the beta-lactamic antibiotics when compared to PBP2, which renders the MRSA strains resistant to multiple antibiotics [1,3,14,16-18].

With the appearance of strains of *S. aureus* resistant to the majority of beta-lactamic antibiotics, including methicillin and glycopeptides, many studies were designed with the aim of detecting risk factors for MRSA carriage [11,19]. A greater risk of colonization and subsequent infection by MRSA is observed in immunodepressed patients exposed to antibiotic therapy, insulin-dependent diabetics, patients who are using catheters, persons in contact with patients colonized or infected with this bacteria [20], patients over 60 years of age [20-22], patients with dermatoses presenting skin rupture [20,21,23], patients with a history of hospitalization [21,23] or surgery [20,21], patients transferred after prolonged hospitalization, and sufferers of chronic diseases [21].

Routine investigation and the adoption of control measures during the admission of patients to areas of high endemicity for MRSA, such as the ICU [24,25] and the dermatology department [26], are considered effective strategies in terms of costs and benefits. Total costs incurred in a control program are reported to be less than the average costs attributed to MRSA infection [27,28].

We evaluated the prevalence of *S. aureus* and MRSA, and risk factors (age, sex, origin, previous hospitalization, corticotherapy and/or immunosuppressive treatment, antibiotic therapy, admission to ICU, and reason for admission to ICU) for nasal or cutaneous colonization by MRSA, in cases introduced into the ICUs of the Oswaldo Cruz University Hospital (HUOC).

Material and Methods

This study was approved by the Ethics Committee of the HUOC, and all patients or their representatives/guardians were informed about the objectives of the study.

Population

A descriptive study was initially carried out to establish the prevalence of *S. aureus*, after which a

case series study was performed to evaluate the prevalence and risk factors of colonization by MRSA in 231 patients over 12 years of age admitted to the ICUs of the HUOC at the University of Pernambuco, Recife, Brazil from January and April 2003.

Laboratory methods

Swabs were taken daily from the anterior nostrils, axillary and perineal regions and from dermatoses presenting skin rupture, to collect biological material from patients within the first 48 hours of their admission to the ICU. Next, samples were inoculated in blood agar medium containing 5% ovine blood and 7.5% mannitol saline agar (Probac do Brasil®), and incubated at 35°C for 24 hours. *Staphylococcus aureus* was identified using Gram staining, a coagulase tube test (Laborclin), and a DNase test (Oxoid®). The coagulase tube test was read after 4 and 24 hours of incubation. In case of discrepancy between the coagulase and the DNase tests, a latex agglutination test (Slidex Staph Test®) was carried out. MRSA was detected by its growth in Mueller Hinton agar medium, to which 4% sodium chloride and 6 mg/L oxacillin (Probac do Brasil®) had been added, following incubation for 24 hours at 35°C. The latex agglutination test (Oxoid®) was used to check for PBP2a.

Variables

The following variables were recorded in the patients during the first 48 hours following admission to the ICU: age, sex, origin (whether admitted to the ICU from hospital or from the community), previous hospitalization, current or previous antibiotic therapy, current or previous corticotherapy and/or immunotherapy, reason for hospitalization (clinical or surgical), type of ICU (cardiac surgery, coronary, general or infectious/parasitic diseases).

For the purposes of analysis, patients' age was divided into four groups: 12-19, 20-39, 40-59 and ≥60 years of age.

The patient was defined as having originated from the hospital when he/she had been in the hospital for at

least 48 hours prior to admission in the ICU. The patient was defined as having originated from the community when he/she was admitted to the ICU from his/her residence or had been admitted to another department of the hospital or to another hospital fewer than 48 hours previously.

Reason for admission to the ICU was classified as clinical or surgical. This variable was defined as surgical when the cure of the disease that was the reason for admission was related to surgical intervention.

Prior hospital admission was defined as the patient presenting a history of hospitalization within the three months preceding admission to the ICU. These same criteria were adopted to define the prior use of antibiotic therapy, corticotherapy and/or immunosuppressive therapy.

Statistical analysis

Data entry was performed using the EPI-INFO software, version 6.04d of November 2002; analysis was carried out considering a significance level of 0.05. The Chi-square test, with and without Yates correction, and Fisher's Exact test for tables in which more than 25% of cases were below 5, were also used in the analysis. For the descriptive analysis, descriptive parameters of mean, standard deviation, median and mode were used, as well as absolute and relative frequency distributions.

Results

Of the 231 patients admitted to the ICUs of the HUOC, 87 (37.7%) tested positive for *S. aureus* and 30 (12.9%) for MRSA.

No association was identified between the risk factors and patients who introduced *S. aureus* into the ICUs.

With reference to the presence of MRSA, there were no significant associations with gender (RR = 0.78; 95% CI 0.44 – 1.39%; $p = 0.538$) or age group ($\chi^2 = 2.15$; $P = 0.542$), the mean age of these patients being 48 years.

Of the 87 patients who tested positive as carriers of *S. aureus*, 41 (47%) and 46 (53%) were admitted for clinical and surgical reasons, respectively. Fifteen of the 41 patients admitted for clinical reasons (37%) and 15 of the 46 patients admitted for surgical reasons (33%) were carriers of MRSA. No association was detected between reason for admission to the ICU and carriage of MRSA (RR = 1.12; 95% CI 0.63 – 2.00; $p = 0.697$).

The origin of the patient prior to admission to the ICU was not identified as being a risk factor for MRSA carriage (RR = 1.30; 95% CI 0.47 – 3.58; $p = 0.590$). A total of 76/87 patients (87%) originated from the hospital and 11/87 (13%) arrived directly from the community. Of these, 27/76 (36%) and 3/11 (27%), respectively, were carriers of MRSA.

Regarding admission to the ICU, more cases of MRSA were found in the general ICU and in the ICU for infectious/parasitic diseases (Table 1).

The use of antibiotics, corticoids and/or systemic immunosuppressants prior to or during the study was not identified as a risk factor for MRSA carriage. Although the relative risk of previous use of corticoids and/or immunosuppressants is reported as 2.28, the confidence interval was large (95% CI 1.20 – 4.34), $p = 0.696$; if the sample size had been greater, we might have been able to identify a significant association.

An association was shown between previous hospitalization and the resistance of *S. aureus* to methicillin (Table 2).

With reference to the site of colonization, we detected a predominance of *S. aureus* over MRSA in the nostrils (Figure 1). When we evaluated the patients regarding the presence of single or multiple sites of colonization, the nostrils continued to be the most prevalent site in both cases (Figure 2).

Discussion

We found cases of *S. aureus* introduced into ICUs by patients, who were specifically classified as originating either from a hospital or from a community source.

The prevalence of 37.7% found for *S. aureus* in our study was greater than the 25.1% observed in a retrospective study carried out between April 1998 and March 2000, involving 565 patients submitted to collection of secretion from the anterior nostrils during the first 24 hours following admission to the ICU [27]. Our figures, however, are close to the 35.7% found in a review study in which the mean rate of positivity for this condition was evaluated at hospital admission [28].

The prevalence of MRSA, detected among carriers of *S. aureus* in our study was greater than that found in ICUs in France, based on reports by Girou et al. and by Lucet et al., who reported rates of 4.1% and 6.9%, respectively [21,25]. However, it was lower than the 46% found in a prospective study carried out in two ICUs in Brazil [16]. Lower rates found in France may be a result of the efficacy of the programs designed for the control of these carriers, which have been classified as advantageous in areas of high endemicity such as ICUs, even considering the elevated costs of such programs [24].

Due to the worldwide increase in the prevalence of MRSA, the hospitalization of carriers is the most frequent way in which the condition is introduced into the health services and, consequently, disseminated [23]. Therefore, identifying it early, within 48 to 72 hours following admission of the carrier to the ICU, provides a way of reducing the risk of infection to the carrier, as well as avoiding cross-transmission to other patients and to health workers.

The prevalence of MRSA in the different age groups also differs from rates found in the literature. Lucet et al. reported that 73% of patients were over 60 years of age, data that is strongly associated with being a carrier of MRSA, independent of any other risk factor [21]. Nevertheless, although Korn et al. reported a frequency of 62% among patients over 60 years of age, no significant association was found between age and being a carrier of MRSA at admission to the ICU [16].

The reason for hospitalization, whether clinical or surgical, was not a risk factor for colonization by MRSA, and these results are in agreement with published studies on the evaluation of patients

Table 1. Distribution of the 87 patients who were carriers of *Staphylococcus aureus* in the intensive care units (ICUs), according to type of ICU and resistance to methicillin – Oswaldo Cruz University Hospital, January to April 2003

ICU	<i>Staphylococcus aureus</i>					
	Resistant		Sensitive		Total	
	N	%	N	%	N	%
Cardiac surgery	13	33.3	26	66.7	39	44.8
Coronary	06	31.6	13	68.4	19	21.8
Infectious/parasitic diseases	04	36.4	07	63.6	11	12.6
General	07	38.9	11	61.1	18	20.7
Total	30	34.5	57	65.5	87	100.0

$\chi^2 = 0.27$, $P = 0.966$; χ^2 = Chi-square test value.

Table 2. Distribution of the 87 patients who were carriers of *Staphylococcus aureus* in the ICUs, with respect to previous hospitalization, according to resistance to methicillin. Oswaldo Cruz University Hospital. January to April 2003

Previous hospitalization	<i>Staphylococcus aureus</i>					
	Resistant		Sensitive		Total	
	N	%	N	%	N	%
Yes	19	45.2	23	54.8	42	48.3
No	11	24.4	34	75.6	45	51.7
Total	30	34.5	57	65.5	87	100.0

$\chi^2 = 4.16$, $p = 0.041$, $RR = 1.85$ (95% CI 1.00 – 3.41). RR = relative risk; CI = confidence interval; χ^2 = Chi-square test value.

Figure 1. Distribution of the 87 patients in the intensive care units, who tested positive in the microbiological exam for *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA), according to sites of colonization. Oswaldo Cruz University Hospital. January to April, 2003.

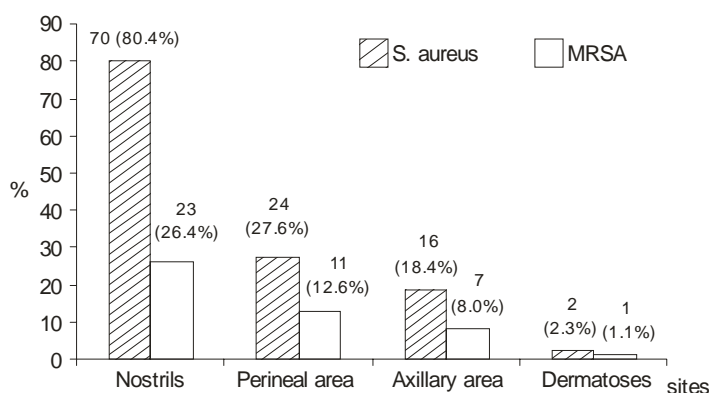
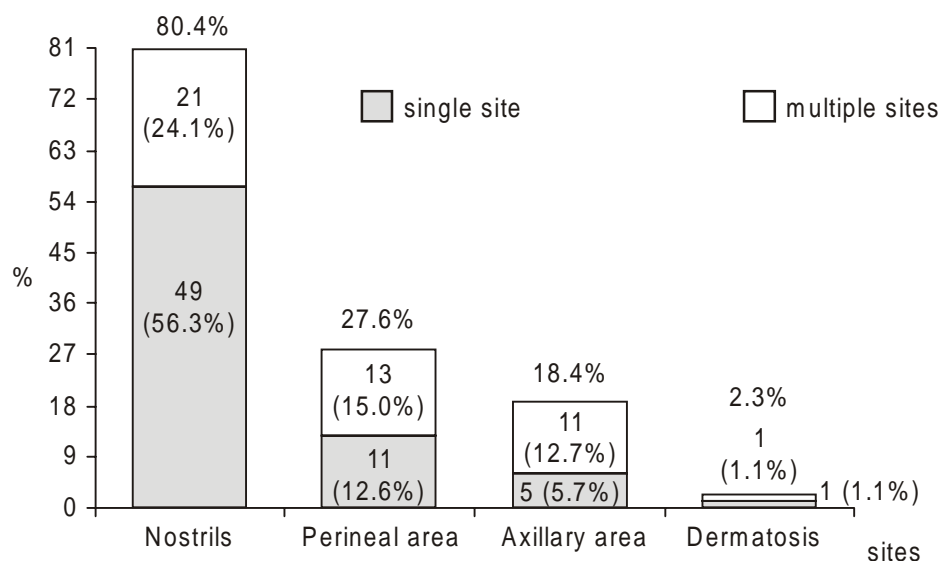


Figure 2. Distribution of the 87 patients in the intensive care units, who tested positive for *Staphylococcus aureus*, according to whether there was a single site of colonization or multiple sites. Oswaldo Cruz University Hospital. January to April, 2003.



transferred from other intra- or extra-hospital departments and of patients coming directly from the community to the ICU [21]. One explanation for the similar prevalence found for the different reasons for admission to the ICU may have been the similarity of risk factors to which patients were submitted in virtue of their poor general condition. They required invasive procedures, as well as multiple antibiotic therapies, regardless of the primary cause of their hospitalization.

With respect to the higher frequency of MRSA carriers in the general ICU and in the ICU for infectious/parasitic diseases, one hypothesis may be that these patients are more seriously ill, and in general, the reason for their hospitalization is inflammatory or infectious processes, more frequently requiring patients to be submitted to multiple antibiotic therapies.

In relation to the origin of these patients, our results are in agreement with those of Korn et al. regarding the fact that hospital origin is not a risk factor for identifying MRSA carriers at the time of admission to the ICU [16], but these results differ from data published by Porter et al., who reported a significant association between being a carrier of *S. aureus*,

including MRSA, and originating from a hospital [29]. On the other hand, our finding that 37.3% of *S. aureus* carriers came directly from the community is similar to results from previous studies [29].

Antibiotic therapy, corticotherapy and the use of immunosuppressants, either prior to or at the time of testing, were not found to be risk factors for positivity to MRSA. Korn et al. reported similar results when they analyzed the previous use of antibiotics in relation to colonization by MRSA [16]. In a univariate study, Lucet et al. also reported no association between antibiotic therapy and immunosuppression as risk factors for positivity to MRSA at the time of admission to the ICU [21]. According to the literature, being a carrier of *S. aureus* and MRSA is a risk factor for the development of infections during hospitalization in an ICU and increases the mortality rates of these patients [1,2,6].

With respect to previous hospitalization, we observed a significant association with MRSA carriers, increasing the risk of colonization by MRSA 1.85 times. These results are not in agreement with those reported by Korn et al., who observed no such association when

they investigated this variable [16]. However, our data are in agreement with results published by Lucet et al. [21].

It is possible that the patients who came from a hospital had been hospitalized for an insufficient period of time for them to become contaminated by *S. aureus* or MRSA. This would explain the similar prevalence rate for the patients coming from a hospital and those coming directly from the community to the ICU. On the other hand, patients who had been previously hospitalized may have been submitted to antibiotic therapy with no provision for methicillin-resistant *S. aureus*, which would favor MRSA.

The most prevalent site of *S. aureus* [19,30] and MRSA [21,25] has been reported to be the anterior nostrils and our data are in agreement with this observation. The second most prevalent site in our study was the perineal area. If the study had been restricted to a single site, that site should have been the anterior nostrils, where there was the greatest prevalence of *S. aureus* and of MRSA. However, it is important to emphasize that if this had been the methodology adopted for this study, 17 patients (19.5%) would not have been diagnosed, permitting the re-colonization of these patients and the dissemination of the bacteria.

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