



Article/Artigo

Relationship between antibiotic consumption, oropharyngeal colonization, and ventilator-associated pneumonia by *Staphylococcus aureus* in an intensive care unit of a Brazilian teaching hospital

Relação entre consumo de antibióticos, colonização de orofaringe e pneumonia associada à ventilação por *Staphylococcus aureus* em um hospital de ensino brasileiro

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ABSTRACT

Introduction: This study evaluated the consumption of major classes of antibiotics, the colonization of the oropharynx of patients on mechanical ventilation, and the risk of ventilator-associated pneumonia (VAP) caused by *Staphylococcus aureus* in an intensive care unit for adults.

Methods: A case-control study was carried out using colonized patients (cases) by oxacillin-resistant *S. aureus* (ORSA) and (controls) oxacillin-sensitive *S. aureus* (OSSA) from May 2009 to August 2010. The occurrence of VAP by *S. aureus* was also evaluated in the same period. Antibiotic consumption was expressed as the number of defined daily doses (DDD)/1,000 patient-days for glycopeptides, carbapenems, and extended-spectrum cephalosporins. **Results:** Three hundred forty-six (56.1%) patients underwent mechanical ventilation with a frequency of oropharyngeal colonization of 36.4%, corresponding to 63.5% for ORSA and 36.5% for OSSA. The risk of illness for this organism was significant ($p \leq 0.05$), regardless of whether colonization/infection was by ORSA or OSSA. The consumption of antibiotics was high, mainly for broad-spectrum cephalosporins (551.26 DDDs/1,000 patient-days). The high density of use of glycopeptides (269.56 DDDs/1,000 patient-days) was related to colonization by ORSA (Pearson $r=0.57/p=0.02$). Additionally, age >60 years, previous antibiotic therapy, and previous use of carbapenems were statistically significant by multivariate analysis. **Conclusions:** There was a significant relationship between the colonization of the oropharyngeal mucosa and the risk of VAP by both phenotypes. The use of glycopeptides was related to colonization by ORSA.

Keywords: *Staphylococcus aureus*. Oropharyngeal colonization. VAP. Antibiotic consumption.

RESUMO

Introdução: Este estudo avaliou o consumo das principais classes de antibióticos, a colonização de orofaringe de pacientes sob ventilação mecânica e o risco de pneumonia associada à ventilação (PAV) causada por *Staphylococcus aureus* em uma unidade de terapia intensiva (UTI) de adultos. **Métodos:** Foi realizado um estudo caso-controle, sendo caso os pacientes colonizados pelo oxacilina-resistente *Staphylococcus aureus* (ORSA), e controle aqueles pelo oxacilina-sensível *Staphylococcus aureus* (OSSA), no período de maio de 2009 a agosto de 2010. A ocorrência de PAVs por *S. aureus* também foi avaliada no mesmo período. O consumo de antibióticos foi expresso pelo número de doses diárias definidas (DDD)/1.000 pacientes-dia para glicopeptídeos, carbapenêmicos e cefalosporinas de amplo espectro. **Resultados:** Trezentos e quarenta e seis (56,1%) dos pacientes foram submetidos à ventilação mecânica com uma frequência de colonização de orofaringe de 36,4%, correspondendo a 63,5% e 36,5% de ORSA e OSSA, respectivamente. O risco de adoecimento por este microrganismo foi significativo ($p \leq 0,05$), considerando se a colonização/infecção foi por ORSA ou OSSA. O consumo de antibióticos foi alto, principalmente para cefalosporinas de amplo espectro (551,26 DDDs/1.000 pacientes-dia). A elevada densidade de uso de glicopeptídeos (269,56 DDDs/1.000) foi relacionada com a colonização pelo ORSA (Pearson $r=0,57/p=0,02$). Adicionalmente, idade > 60 anos, terapia antibiótica prévia e uso prévio de carbapenêmicos foram estatisticamente significantes por análise multivariada. **Conclusões:** Foi observada uma relação significativa entre a colonização da mucosa de orofaringe e o risco de PAV por ambos fenótipos. O uso de glicopeptídeos foi relacionado com a colonização pelo ORSA.

Palavras-chaves: *Staphylococcus aureus*. Colonização de orofaringe. PAV. Consumo de antibióticos.

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Received in 29/06/2011

Accepted in 03/08/2011

INTRODUCTION

Nosocomial infections pose a significant threat to patients worldwide, and antibiotic-resistant bacteria are related to a greater impact on morbidity/mortality and the costs of such infections¹⁻³. Ventilator-associated pneumonia (VAP) is the most frequent infection acquired in the intensive care unit (ICU), occurring in 9% to 24% of patients intubated for longer than 48h^{4,5}. It is associated with increased morbidity, prolonged hospitalization, and increased healthcare costs⁶.

Each antibiotic prescription has an environmental and ecological consequence^{9,10}. Many case-control studies demonstrate prior antibiotic exposure, particularly with cephalosporins and fluoroquinolones, as a risk factor for both oxacillin-resistant *Staphylococcus aureus* (ORSA) colonization and infection^{2,10}. Antibiotics such as fluoroquinolones and cephalosporins are well known to increase the expression of fibronectin adhesions, facilitating adherence and ability to colonize, and many other virulence factors such as toxins that might cause colonization and develop into infection¹⁰⁻¹².

Increased numbers of ORSA were demonstrated in the noses of carriers receiving fluoroquinolones or β -lactams compared with controls¹³. Presumably this is due to a competitive advantage achieved by antibiotic administration, ablating the normal protective commensal flora, including oxacillin-sensitive *S. aureus* (OSSA), allowing multiplication of the ORSA with increased potential for contaminating the environment¹⁰.

The aim of this study was to evaluate the consumption of major classes of antibiotics, the colonization of the oropharynx of patients on mechanical ventilation (MV), and the risk of VAP caused by *S. aureus* susceptible or resistant to oxacillin, as well as other risk factors in patients in an adult mixed ICU from a Brazilian university hospital.

METHODS

Setting

The Uberlândia Federal University-Hospital Clinic (UFU-HC) is a teaching hospital with 500 beds and a clinical-surgical ICU for adults with 15 beds.

Study design

A case-control study was carried out using colonized patients by ORSA (case) and OSSA (control) from May 2009 to August 2010, when 617 patients were admitted. The cultures of oropharyngeal secretions were taken at baseline (< 24h in the ICU) and every 2 days until the confirmation of colonization or discharge. In the same period the occurrence of VAPs by *S. aureus* was also evaluated.

The patients with VAP were under MV for a period ≥ 48 h after being admitted to the ICU, with new and/or progressive radiological infiltrate and experiencing at least two of the following conditions: purulent sputum, temperature higher than 38.5°C or lower than 35°C, and leukocyte count higher than 10,000/ μ L with deviation to the left or lower than 3,000/ μ L; and positive quantitative culture of the endotracheal aspirate (count $\geq 10^6$ CFU/ml)¹⁴⁻¹⁶.

Antibiotic consumption was expressed as the number of defined daily doses (DDD)/1,000 patient-days for the following classes: glycopeptides (vancomycin and teicoplanin), carbapenems (imipenem, meropenem, and ertapenem), and extended-spectrum cephalosporins (ceftriaxone and cefepime).

Additionally, the following patient data were collected: age, gender, invasive procedures, admission diagnosis, previous use of antibiotics and corticoids, hospitalization, and MV times.

Microbiological techniques

The collection of clinical specimen obtained from the oropharynx was made with a swab, and the specimen was transported in sterile tube with 1ml of trypticase soy broth supplemented with 6.5% NaCl. Endotracheal aspirate was collected by probe n 12 early in the morning by the health professionals (physiotherapists and nurses) in charge of the procedure, and was transported in a sterile tube to the Microbiology Laboratory of UFU. Oropharynx and endotracheal aspirate samples were cultivated in salty mannitol agar (Biobrás, Brazil) by qualitative and quantitative techniques, respectively. The *S. aureus* isolates were identified by mannitol fermentation, Gram stain, and catalase, free coagulase, and bond coagulase tests. Resistance to

oxacillin was detected by means of a screening test in salty mannitol agar (Biobrás, Brazil) incorporated with 6 μ g/ml of oxacillin and 4.5% of NaCl.

Statistical analysis

Univariate comparisons were carried out using the Qui-Square (X^2) and Fisher's exact tests. Multivariate analysis was carried out by simple or multiple logistic regression when appropriate, and the values were included when significance was <0.25 in univariate analysis or in case of clinical relevance. Pearson's correlation coefficient was used to determine the relationship between antibiotic consumption and trends in resistance. The results were considered statistically significant at a level of 5%. The epidemiological data were analyzed through the programs Epi-Info version 5.0¹⁷, GraphPad prism 3.0¹⁸, and BioEstat 5.0¹⁹.

Ethical considerations

The Ethics Committee for Human Research of UFU approved the project.

RESULTS

From May 2009 to August 2010 a total of 617 adult patients were admitted to the adult ICU of the UFU-HC. Three hundred forty-six (56.1%) patients who were submitted to MV. All these patients were surveyed, and 126 (36.4%) were colonized in the oropharynx with *S. aureus*; 63.5% were ORSA and 36.5% were OSSA, corresponding to 10.97 ± 6.67 and 6.26 ± 4.49 colonizations/1,000 patient-days for these microorganisms, respectively. Among the 320 (51.9%) patients submitted to MV for longer than 48h, VAP was diagnosed in 81 (25.3%) patients, the main agents being represented by *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Although high frequencies of colonization of the mucous membranes of the oropharynx by *S. aureus* (126/346; 36.4%), ORSA (80/346; 23.1%), and OSSA (46/346; 13.3%) were found, the participation of these microorganisms in VAPs was low at 11 (13.6%) of 81, 4 (4.9%) of 81, and 7 (8.6%) of 81, respectively.

The risk of illness in patients colonized in oropharynx was 7 (5.5%) of 126, 4 (5%) of 80, and 3 (6.5%) of 46 for *S. aureus*, ORSA, and OSSA, respectively. Four patients had episodes of VAP (4 OSSA) without previous colonization by this microorganism. The risk of pneumonia was significant ($p \leq 0.05$) by univariate analysis, regardless of whether colonization/infection was by ORSA or OSSA (Table 1).

TABLE 1 - Mechanically ventilated patients colonized by *Staphylococcus aureus*, ORSA, and OSSA and the risk of acquiring VAP in the adult ICU of the UFU-HC.

	Colonized	Not colonized		Odds	Confidence
<i>Staphylococcus aureus</i>	n=126 (%)	n=220 (%)	P	ratio	interval
VAP	7 (5.5)	4 (1.8)	0.11	3.18	0.81-13.20
ORSA	colonized by ORSA	not colonized by ORSA		Odds	Confidence
	n= 80 (%)	n= 266 (%)	P	ratio	interval
VAP by ORSA	4 (5.0)	0	0.003	-	-
OSSA	colonized by OSSA	not colonized by OSSA		Odds	Confidence
	n= 46 (%)	n= 300 (%)	P	ratio	interval
VAP by OSSA	3 (6.5)	4 (1.3)	0.05	5.16	0.88-28.55

ORSA: oxacillin-resistant *Staphylococcus aureus*; **OSSA:** oxacillin-sensitive *Staphylococcus aureus*; **VAP:** ventilator-associated pneumonia; **ICU:** intensive care unit; **UFU-HC:** Uberlândia Federal University-Hospital Clinic.

The average time to colonization was slightly longer for ORSA (5.8 days) than for OSSA (5.0 days) (Table 2). Oropharyngeal colonization was associated with the length of stay in the ICU ($p = 0.0001$), with rates of 93.7% (75/80 patients) for ORSA and 65.2% (30/46 patients) for OSSA, from the first week in the unit (Figure 1); 6.3% (5/80) and 34.8% (16/46) of patients were admitted to the ICU with ORSA and OSSA, respectively.

The consumption of antibiotics was high, mainly for broad-spectrum cephalosporins (551.26 DDDs/1,000 patient-days). The high density of use of glycopeptides (269.56 DDDs/1,000 patient-days) was related to colonization by ORSA (Pearson $r = 0.57/p = 0.02$) (Figure 2). Additionally, age >60 years ($p = 0.04$), previous antibiotic therapy ($p = 0.04$), and previous use of carbapenems ($p = 0.03$) were statistically significant by univariate and multivariate analysis (Tables 2 and 3).

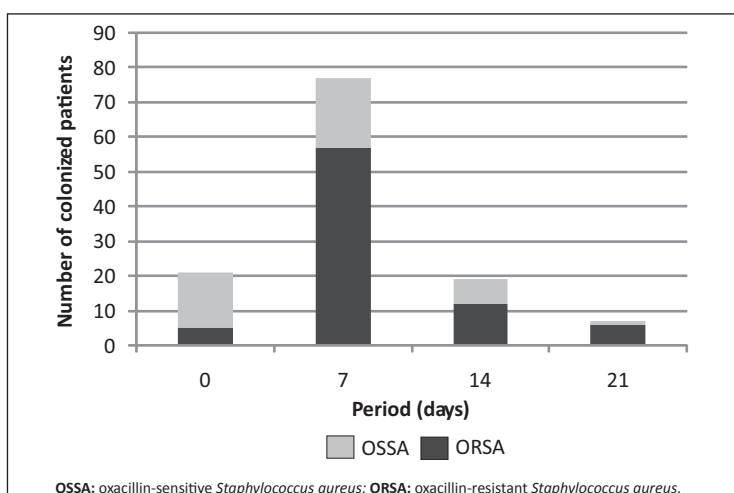


FIGURE 1 - Time for the development of oropharyngeal colonization by *Staphylococcus aureus* in hospitalized patients on mechanical ventilation in the adult Intensive Care Unit of the Uberlândia Federal University-Hospital Clinic.

TABLE 2 - Characteristics of oropharynx colonization by *Staphylococcus aureus* with resistance (ORSA) or sensitivity (OSSA) to oxacillin in interned patients on mechanical ventilation in ICU for adult patients of the UFU-HC

Characteristics	ORSA n=80 (%)	OSSA n=46 (%)	P	Odds ratio	Confidence interval
Gender					
male	54 (67.5)	37 (80.4)	0.18	0.51	0.19-1.29
female	26 (32.5)	9 (19.6)	0.18	1.98	0.77-5.16
Age > 60 years	25 (31.3)	6 (13.0)	0.04 [#]	3.03	1.05-9.14
Invasive procedures					
tracheostomia	9 (11.3)	3 (6.5)	0.53	1.82	0.42-9.01
CVC	73 (91.3)	41 (89.1)	0.76	1.27	0.32-4.85
VP	78 (97.5)	44 (95.6)	0.62	1.77	0.17-18.41
NP	75 (93.7)	44 (95.6)	1.00	0.68	0.09-4.22
drain	11 (13.7)	6 (13.0)	0.87	1.06	0.33-3.53
Use of corticoids	24 (30.0)	10 (21.8)	0.42	1.54	0.61-3.94
Previous antibiotic therapy	72 (90.0)	33 (71.7)	0.02 [#]	3.55	1.22-10.49
Admission diagnosis					
clinical	34 (42.5)	10 (21.8)	0.03 [#]	2.66	1.08-6.65
surgical	11 (13.7)	6 (13.0)	0.87	1.06	0.33-3.53
trauma	35 (43.8)	30 (65.2)	0.06	0.47	0.21-1.05
Infection (VAP)	4 (5.0)	3 (6.5)	0.70	0.75	0.13-4.50
Use of antibiotics					
≥ 3	27 (33.7)	10 (21.8)	0.22	1.83	0.74-4.64
carbapenems	24 (30.0)	5 (10.9)	0.02 [#]	3.51	1.14-11.53
glycopeptides	27 (33.7)	13 (13.1)	0.66	1.29	0.55-3.08
3 rd and 4 th generations cep ^Y	42 (52.5)	25 (54.3)	0.99	0.93	0.42-2.05
Penicillins					
amphicillin	11 (13.7)	9 (19.6)	0.54	0.66	0.23-1.91
oxacillin	3 (3.7)	6 (13.0)	0.07	0.26	0.05-1.26
fluoroquinolones	14 (17.5)	4 (8.7)	0.27	2.230	62-8.65
others*	42 (52.5)	21 (45.6)	0.58	1.32	0.60-3.91
Mean time to colonization (days)	5.8 ± 4.1	5.0 ± 6.5	-	-	-

ORSA: oxacillin-resistant *Staphylococcus aureus*; OSSA: oxacillin-sensitive *Staphylococcus aureus*; ICU: intensive care unit; UFU-HC: Uberlândia Federal University-Hospital Clinic; CVC: central vascular catheter; VP: vesical probe; NP: nasogastric probe; VAP: ventilator-associated pneumonia; Y: cephalosporins; *cephazolin, clindamycin, chloramphenicol, erythromycin, fluconazol, gentamicin, metronidazol, nistatin, rifampin, # $p \leq 0.05$.

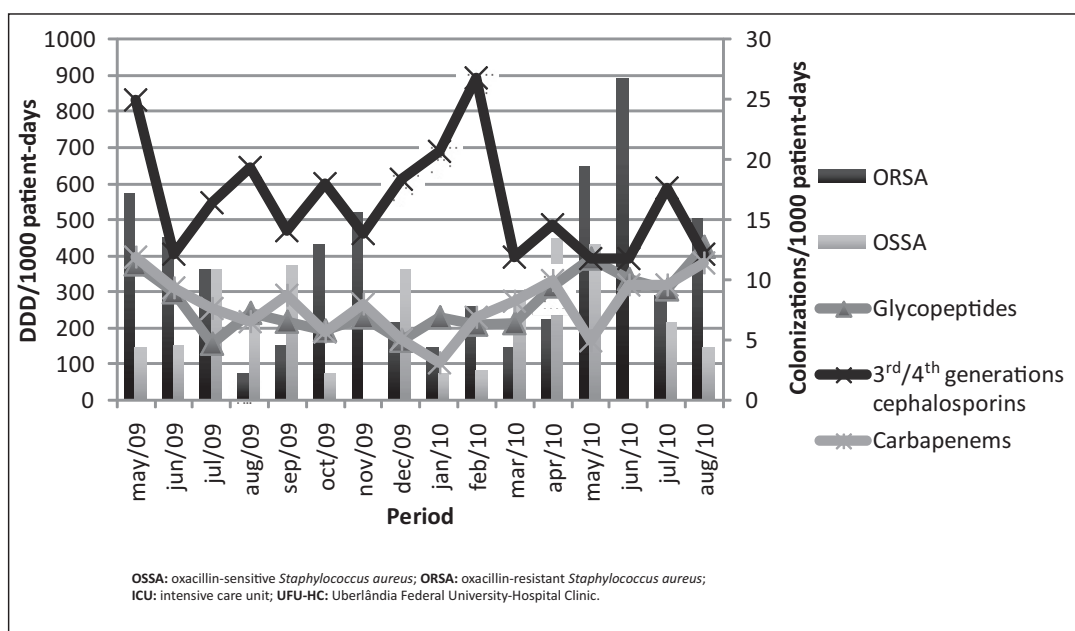


FIGURE 2 - Relationship between the density of antibiotic use and density of oropharyngeal colonization by *Staphylococcus aureus* in hospitalized patients on mechanical ventilation in adult ICU of the UFU-HC.

TABLE 3 - Multivariable regression analysis of factors associated with oropharynx colonization by *Staphylococcus aureus* with resistance (ORSA) to oxacillin in interned patients on mechanical ventilation in ICU for adult patients of the UFU-HC.

Variables	P	Odds ratio	Confidence interval
Age > 60	0.04	3.23	1.06-9.88
Previous antibiotic therapy	0.04	3.31	1.07-10.20
Previous use of carbapenems	0.03	3.68	1.10-12.32
Clinical diagnosis admission	0.07	3.14	0.89-11.07
Trauma diagnosis admission	0.40	1.64	0.52-5.13
Use of three or more antibiotics	0.49	0.72	0.28-1.84
Previous use of fluoroquinolones	0.95	1.04	0.26-4.15

ICU: intensive care unit; UFU-HC: Uberlândia Federal University-Hospital Clinic; P: p-value.

DISCUSSION

Staphylococcus aureus was colonizing the oropharynx in 36.4% of patients undergoing MV at the ICU of the UFU-HC, with 63.5% corresponding to ORSA and 36.5% to OSSA. The colonization of mucous membranes of the upper respiratory tract with potentially pathogenic microorganisms is particularly common in critically ill patients, especially those with decreased level of consciousness, which occurs frequently in ICUs²⁰. For these patients, especially in the ICU, the normal microbiota undergoes drastic changes due to the use of antibiotics^{21,22} and the action of proteases that decrease immunoglobulin and fibronectin present in the mucosa, preventing the adherence of microorganisms of the normal microbiota, favoring Gram-negative bacilli and *S. aureus*^{22,23}.

Oropharyngeal and tracheal colonization plays a central role in the pathogenesis of VAP^{16,24}. In studies of intensive-care patients, ORSA colonizes the throat more frequently than it does either the nose or the groin/axilla²⁵⁻²⁸, and nasal colonization with ORSA is a poor predictor for the subsequent occurrence of ORSA lower respiratory tract infections that require antimicrobial

treatment²⁹. On the other hand, there is less information on the etiopathogenesis for the development of VAP by ORSA. DNA genomic analysis demonstrated that an identical strain was isolated from oropharyngeal or gastric samples and bronchial samples in all but three cases of pneumonia, due to *S. aureus*^{30,31}. In our study 5.5% of patients colonized by *S. aureus* developed VAP as opposed to just 1.8% of patients not colonized ($p > 0.05$), and among individuals colonized by phenotypes ORSA and OSSA, 5% and 6.5% developed VAP, respectively, as opposed to just 0% and 1.3% ($p = 0.003/p = 0.05$) of patients not colonized for these phenotypes, respectively. However, *S. aureus* was isolated in only 13.6% of episodes of VAP, the main agents being represented by *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

In a recent multi-centric study including 1,290 patients infected in the ICUs of hospitals in South and Central America, accounting for 60.3% of inpatients, pneumonia accounted for most (66%), predominated (70.9%) by Gram-negative bacteria, while ORSA accounted for just 10.4%³². Recent data from the European Antimicrobial Resistance Surveillance Network show that six countries reported decreasing trends in the proportion of ORSA among *S. aureus* isolates from invasive infections for the period 2006-2009. This is likely due to sustained efforts to contain the spread of ORSA in hospitals and other healthcare facilities³³. In three ICUs in France the acquisition of ORSA was reduced from 7% to 3% through multiple interventions, including active surveillance cultures, contact precautions, and the use of alcohol hand rub^{34,35}. In other regions/countries, however, *S. aureus*, particularly ORSA, remains endemic in larger hospitals as a major cause of bloodstream infections and pneumonia^{32,36,37}. In this investigation, their participation in VAPs was small compared with that of Gram-negative bacilli, although the rates of oropharyngeal colonization are significant, representing a potential risk for developing this infection.

With a hospital stay of 5 days or longer, the patient is at greater risk of acquiring more resistant pathogens, and empirical treatment with broad-spectrum antimicrobial agents should be prescribed³⁸. In our

study, the mean time to colonization was 5.8 for ORSA and 5.0 for OSSA, and oropharyngeal colonization occurred in 93.7% of patients with ORSA and 65.2% with OSSA, from the first week in the unit.

Antibiotic pressure has been described as a risk factor for the acquisition of ORSA^{39,41}, and the use of antibiotics during and prior to hospitalization in ICU has been shown to increase the number of ORSA infections⁴¹⁻⁴⁴. Our results show an increased risk of acquiring ORSA if antibiotics are administered prior and during hospitalization in ICU ($p \leq 0.05$). A meta-analysis has shown that subjects who have been exposed to antibiotic therapy have an almost 2-fold chance of acquiring ORSA as opposed to non-exposed subjects. This risk is almost three times greater after the use of fluoroquinolones and glycopeptides beyond the cephalosporins². Aldeyab et al.⁴⁵ built a multivariate ARIMA model (time series analysis) to relate ORSA incidence with antibiotic usage. Retrospective analysis of a 5-year data set showed that temporal variation in ORSA incidence followed temporal variations in the use of fluoroquinolones, broad-spectrum cephalosporins, macrolides, and amoxicillin/clavulanic acid. In our study the use of antibiotics was high compared with that in American and European studies^{46,47}, mainly for broad-spectrum cephalosporins, with higher recovery of ORSA; however, the high density of use of glycopeptides in the ICU was significantly related to the acquisition of the multidrug-resistant phenotype by patients, as well as previous use of carbapenems. In addition, patients who had prior use of fluoroquinolones were two times more likely to acquire ORSA ($p > 0.05$). The need to improve antibiotic prescribing in hospitals worldwide is urgent. Guidelines from the Society of Hospital Epidemiologists of America and the Infectious Diseases Society of America strongly support the wide diffusion of antibiotic stewardship programs in healthcare settings^{48,49}. Changing hospital antibiotic use is a challenge of formidable complexity. Many determinants (cultural, contextual, and behavioral) affect antibiotic use in hospitals. The great diversity of the determinants dictates that measures or strategies to improve antibiotic use need to be equally diverse⁵⁰.

Age >60 years was considered an independent risk factor for the acquisition of ORSA ($p = 0.04$), as were previous antibiotic therapy ($p = 0.04$) and previous use of carbapenems ($p = 0.03$). These risk factors correspond to what is reported in the literature, which includes underlying diseases, prior hospitalization, duration of hospitalization, admission to ICU, central venous catheterization, enteral feeding, nursing staff workload, and poor compliance with hand hygiene^{2,39,51,52}.

There was a significant relationship between colonization of the oropharyngeal mucosa and the risk of VAP by ORSA or OSSA. The consumption of antibiotics was high mainly for broad-spectrum cephalosporins, and the high density of use of glycopeptides was associated with colonization by ORSA. Age >60 years, previous antibiotic therapy, and previous use of carbapenems were considered independent risk factors for acquisition of ORSA. However, despite a high rate of colonization by this microorganism, its involvement in the etiology of VAPs was small, overcome by Gram-negative bacilli.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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