Risk Factors, Length of Stay and In-Hospital Mortality of Methicillin-Resistant *Staphylococcus aureus* Infections: A Case-Control Study

Fatores de Risco, Tempo de Internamento e Mortalidade Intra-Hospitalar de Infeções por *Staphylococcus aureus* Resistentes à Meticilina: Um Estudo Caso-Controlo

Rúben RODRIGUES✉[1], Rui PASSADOURO[1], Odete GOMES[2], Ricardo CASTRO[3]
Acta Med Port 2020 Mar;33(3):174-182 ▪ https://doi.org/10.20344/amp.10952

ABSTRACT

Introduction: The emergence of strains of methicillin-resistant *Staphylococcus aureus* is a serious therapeutic challenge in healthcare provision. With this study, we aimed to investigate the risk factors and clinical outcomes (mortality and length of hospital stay) associated with methicillin-resistant *Staphylococcus aureus* infections in patients admitted to a district hospital in Portugal.

Material and Methods: A case-control study was performed in 96 cases and 122 controls, selected, respectively, as function of antibiotic resistance or sensitivity to methicillin. Data were obtained through consultation of clinical records and subjected to multivariate statistical analysis.

Results: We identified the following independent risk factors for the occurrence of methicillin-resistant *Staphylococcus aureus* infection: urinary catheter (aOR = 10.62, 95% CI 3.66 – 30.78), prior use of antibiotics in the last 30 days (aOR = 5.60, 95% CI 2.15 – 14.62), exposure to 5 - 7 days of hospitalization (aOR = 4.99, 95% CI 1.20 – 20.79) or to ≥ 8 days (aOR = 5.34, 95% CI 1.18 – 24.22), chronic obstructive pulmonary disease (aOR = 4.27, 95% CI 1.64 – 11.13) and recent hospitalization (aOR = 2.66, 95% CI 1.14 – 6.23). Compared to infections due to methicillin-susceptible *Staphylococcus aureus*, we found increased probability of having a longer hospital stay (aHR = 1.74, 95% CI 1.11 – 2.71) and in-hospital mortality was significantly higher (p = 0.001) between patients infected by methicillin-resistant *Staphylococcus aureus*.

Discussion: The results demonstrate that methicillin resistance is associated with an increased clinical risk to patients infected by *Staphylococcus aureus*, in particular, a raised mortality and prolonged hospitalization.

Conclusion: Our study underlines the additional burden imposed by methicillin resistance in *Staphylococcus aureus* infections. This highlights an urgent need to reinforce and optimize prevention, control, timely detection and effective treatment strategies for multidrug-resistant *Staphylococcus aureus* strains.

Keywords: Hospital Mortality; Length of Stay; Methicillin-Resistant Staphylococcus aureus; Portugal; Risk Factors; Staphylococcal Infections

RESUMO

Introdução: A emergência de estirpes de *Staphylococcus aureus* resistentes à meticilina constitui um sério desafio terapêutico na prestação de cuidados de saúde. Com esta investigação, pretendeu-se estudar os fatores de risco e os resultados clínicos (mortalidade e tempo de internamento) associados a infeções por *Staphylococcus aureus* resistentes à meticilina em doentes internados numa unidade hospitalar em Portugal.

Material e Métodos: Realizou-se um estudo caso-controlo. Integraram o estudo 96 casos e 122 controlos, selecionados, respetivamente, em função da resistência ou sensibilidade antibiótica à meticilina. Os dados obtidos, por consulta de registos clínicos, foram alvo de análise estatística multivariada.

Resultados: Identificaram-se os seguintes fatores de risco independentes para desenvolvimento de infeção por *Staphylococcus aureus* resistentes à meticilina: cateter urinário (aOR = 10,62, IC 95% 3,66 – 30,78), antibioterapia nos últimos 30 dias (aOR = 5,60, IC 95% 2,15 – 14,62), exposição a cinco a sete dias de internamento (aOR = 4,99, IC 95% 1,20 – 20,79) ou a oito ou mais dias (aOR = 5,34, IC 95% 1,18 – 24,22), doença pulmonar obstrutiva crónica (aOR = 4,27, IC 95% 1,64 – 11,13) e internamento hospitalar recente (aOR = 2,66, IC 95% 1,14 – 6,23). Comparativamente a infeções por *Staphylococcus aureus* sensíveis à meticilina, constatou-se uma probabilidade acrescida de prolongamento do internamento (aHR = 1,74, IC 95% 1,11 – 2,71) e uma mortalidade intra-hospitalar significativamente superior (p = 0,001) em doentes infetados por *Staphylococcus aureus* resistentes à meticilina.

Discussão: Os resultados demonstram que a resistência à meticilina se encontra associada a um risco clínico acrescido para os doentes infetados por *Staphylococcus aureus*, com tradução no aumento da mortalidade e no prolongamento do tempo de internamento.

Conclusão: Este estudo salienta a sobrecarga adicional associada à resistência à meticilina em infeções por *Staphylococcus aureus*. Urge, portanto, reforçar e optimizar estratégias de controlo, prevenção, deteção atempada e tratamento efetivo de estirpes multirresistentes de *Staphylococcus aureus*.

Palavras-chave: Factores de Risco; Infecções Estafilocócicas; Mortalidade Hospitalar; Portugal; Staphylococcus aureus Resistente à Meticilina; Tempo de Internamento

1. Unidade de Saúde Pública. Agrupamento de Centros de Saúde do Pinhal Litoral. Leiria. Portugal.
2. Serviço de Medicina Intensiva. Centro Hospitalar de Leiria. Leiria. Portugal.
3. Serviço de Patologia Clínica. Centro Hospitalar de Leiria. Leiria. Portugal.
✉ Autor correspondente: Rúben Rodrigues. rubenjbr@gmail.com
Recebido: 18 de junho de 2018 - Aceite: 14 de outubro de 2019 | Copyright © Ordem dos Médicos 2020

INTRODUCTION

Staphylococcus aureus is a major cause of community- and healthcare-associated infections worldwide.[1–3] Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has made treatment less effective, putting into question our ability to treat infections.[3,4] Especially alarming is the rapid spread of multiresistant strains of this pathogen, particularly within healthcare settings.[2,5]

MRSA infections are associated with increased morbidity and mortality, extra length of hospital stay and excess costs,[4,6–8] presenting a serious threat to the patient safety and a serious challenge to the functioning of health systems.[9] The European Centre for Disease Prevention and Control (ECDC) estimated, in 2007, that a total number of 171 200 nosocomial MRSA infections are acquired annually in the European Union (EU) member states, Iceland and Norway; overall, MRSA accounts for 44.3% of healthcare-associated infections due to selected antibiotic-resistant bacteria, being responsible for 5 400 (21.5%) attributable extra deaths and more than one million (41.4%) excess days of hospitalization, with an increase in hospital costs of approximately 380 million euros.[10]

Portugal is one of several European countries with high endemicity of MRSA. According to the data from the European Antimicrobial Resistance Surveillance Network (EARS-Net), in 2015, Portugal presented a high proportion of methicillin-resistant *S. aureus* (46.8%), about two and a half times higher than the average weighted by the EU population (16.8%); value only surpassed, in the European context, by Malta (48.3%) and Romania (57.2%)[11]. Even though there has been a gradual improvement (compared to 54.6% in 2011),[12] the current estimate is still high and continues to generate concern; specifically, faced with the possibility of occurrence and spread of more cases of vancomycin-resistant *S. aureus* (VRSA), as a therapeutic alternative to methicillin for multiresistant strains (the first European case of VRSA was isolated in Portugal in 2013).[13] Moreover, MRSA isolates are often also resistant to fluoroquinolones, further limiting the treatment options available for severe infections. MRSA remains a public health priority in Portugal.[11]

In order to contribute to a better knowledge and to help prevent and control these infections, we launched this study to identify the risk factors and analyse the clinical outcomes (mortality and length of stay) associated with MRSA infections among hospitalized adult patients in a district hospital in Portugal.

MATERIAL AND METHODS

Study design and setting

A retrospective case-control design was adopted. The study was conducted at Santo André Hospital, a 464-bed district hospital located in Leiria, Portugal.

Selection of participants

From microbiology laboratory records, we identified retrospectively all patients with a microbiological culture positive for *S. aureus* who had been hospitalized between September 1st, 2015 and August 31st, 2016. *S. aureus* was identified in cultures of clinical samples taken at the request of the patient's physician. Antibiotic susceptibility was determined by the hospital microbiology laboratory, according to consensus protocols published in the ECDC manual.[14]

Eligible participants were all adult patients (≥ 18 years old) with a laboratory-confirmed diagnosis of *S. aureus* infection. Patients who met the inclusion criteria for the period of study were further evaluated with the following exclusion criteria: (i) absence of the medical record with confirmation of clinical and laboratory diagnosis of *S. aureus* infection (in order to exclude colonization), (ii) laboratory identification of a pathogen other than *S. aureus* in the same sample or in another sample of the same type that has been harvested at the same time and (iii) hospital discharge within 24 hours (the official definition of 'ambulatory').[15] The decision to include/exclude patients was based on the information available in clinical records (provided through the electronic hospital information system) and covering the whole period of hospitalization.

The selected participants were classified in two groups (cases and controls), according to results of susceptibility tests. Hospitalized patients with a laboratory-confirmed MRSA infection were included in the case group. On the other hand, patients infected with methicillin-susceptible *Staphylococcus aureus* (MSSA) were classified as controls. As a note, in cases where the culture of two or more samples of the same type occurred, we determined that at least one positive sample was sufficient to consider infection.

Data collection and measurements

As mentioned, data were initially retrieved from the laboratory information system and later through a review of patients' electronic clinical records (including admission and progress notes, drug prescriptions, nursing and operation records and discharge summaries). A structured form was applied to collect patients' data.

Once the available literature was reviewed and the research hypotheses were formulated, we assessed potential risk factors by gathering exposure information regarding patient demographics as well as clinical variables, including: sex, age, patients' place of provenance (community/patient's house, nursing home for elderly people, continuing care unit, other), in-hospital exposure time (time period of hospitalization defined by the number of days between the hospital admission and the infection onset), underlying chronic diseases (chronic obstructive pulmonary disease [COPD], insulin-dependent diabetes mellitus, malignancy, chronic renal insufficiency), recent hospitalization (in the previous 30 days before admission), prior antibiotic therapy (within 30 days preceding *S. aureus* infection), invasive procedures (surgery, haemodialysis) and presence of indwelling devices (central or peripheral vascular catheter, intubation or tracheostomy, urinary catheter). Data on risk factors occurring during the hospital stay were recorded for

patients with *S. aureus* infection if they occurred at any time between admission and the sample date of the first positive culture. As such, the day of infection onset was defined as the sample date of the initial MRSA or MSSA isolate (for cases and controls, respectively). Biological sample/specimen source (blood, urine, respiratory specimen, exudate sample, and others) and inpatient ward (where the patient was hospitalized at the time of the culture request) were also documented, but not included in the analysis, since we considered them as potentially interfering variables.

Additional information was obtained regarding the following clinical outcomes: in-hospital mortality and length of stay (after the onset of *S. aureus* infection).

Data analysis

Data from completed forms were entered and analyzed using SPSS v24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.).

A descriptive analysis was performed to analyse patients' sociodemographic and clinical characteristics as well as clinical outcomes following infection. Quantitative variables were described by mean value and standard deviation. Absolute and relative frequencies were used to summarize qualitative variables.

For the inferential analysis of research hypotheses, we performed the statistical study in two stages (Fig. 1). In the first one we focused on identifying potential risk factors. In order to explore possible statistical differences between the two independent groups (MRSA *versus* MSSA) we started by estimating the statistical significance for each potential risk factor separately. Quantitative variables were analyzed using the non-parametric Mann–Whitney U test, since the assumptions of both normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test) could not be met. Qualitative variables were analyzed using the chi square test or Fisher's exact test, as appropriate. Crude odds ratios (OR) with 95% confidence intervals (CI) were calculated as measures of association. Logistic regression modelling was subsequently employed to control for the confounding effect of each variable. Variables with p values < 0.1 were considered for multivariate analysis. At this level, results are presented as adjusted odds ratios (aOR) along with 95% CI. Validation of the model was managed using the Hosmer–Lemeshow goodness-of-fit test.

In a second stage, we approached the clinical outcomes associated with methicillin resistance in *S. aureus* infections. In-hospital mortality was defined as the proportion of MRSA and MSSA patients who died during the period of hospitalization (after the onset of infection). Comparison of mortality between groups was performed using the chi square test. The hypothetical effect of methicillin resistance on increasing the length of hospital stay (among patients who survived *S. aureus* infection) was addressed by Cox regression model with adjustment for the independent risk factors previously identified in the multivariate analysis. The hazard ratio (HR), which is derived from this model, provides an estimate of relative risk of a certain clinical event based on comparison of event rates between groups (cases *versus* controls); in this case, an increase in the length of hospital stay as a hypothetical result of methicillin resistance in *S. aureus* infections. Here, the strength of the association was quantified by adjusted hazard ratio (aHR) and 95% CI. The Cox regression model analyzed time to hospital discharge as duration data, not as a continuous outcome (despite its discrete nature). Thus, patients who

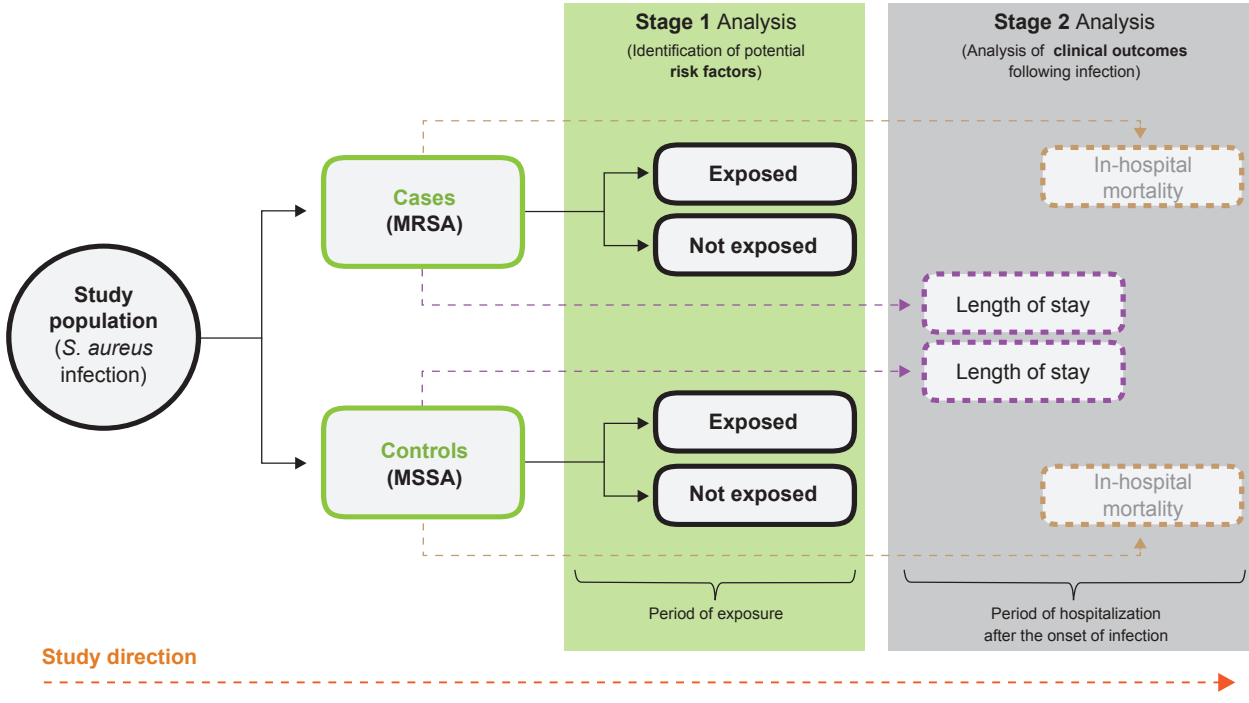

Figure 1 – Flowchart summarizing statistical study design and data analysis

S. aureus: *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *Staphylococcus aureus*

died post-infection onset, but prior to hospital discharge or that in the meantime were transferred to another hospital (not considered as such intra-hospital patient transfers/ inter-service handoffs) were censored in this model. Collinear variables were not included.

Lastly, the level of statistical significance was set at 5% ($p < 0.05$) for all patient-based analyses.

Ethical issues

This study received prior approval from the National Committee for Data Protection (Comissão Nacional de Proteção de Dados) and the hospital Ethics Committee. The research was conducted in accordance with the national standards and conditions applicable to the processing of personal data for clinical research. Patients' privacy and confidentiality were ensured by the lack of identifiers in the database (only a unique identification number was attributed to each participant). Recorded data were kept anonymous.

RESULTS

A total of 218 individuals (127 males and 91 females) were included in this study. The mean age was 70.83 years (± 16.25) in a range between 28 and 95 years.

Overall, 96 cases (with MRSA infection) and 122 controls (with MSSA infection) were selected for the study. The respiratory tract was the most common source of *S. aureus* isolates (101; 46.33%). Most of the cultures were requested in the Emergency Department (106; 48.62%). Additional information regarding patient sociodemographic and clinical variables is summarized in Table 1.

Risk factors

Sex, age, place of provenance, in-hospital exposure time, underlying chronic disease(s), recent hospitalization, prior antibiotic therapy, invasive procedures and indwelling devices were included for analysis as potential risk factors for development of infection by a methicillin-resistant strain of *S. aureus*.

In comparison to MSSA infections, we firstly found statistically significant associations between the occurrence of MRSA infection and the following independent variables: age ($p = 0.001$), patients' place of provenance ($p = 0.032$), in-hospital exposure time ($p < 0.001$), underlying COPD ($p = 0.002$) and chronic renal insufficiency ($p = 0.012$), recent hospitalization ($p < 0.001$), prior antibiotic therapy ($p < 0.001$), surgery within 30 days preceding infection ($p = 0.024$) and indwelling devices (central vascular catheter: $p = 0.037$; peripheral vascular catheter: $p = 0.012$; intubation or tracheostomy: $p = 0.015$; and urinary catheter: $p < 0.001$). The remaining variables (sex, insulin-dependent diabetes mellitus, malignancy, and haemodialysis) were not associated with MRSA infections. The results of bivariate analyses are also available in Table 1.

Once performed the adjustment of the logistic regression model (with the inclusion of the variables that, in relation to the dependent variable, originated p values < 0.10), only five variables remained statistically significant. The independent associations were observed for the presence of urinary catheter (aOR = 10.62, 95% CI 3.66 – 30.78), prior antibiotic therapy within 30 days preceding infection (aOR = 5.60, 95% CI 2.15 – 14.62), in-hospital exposure time of 5 - 7 days (aOR = 4.99, 95% CI 1.20 – 20.79) or ≥ 8 days (aOR = 5.34, 95% CI 1.18 – 24.22), COPD (aOR = 4.27, 95% CI 1.64 – 11.13) and recent hospitalization (aOR = 2.66, 95% CI 1.14 – 6.23) in the previous 30 days before admission (Table 2).

It should also be noted that almost all patients with MRSA infection (93 of 96 cases, 96.88%) had at least one of the five identified risk factors; compared with 68.85% among MSSA infected patients (84 of 122 controls).

Clinical outcomes

Within the study population, 56 patients had died in the course of hospitalization after the onset of *S. aureus* infection; 35 deaths occurred among cases (proportional mortality: 36.46%) and 21 among controls (proportional mortality: 17.21%). We found a statistically significant difference in relation to the number of in-hospital deaths that occurred in the two groups. Compared to MSSA infections, in-hospital mortality was significantly higher ($p = 0.001$) in the group of MRSA infected patients (Table 3).

Among survivors, there was an increased likelihood (74%) of having an extended length of hospital stay associated with MRSA infections (HR = 1.74, 95% CI 1.23 – 2.44), comparatively with MSSA infections. This association remained statistically significant after adjustment of the Cox regression model for the independent risk factors previously identified for MRSA infections (aHR = 1.74, 95% CI 1.11 – 2.71) (Table 4 and Fig. 2).

Indeed, the average length of stay (after the infection onset) was significantly higher in the group of cases compared to the group of controls (15.64 *vs* 10.33 days, respectively; $p = 0.002$) (Table 3).

DISCUSSION

Methicillin resistance in *S. aureus* represents a serious health concern in Portugal.[13,16] Yet, and as far as we are aware, this is the first study to specifically investigate risk factors for MRSA infections and the associated clinical outcomes (in-hospital mortality and length of stay) in a hospital setting at national level.

Risk factors

Among the characteristics evaluated in the current study, the presence of urinary catheter (during hospital stay) was independently associated with MRSA infections (aOR = 10.62, 95% CI 3.66 – 30.78). Although urine has not been the most common specimen source, the association found is not surprising, since a few other studies have obtained similar results.[17,18] Furthermore, it is associated not only with the occurrence of MRSA urinary tract infections, but also with bacteraemia. For instance, Carnicer-Pont *et al* demonstrated the independent association between

Table 1 – Sociodemographic and clinical characteristics associated with the occurrence of MRSA infections (compared with MSSA infections)

	Infection					
	MRSA	MSSA	Total	OR	(95% CI)	*p* value *
n (%)	96 (44.0)	122 (56.0)	218 (100.0)	–	–	–
Sociodemographic data						
Sex						0.395
Male, n (%)	59 (46.5)	68 (53.5)	127 (100.0)	1.00	[reference]	–
Female, n (%)	37 (40.7)	54 (59.3)	91 (100.0)	0.79	(0.46 – 1.36)	–
Age (years), mean ± *SD*	75.11 ± 14.19	67.45 ± 17.01	70.83 ± 16.25	1.03#	(1.01 – 1.05)#	0.001**
Place of provenance						0.032
Community, n (%)	58 (38.2)	94 (61.8)	152 (100.0)	1.00	[reference]	
Nursing home for elderly people, n (%)	19 (51.4)	18 (48.6)	37 (100.0)	2.24	(1.09 – 4.57)	
Continuing care unit, n (%)	12 (70.6)	5 (29.4)	17 (100.0)	4.15	(1.39 – 12.39)	
Other, n (%)	7 (58.3)	5 (41.7)	12 (100.0)	2.42	(0.73 – 7.99)	
Clinical data						
In-hospital exposure time until infection onset						< 0.001
0 – 2 days, n (%)	53 (37.3)	89 (62.7)	142 (100.0)	1.00	[reference]	
3 – 4 days, n (%)	6 (24.0)	19 (76.0)	25 (100.0)	0.53	(0.20 – 1.41)	
5 – 7 days, n (%)	16 (66.7)	8 (33.3)	24 (100.0)	3.36	(1.35 – 8.38)	
≥ 8 days, n (%)	21 (77.8)	6 (22.2)	27 (100.0)	5.88	(2.23 – 15.49)	
Inpatient ward						
Emergency Department, n (%)	43 (40.6)	63 (59.4)	106 (100.0)	–	–	–
Intensive Care, n (%)	9 (39.1)	14 (60.9)	23 (100.0)	–	–	–
Medicine, n (%)	16 (59.3)	11 (40.7)	27 (100.0)	–	–	–
Surgery, n (%)	8 (66.7)	4 (33.3)	12 (100.0)	–	–	–
Orthopaedics, n (%)	7 (26.9)	19 (73.1)	26 (100.0)	–	–	–
Other, n (%)	13 (54.2)	11 (45.8)	24 (100.0)	–	–	–
Biological sample						
Blood, n (%)	15 (42.9)	20 (57.1)	35 (100.0)	–	–	–
Urine, n (%)	8 (61.5)	5 (38.5)	13 (100.0)	–	–	–
Respiratory specimen, n (%)	51 (50.5)	50 (49.5)	101 (100.0)	–	–	–
Exudate sample, n (%)	10 (25.6)	29 (74.4)	39 (100.0)	–	–	–
Other, n (%)	12 (40.0)	18 (60.0)	30 (100.0)	–	–	–
Underlying chronic disease(s)						
COPD, n (%)	28 (65.1)	15 (34.9)	43 (100.0)	2.94	(1.46 – 5.90)	0.002
Insulin-dependent DM, n (%)	16 (50.0)	16 (50.0)	32 (100.0)	1.33	(0.63 – 2.81)	0.462
Malignancy, n (%)	21 (45.7)	25 (54.3)	46 (100.0)	1.09	(0.57 – 2.09)	0.804
Chronic renal insufficiency, n (%)	46 (54.8)	38 (45.2)	84 (100.0)	2.03	(1.17 – 3.54)	0.012
Recent hospitalization within 30 days before admission, n (%)	55 (61.1)	35 (38.9)	90 (100.0)	3.33	(1.90 – 5.86)	< 0.001
Prior antibiotic therapy within 30 days preceding infection, n (%)	79 (59.0)	55 (41.0)	134 (100.0)	5.66	(3.00 – 10.67)	< 0.001
Invasive procedures within 30 days preceding infection						
Surgery, n (%)	28 (58.3)	20 (41.7)	48 (100.0)	2.10	(1.10 – 4.03)	0.024
Haemodialysis, n (%)	3 (75.0)	1 (25.0)	4 (100.0)	3.90	(0.40 – 38.13)	0.323***
Presence of indwelling devices during hospital stay, until infection onset						
Central vascular catheter, n (%)	19 (61.3)	12 (38.7)	31 (100.0)	2.26	(1.04 – 4.93)	0.037
Peripheral vascular catheter, n (%)	65 (51.2)	62 (48.8)	127 (100.0)	2.03	(1.16 – 3.54)	0.012
Intubation or tracheostomy, n (%)	25 (61.0)	16 (39.0)	41 (100.0)	2.33	(1.16 – 4.68)	0.015
Urinary catheter, n (%)	53 (77.9)	15 (22.1)	68 (100.0)	8.79	(4.48 – 17.25)	< 0.001

MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *Staphylococcus aureus*; OR: odds ratio; CI: confidence interval; SD: standard deviation; COPD: chronic obstructive pulmonary disease; DM: diabetes mellitus.

* Chi square test; ** Mann–Whitney U test (for independent samples); *** Fisher's exact test, # Odds ratio calculated for one unit increase in the variable (i.e. 1-year increase in age).

Table 2 – Logistic regression analysis of risk factors for methicillin-resistant *Staphylococcus aureus* infections

	Bivariate analysis			Multivariate analysis		
	OR	(95% CI)	*p* value	aOR	(95% CI)	*p* value
Presence of indwelling devices during hospital stay, until infection onset						
Urinary catheter	8.79	(4.48 – 17.25)	< 0.001	10.62	(3.66 – 30.78)	< 0.001
Prior antibiotic therapy within 30 days preceding infection	5.66	(3.00 – 10.67)	< 0.001	5.60	(2.15 – 14.62)	< 0.001
In-hospital exposure time until infection onset						
0 – 2 days	1	[reference]		1	[reference]	
3 – 4 days	0.53	(0.20 – 1.41)	0.204	1.55	(0.40 – 6.06)	0.530
5 – 7 days	3.36	(1.35 – 8.38)	0.009	4.99	(1.20 – 20.79)	0.027
≥ 8 days	5.88	(2.23 – 15.49)	< 0.001	5.34	(1.18 – 24.22)	0.030
Underlying chronic disease(s)						
COPD	2.94	(1.46 – 5.90)	0.002	4.27	(1.64 – 11.13)	0.003
Recent hospitalization within 30 days before admission	3.33	(1.90 – 5.86)	< 0.001	2.66	(1.14 – 6.23)	0.024

OR: odds ratio; CI: confidence interval; aOR: adjusted odds ratio; COPD: chronic obstructive pulmonary disease.
[Hosmer–Lemeshow goodness-of-fit test: $p = 0.502$ (> 0.05)]
Note: The following variables were also included in the logistic regression analysis: age, place of provenance (community, nursing home for elderly people, continuing care unit, other), underlying chronic disease (chronic renal insufficiency), invasive procedures (surgery) and presence of indwelling devices (central vascular catheter, peripheral vascular catheter, intubation or tracheostomy).

hospital-acquired MRSA bacteraemia and the use of urinary catheters (aOR = 37.1, 95% CI 7.1 – 193.2).[19]

Prior antibiotic therapy (within 30 days preceding infection) was shown to be another risk factor independently associated with MRSA infections in multivariate analysis (aOR = 5.60, 95% CI 2.15 – 14.62). Indeed, previous exposure to antibiotics, particularly broad-spectrum fluoroquinolones and third-generation cephalosporins, is considered an independent risk factor for acquisition of infections by methicillin-resistant *S. aureus* strains in numerous studies.[20–23]

In-hospital exposure time (until infection onset) of 5 - 7 days or ≥ 8 days (aOR = 4.99, 95% CI 1.20 – 20.79 and aOR = 5.34, 95% CI 1.18 – 24.22, respectively) and history of recent hospitalization (in the previous 30 days before admission) (aOR = 2.66, 95% CI 1.14 – 6.23) were also confirmed as independent risk factors for MRSA infections. These findings are consistent with those published in the literature,[17,24–27] despite some methodological differences between studies with regard to specific technical definitions such as the number of previous hospitalizations (1 to ≥ 3) and the time frame preceding hospital admission (from 1 to 24 months).

It was still observed in this study that COPD (aOR = 4.27, 95% CI 1.64 – 11.13) was independently associated with an increased risk of subsequent MRSA infection. This result is supported by growing evidence which implies that comorbid COPD is a risk factor for MRSA infections.[25,28]

Overall, we confirmed five of the potential risk factors addressed in our study population. Interestingly, other factors that found to be significant in the first stage of the inferential analysis, including, for example, surgery (within 30 days preceding infection) or endotracheal intubation/tracheostomy, failed to reach statistical significance in the multivariate analysis. Even though not all the selected variables have been scientifically recognized in previous studies as being risk factors for MRSA infections, these results may be a reflection of the low absolute frequencies verified in some of the characteristics analyzed, precluding meaningful statistical comparisons. We also consider that the operational definitions adopted for a few variables could have affected the significance and association, or lack of association between the potential risk factors assessed (e.g., haemodialysis) and the occurrence of MRSA infection.

Further studies will be necessary to extend the scope to other settings or populations, such as nursing homes. Those studies would be particularly useful if standardized

Table 3 – Clinical outcomes associated with the development of MRSA infections (compared with MSSA infections)

	Infection			
	MRSA	MSSA	Total	*p* value
Clinical outcomes				
In-hospital mortality after infection onset, n (%)	35 (62.5)	21 (37.5)	56 (100.0)	0.001*
Length of stay after infection onset (days)§, mean ± *SD*	15.64 ± 12.43	10.33 ± 7.04	12.44 ± 9.86	0.002**

MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *Staphylococcus aureus*; SD: standard deviation.
* Chi square test; ** Mann–Whitney U test (for independent samples); § Excluded the patients who died during hospitalization (after the infection onset) or that were transferred to another hospital.

Table 4 – Cox regression analysis of the relationship between resistant to methicillin and length of stay

	Bivariate analysis			Multivariate analysis#		
	HR	(95% CI)	*p* value	aHR	(95% CI)	*p* value
Length of stay after infection onset (days)§	1.74	(1.23 – 2.44)	0.002	1.74	(1.11 – 2.71)	0.015

HR: hazard ratio; CI: confidence interval; aHR: adjusted hazard ratio.
Included for adjustment the independent risk factors identified for the occurrence of MRSA infections: presence of urinary catheter, prior antibiotic therapy, in-hospital exposure time of 5 - 7 days and ≥ 8 days, COPD and recent hospitalization; § Excluded the patients who died during hospitalization (after the infection onset) or that were transferred to another hospital.

and strict criteria were used to include participants and data were collected in a similar way, allowing more accurate data analysis and helping us to get a better interpretation of results.

Clinical outcomes

Another aim of the study was to analyse the clinical impact of methicillin resistance among patients infected with *S. aureus*. In accordance with previous studies,[25,29–31] patients with MRSA infection had a significantly higher in-hospital mortality compared with those in the control group (infected with MSSA) (p = 0.001); and, in fact, the descriptive analysis by itself has shown that more MRSA than MSSA infected patients died during the hospital stay.

Moreover, methicillin resistance was independently associated with a significantly extended length of hospital stay following the infection onset. After adjustment for risk factors (previously identified), our observations revealed a nearly 1.7-fold increased odds of prolonged length of stay in patients with MRSA infections (aHR = 1.74, 95% CI 1.11 – 2.71). This result indicates an increased hazard of hospital discharge delay, hence a longer time to discharge. Therefore, an effect attributable to methicillin resistance can be discerned for the length of hospital stay. Two other studies (conducted in China and USA, respectively)[25,32] also identified MRSA infection as a predictor of increased length of stay. Even so, it should remain clear that a hazard ratio does not convey information about how much longer this event may occur, but instead estimates the risk of it occurring (in relative terms, as a ratio of risks).

These findings corroborate our hypothesis that methicillin resistance in *S. aureus* infections are associated with worst clinical outcomes among hospitalized patients. Additional research will also be important to evaluate the health impact of MRSA spread in community settings.

Study's limitations and strengths

As any retrospective study, our research has certain limitations that should be acknowledged. Specifically, we could not follow patients after discharge, which does not allow us to analyse infection recurrence as well as to determine mortality within a certain period of follow-up (e.g., 30 days after infection onset). Also, we were unable to assess patients' causes of death (inaccessible data). Moreover, we didn't differentiate community-acquired from hospital-acquired *S. aureus* infections. And obviously, as with all observational studies, we cannot entirely exclude any confounding or residual bias. Particularly, we assume the possibility of information bias due to misclassification of patients' exposure status and outcomes, even if some errors (e.g., typos in the clinical records consulted) possibly have occurred in both groups (and conceivably tended to minimize any true differences between them: bias toward the null).

Still, some strengths are also recognized. Firstly, the study design employed allowed us to examine, simultaneously, multiple risk factors associated with the problem under study (methicillin resistance in *S. aureus* infections), in line with the current scientific evidence supporting its multifactorial nature. Secondly, the option to study all hospitalized patients with *S. aureus* infections (within the timeframe set) also contributed to the strength of this evaluation, in the sense that both cases and controls were selected on uniform criteria without any relationship with the exposure, helping to minimize one of the limitations usually associated with a case-control study: selection bias. A third strength of this study is the relatively high number of participants. Furthermore, data collection occurred through a structured review of electronic medical records rather than self-reported questionnaires, thus reducing recall bias and, therefore, ensuring the accuracy of our data (despite the possible information bias outlined above).

At last, our study is internally valid as we used a population-based design. Although the generalization of the results obtained should naturally be considered with due care, the identified risk factors and the clinical outcomes

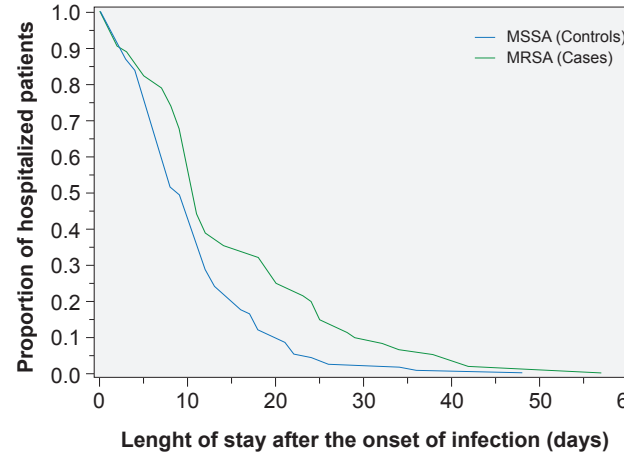

Figure 2 – Length of hospital stay among patients with methicillin-resistant or methicillin-susceptible *Staphylococcus aureus* infections. Adjusted for the independent risk factors identified for the occurrence of MRSA infections (presence of urinary catheter, prior antibiotic therapy, in-hospital exposure time of 5 - 7 days and ≥ 8 days, COPD and recent hospitalization).
MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *Staphylococcus aureus*

evaluated for MRSA infections are broadly in line with the evidence available in previous studies.

CONCLUSION

In summary, this study shows that the presence of urinary catheter, prior antibiotic therapy (in the last 30 days), in-hospital exposure time of five or more days, underlying COPD, and recent hospitalization are independent risk factors for MRSA infections. Furthermore, our findings underline the additional burden imposed by methicillin resistance since it is associated with an increased risk of prolonged length of stay as well as a significantly higher risk of in-hospital death. Thus, it continues to be necessary to optimize strategies to prevent and control nosocomial emergence and dissemination of multidrug-resistant strains of *S. aureus*. In that sense, recognition of the variables that identify patients at higher risk of developing MRSA infection may help physicians target preventive measures and optimising antibiotic use, in order to reduce morbidity and mortality in hospital settings. The combined focus on centralized information systems supporting clinical decision-making and new laboratory technologies for the early identification of MRSA could be the key to controlling the problem. Moreover, the health impact assessment of the spread to the community of this pathogen may also be important for preventing infections.

PROTECTION OF HUMAN SUBJECTS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration issued by World Medical Association.

CONFIDENTIALITY OF DATA

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

FINANCIAL SOURCES

No funding was received for this research.

REFERENCES

1. DeLeo FR, Chambers HF. Reemergence of antibiotic-resistant Staphylococcus aureus in the genomics era. J Clin Invest. 2009;119:2464–74.
2. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, et al. Meticillin-resistant Staphylococcus aureus (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents. 2012;39:273–82.
3. World Health Organization. Antimicrobial resistance: global report on surveillance. Geneva: WHO; 2014.
4. Carbon C. Costs of treating infections caused by methicillin-resistant staphylococci and vancomycin-resistant enterococci. J Antimicrob Chemother. 1999;44:S31–6.
5. Holden MT, Hsu LY, Kurt K, Weinert LA, Mather AE, Harris SR, et al. A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant Staphylococcus aureus pandemic. Genome Res. 2013;23:653–64.
6. Kraker ME, Wolkewitz M, Davey PG, Grundmann H. Clinical impact of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay related to methicillin-resistant Staphylococcus aureus bloodstream infections. Antimicrob Agents Chemother. 2011;55:1598–605.
7. Cosgrove SE. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital dtay, and health care costs. Clin Infect Dis. 2006;42:S82-9.
8. Coia JE, Duckworth GJ, Edwards DI, Farrington M, Fry C, Humphreys H, et al. Guidelines for the control and prevention of meticillin-resistant Staphylococcus aureus (MRSA) in healthcare facilities. J Hosp Infect. 2006;63:S1–44.
9. European Centre for Disease Prevention and Control. Last-line antibiotics are failing: options to address this urgent threat to patients and healthcare systems. Stockholm: ECDC; 2016.
10. European Centre for Disease Prevention and Control, European Medicines Agency. The bacterial challenge: time to react. Stockholm: ECDC/EMEA; 2009.
11. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2015. Stockholm: ECDC; 2017.
12. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2014. Stockholm: ECDC; 2015.
13. Direção-Geral da Saúde. Prevenção e controlo de colonização e infeção por Staphylococcus aureus resistente à meticilina (MRSA) nos hospitais e unidades de internamento de cuidados continuados integrados. Norma N.º 018/2014. Lisboa: DGS; 2015.
14. European Centre for Disease Prevention and Control. Antimicrobial resistance reporting protocol. The European Surveillance System. Stockholm: ECDC; 2015.
15. Ministério da Saúde. Portaria n.º 132/2009. Diário da República, I série, nº 21 (2009/01/30). p. 660–758.
16. Fernandes PA, Silva MG, Cruz AP, Paiva JA, Nogueira PJ, Farinha CS, et al. Portugal: prevenção e controlo de linfeções e de resistência aos antimicrobianos em números - 2015. Lisboa: Direção Geral de Saúde; 2016.
17. European Centre for Disease Prevention and Control. Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals 2011-2012. Stockholm: ECDC; 2013.
18. Callejo-Torre F, Eiros Bouza JM, Astigarraga PO, Del Corral MJ, Martínez MP, Alvarez-Lerma F, et al. Risk factors for methicillin-resistant Staphylococcus aureus colonisation or infection in intensive care units and their reliability for predicting MRSA on ICU admission. Infez Med. 2016;24:201–9.
19. Carnicier-Pont D, Bailey KA, Mason BW, Walker AM, Evans MR, Salmon RL. Risk factors for hospital-acquired methicillin-resistant Staphylococcus aureus bacteraemia: a case-control study. Epidemiol Infect. 2006;134:1167–73.
20. MacDougall C, Powell JP, Johnson CK, Edmond MB, Polk RE. Hospital and community fluoroquinolone use and resistance in Staphylococcus aureus and Escherichia coli in 17 US hospitals. Clin Infect Dis. 2005;41:435–40.
21. Dziekan G, Hahn A, Thüne K, Schwarzer G, Schäfer K, Daschner FD, et al. Methicillin-resistant Staphylococcus aureus in a teaching hospital: investigation of nosocomial transmission using a matched case-control study. J Hosp Infect. 2000;46:263–70.
22. Campillo B, Dupeyron C, Richardet JP. Epidemiology of hospital-acquired infections in cirrhotic patients: effect of carriage of methicillin-resistant Staphylococcus aureus and influence of previous antibiotic therapy and norfloxacin prophylaxis. Epidemiol Infect. 2001;127:443–50.
23. Monnet DL, MacKenzie FM, López-Lozano JM, Beyaert A, Camacho M, Wilson R, et al. Antimicrobial drug use and methicillin-resistant Staphylococcus aureus, Aberdeen, 1996–2000. Emerg Infect Dis. 2004;10:1432–41.
24. Gieffers J, Ahuja A, Giemulla R. Long term observation of MRSA prevalence in a German rehabilitation center: risk factors and variability of colonization rate. GMS Hyg Infect Control. 2016;11:1–5.
25. Yao Z, Peng Y, Chen X, Bi J, Li Y, Ye X, et al. Healthcare associated infections of methicillin-resistant staphylococcus aureus: a case-control-control study. PLoS One. 2015;10:1–9.

26. Salgado CD, Farr BM, Calfee DP. Community-acquired methicillin-resistant Staphylococcus aureus: a meta-analysis of prevalence and risk factors. Clin Infect Dis. 2003;36:131–9.
27. Harinstein L, Schafer J, D'Amico F. Risk factors associated with the conversion of meticillin-resistant Staphylococcus aureus colonisation to healthcare-associated infection. J Hosp Infect. 2011;79:194–7.
28. Sganga G, Tascini C, Sozio E, Carlini M, Chirletti P, Cortese F, et al. Focus on the prophylaxis, epidemiology and therapy of methicillin-resistant Staphylococcus aureus surgical site infections and a position paper on associated risk factors: the perspective of an Italian group of surgeons. World J Emerg Surg. 2016;11:1–13.
29. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. Clin Infect Dis. 2003;36:53–9.
30. Kaasch AJ, Barlow G, Edgeworth JD, Fowler VG, Hellmich M, Hopkins S, et al. Staphylococcus aureus bloodstream infection: a pooled analysis of five prospective, observational studies. J Infect. 2014;68:242–51.
31. Whitby M, McLaws ML, Berry G. Risk of death from methicillin-resistant Staphylococcus aureus bacteraemia: a meta-analysis. Med J Aust. 2001;175:264–7.
32. Cosgrove SE, Qi Y, Kaye KS, Karchmer AW, Carmeli Y. The impact of methicillin resistance in Staphylococcus aureus bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infect Control Hosp Epidemiol. 2005;26:166–74.