Epidemiology and Antimicrobial Susceptibility Trends of Methicillin-Resistant *Staphylococcus aureus* in a Tertiary Hospital

ORIGINAL

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Abstract

Introduction: *Staphylococcus aureus* is a major cause of health care associated infections worldwide.

Objective: The aim of this work was to evaluate epidemiological characteristics and antimicrobial susceptibility of Methicillin-resistant *S. aureus* (MRSA) strains isolates from infections in a Brazilian tertiary hospital.

Methods: Clinical and epidemiological data of the patients were collected. Bacterial strains were isolated and identified using the classical identification tests. Antimicrobial susceptibility assays were performed using the disc-diffusion method.

Findings: A total of 590 samples of *S. aureus* were isolated from patients and 42.5% were characterized as MRSA. Considering the clinical specimens, most of samples were isolated from blood and tracheal secretion, catheter tip, surgical site swabs, wound secretion, exudates and urine. Overall, a high frequency of resistance was observed against ciprofloxacin, clindamycin, erythromycin, amikacin, gentamicin, trimethoprim-sulfamethoxazole, tetracycline and chloramphenicol. Association between death and multidrug-resistance in elderly patients, and death and occurrence of bacteremia by multidrug-resistant MRSA was observed.

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Conclusions: Our data are highly relevant for surveillance systems and to map on a wider scale the dynamics of circulation of MRSA and raise discussions on containment strategies and rational use of empiric chemotherapy.

Keywords

Staphylococcus aureus,
antimicrobial susceptibility,
methicillin-resistance.

Introduction

The Gram positive cocci *Staphylococcus aureus* is one of the most common microorganisms isolated from infections related to health care around the world. This scenario has gotten worse over time due to an increase in antimicrobial resistance rate, especially to methicillin, a penicillinase-resistant semi-synthetic penicillin, antimicrobial of choice for the treatment of infections caused by antimicrobial-resistant *S. aureus* [1-3].

Methicillin-resistant *S. aureus* (MRSA) are resistant to other penicillins other β -lactams such as cephalosporins, carbapenems and monobactams [4]. These bacteria, initially related to infections associated to health care (hospital-acquired methicillin-resistant *Staphylococcus aureus* HA-MRSA), nowadays also represent a major problem in the community (community-associated methicillin-resistant *Staphylococcus aureus* CA-MRSA) [5,6]. While the community-associated MRSA diseases are related to skin infections, the more severe clinical infections are more frequently related to hospitalized patients [7].

Although in Brazil, researches show a high frequency of MRSA colonization in patients with bacteremia, [8,9] the most of the scientific data considering epidemiology and antimicrobial susceptibility patterns of MRSA to other antimicrobial, are originated in other countries. Thus, the available information is not, indeed, representative of the Brazilian reality, which makes difficult to guide the empirical chemotherapy. In this respect, considering the worldwide spread of MRSA and the lack of regional data on HA-MRSA epidemiology and antimicrobial

susceptibility patterns, the aim of this study was to describe epidemiological characteristics and antimicrobial susceptibility trends of MRSA strains associated to patients admitted to a tertiary hospital, between 2005 and 2010, in Brazil.

Methods

The study was conducted at a teaching hospital in Juiz de Fora, MG, Brazil. It is a tertiary institution with 146 beds, and six of them are in an Intensive Care Unit (ICU). A total of 590 samples of *S. aureus* were isolated and identified from different clinical specimens from patients admitted between 2005 and 42.5% (n=251) were characterized as MRSA. Considering only the non-replicate isolates, 103 MRSA strains were considered in this study (**Table 1**), which was approved by the Ethics Committee of the Federal University of Juiz de Fora (certificate no. 267/2011). Clinical and epidemiological data of the patients were collected from medical records and record books from the clinical microbiology laboratory Prof. Maurilio Baldi at the UFJF Teaching Hospital.

All the clinical specimens were inoculated in Mannitol Salt Agar (Difco, USA) with a 0.001 mL sterile loop and incubated at 35.5°C for up to 48 hours. Bacterial strains (3 to 5 colonies/clinical specimens) in monomicrobial cultures were isolated and identified using the classical identification tests [10]. Antimicrobial susceptibility assays were performed on Mueller–Hinton agar (HiMedia) using the disc-diffusion method and growth inhibition zones were interpreted according to the Clinical and Laboratory

Table 1. Epidemiological parameters and frequency of antimicrobial resistance in MRSA strains associated to patients admitted to a tertiary hospital over a 6-year period.

Epidemiological parameters	Sampled period					
	2005	2006	2007	2008	2009	2010
	(n=25)	(n=13)	(n=27)	(n=21)	(n=10)	(n=07)
Gender						
Male	72.0%	69.2%	74%	66.6%	70.0%	71.4%
Female	28.0%	30.8%	26%	33.4%	30.0%	28.6%
Average age (years)	50.2	54.9	52.2	56.5	58.5	64.4
Type of Infection						
Bacteremia	4.0%	23.1%	22.2%	33.3%	30.0%	14.3%
Catheter bacteremia	12.0%	30.7%	11.1%	14.3%	10.0%	0.0%
Surgical Site	28.0%	0.0%	18.5	14.3%	0.0%	0.0%
Wound infection	0.0%	0.0%	11.1%	4.8%	10.0%	14.3%
Digestive system	8.0%	0.0%	7.4%	0.0%	0.0%	0.0%
Musculoskeletal	8.0%	0.0%	3.8%	0.0%	0.0%	0.0%
Respiratory system	36.0%	46.2%	18.5%	23.8%	50.0%	71.4%
UTI	4.0%	0.0%	7.4%	9.5%	0.0%	0.0%
Clinical specimens						
Blood	20.0%	15.4%	18.5%	38.1%	30.0%	14.3%
Tracheal secretion	20.0%	30.8%	18.5%	14.3%	50.0%	71.4%
Urine	4.0%	0.0%	7.5%	9.5%	0.0%	0.0%
Catheter tip	16.0%	30.8%	11.1%	19.0%	10.0%	0.0%
Exudates	12.0%	23.0%	22.2%	4.8%	10.0%	14.3%
Surgical Site Swabs	28.0%	0.0%	22.2%	14.3%	0.0%	0.0%
Patient evolution						
Discharged	56.0%	46.1%	55.5%	61.9%	30.0%	57.1%
Death	36.0%	53.9%	44.5%	38.1%	60.0%	42.9%
Transference	8.0%	0.0%	0.0%	0.0%	10.0%	0.0%
Hospital section						
Infirmary	24.0%	23.1%	22.2%	42.9%	40.0%	14.3%
Surgery	36.0%	23.1%	29.6%	14.3%	0.0%	14.3%
Intensive care	36.0%	53.8%	44.4%	33.3%	50.0%	71.4%
Pediatric	4.0%	0.0%	0.0%	9.5%	10.0%	0.0%
BMT	0.0%	0.0%	3.8%	0.0%	0.0%	0.0%
UTI (urinary tract infection); BMT (Bone marrow transplantation).						

Standards Institute (CLSI) [11]. Antimicrobial discs amikacin (30µg), ciprofloxacin (5µg), clindamycin (2µg), chloramphenicol (30µg), erythromycin (15µg), gentamicin (10µg), trimethoprim-sulfamethoxazole (25µg), tetracycline (30µg) and were of commercial grade (Laborclin Ltda, Brazil). To evaluate the resistance to vancomycin E-test® was used (AB Biodisk, Solna, Sweden). MRSA was detected by resistance to ceftiofur (30µg) and oxacillin (1µg) disks. *Staphylococcus aureus* ATCC 25923 was used for quality control. Besides the descriptive analysis, univariate analysis was performed estimating the odds ratios (OR) with their respective 95% confidence intervals (CI) to verify the associations between microbial resistance with discharged, transferred and deceased patients. The same analysis was performed among the type of MRSA infection and patient's age and outcome ($p < 0.05$). For means comparisons the Mann-Whitney test was used. The comparison of each factor was done by the non parametric Fisher's exact test.

Results

Detailed clinical and epidemiological characteristics of the patients are presented in table 1. Overall, 71% of bacteria were recovered from male patients and 29% from female. The average age was 54.4 years old. Overall, positive correlations between age or gender, and MRSA infected patients were not observed ($p = 1.00$). Although mainly associated to patients admitted in intensive care unit (43.7%), MRSA were also isolated from patients admitted to adult infirmary (28.1%), surgical unit (23.3%), pediatrics (3.9%) and bone marrow transplantation unit (1%). Considering the associated infections, 34% and 20.4% of the samples were isolated from respiratory tract and bacteremia infections, respectively. MRSA was also isolated from surgical site, catheter-related bacteremia, wound infections, infections of the musculoskeletal system, tract urinary and digestive system.

Considering the clinical specimens, most of samples were isolated from tracheal secretion and blood (26.2% and 23.3% respectively) followed by surgical site swabs, catheter tip, exudates, wound secretion and urine (**Table 1**). Regarding to the patients, 53.4% were discharged, 43.6% died and 3% were transferred to other hospitals.

Overall, a high frequency of resistance was observed against ciprofloxacin (92.5%), clindamycin (89.7%), erythromycin (86.9%), amikacin (73.8%), gentamicin (70%), trimethoprim-sulfamethoxazole (63.5%), tetracycline (60.7 %) and chloramphenicol (52.3%). No bacterial resistance was observed against vancomycin (**Figure 1**).

Association between death and multiresistant MRSA was observed in elderly patients (OR=3.57-95% CI: 0.3-38.8). Association was also observed between death and the occurrence of bacteremia in patients with multiresistant MRSA (OR=1.57-95% CI: 0.2-10.4). However these associations were not considered significant.

When considering MRSA occurrence over time and their antimicrobial susceptibility, alterations in the resistance patterns were observed. In 2005, from the tested drugs, the most effective antimicrobial was chloramphenicol, and high resistance levels were observed against amikacin, ciprofloxacin, clindamycin, erythromycin, gentamicin, trimethoprim-sulfamethoxazole and tetracycline. In 2006, increased resistance was observed against all tested drugs with rates of 100% resistance against ciprofloxacin, clindamycin, gentamicin, and trimethoprim-sulfamethoxazole. In the subsequent two years, decreased resistance was observed, especially in 2008, with exception for chloramphenicol in 2007. In 2009 it was observed high levels of sensitivity to amikacin, gentamicin, trimethoprim-sulfamethoxazole and tetracycline (90%). In 2010, there was a re-emergence of high resistance levels (100%), especially against ciprofloxacin, clindamycin and erythromycin. Considering amikacin, gentamicin, trimethoprim-sulfamethoxazole and tetra-

Figure 1: Antimicrobial susceptibility of Methicillin-resistant *Staphylococcus aureus* (MRSA) in a tertiary hospital over a 6-year period. AK, amikacin; CP, ciprofloxacin; CD, clindamycin; CL, chloramphenicol; EM, erythromycin; GM, gentamicin; TS, trimethoprim-sulfamethoxazole; TE, tetracycline; VA, vancomycin.

cycline, although it has been observed an increased resistance compared to the previous year, the resistance rates remained relatively low (28.6%).

Discussion

Methicillin-resistant *Staphylococcus aureus* has become a global problem and active surveillance for this pathogen, with appropriate prevention measures, can reduce its infections rates and reduce treatment costs [12]. This study showed the clinical epidemiology of MRSA isolates from inpatients, identifying the distribution of this pathogen in different sections, associated with gender and average and more age type of infection, clinical specimens, and patient evolution of a teaching hospital. The results obtained demonstrate there was no statistically significant difference when the mean age of patients was compared among the years

of the study ($p > 0.05$). The incidence of MRSA infections is among the highest in the elderly population [13]. Study conducted in Sacramento, EUA, demonstrated that the average age of health care associated-MRSA patients was 54, which was 15 years older than the average age of patients with community associated-MRSA [14].

It is accepted that morbidity and mortality rates especially from infections with MRSA in bloodstream tend to be even greater in the elderly due to their physiological conditions and immunological status [15]. The impact of methicillin-resistance on mortality among patients infected with *S. aureus* has been primarily evaluated in patients with bacteremia, and the results were variable [8,16]. A meta-analysis involving studies with mortality data published between the years 1980 to 2000 was performed. Among 31 cohort studies including 3.963 patients, of whom 34% were infected with

MRSA, there was a significant increase in mortality associated with bacteremia [17]. Although there are few national data to compare our results, according to the literature methicillin-resistance significantly increased in the early 2000s in developed regions, such as the European countries [18] and the USA [19] and developing regions such as Latin America [20].

In Brazil, oxacillin is used as penicillinase-resistant semisynthetic penicillin, being oxacillin-resistant *S. aureus* (ORSA) equivalent to the MRSA strains in other regions. Reports from different hospitals between 1997 and 2001 with totalizing 1.516 *S. aureus* samples showed resistance rates of oxacillin resistance as of 3.8% [21]. Reports comprising the period between 2005 and 2008, involving 2.218 samples, showed ORSA occurrence of 31.0%, and most of these bacteria were also resistant against erythromycin, clindamycin, ciprofloxacin, and levofloxacin [22]. With regard to the ORSA observations, our data corroborate these previous studies. An increase in MRSA-associated hospital infections has been reported in intensive care units in USA, which ranged from only 2% in 1974 to rates of 22% in 1995 and 64% in 2004 [23]. Subsequent studies showed stabilization in this tendency with rates of 56% between 2006 and 2007 [24].

Another study conducted in a teaching hospital in the USA, which evaluated MRSA colonization or infections in hospitalized patients revealed that the most common types of infections were bacteremia (28%), pneumonia (20%), soft tissue infections (16%), and bones or joints infections (16%) [25]. Overall, the most frequently reported risk factors related to the acquisition of MRSA bacteremia include previous antibiotic chemotherapy, prolonged stay in ICU, previous MRSA infection and colonization, use of invasive devices such as central venous catheters and urinary catheter, previous hospitalization and presence of co-morbidities [9, 26].

The therapy used for MRSA infections is still rather limited. Some drugs have been used in recent years

such as dalfopristin, linezolid, tigecycline and daptomycin [27, 28]. However, the use of vancomycin is still the choice for treatment of these infections. The increase use of glycopeptides has facilitated the selection of resistance and the high toxicity caused in the human body, have shown the necessity of the restricted use of these antimicrobials [29, 30].

As a whole, the dynamics of antimicrobial resistance, observed in this study, show that the widespread use of ciprofloxacin, clindamycin and erythromycin should not be encouraged as the first empiric therapy option. By the other hand, the effectiveness of drugs such as chloramphenicol might be related to its low prescription over the years in our region. Based on results from antimicrobial susceptibility testing, the use of amikacin, gentamicin, tetracycline and trimethoprim-sulfamethoxazole should be sustained due to the variations observed for their effectiveness among the isolated bacteria.

We observed vancomycin as the most effective drug; other authors also suggest that it should be used only for serious infections, when other antimicrobials are without therapeutic effect [29,30]. Historically, the occurrence of antimicrobial resistance is inevitable. This is due to the natural species evolution caused by environmental selective pressures. In this respect, retrospective studies on antimicrobial susceptibility patterns should improve strategies to limit the emergence of multi-drug-resistant bacterial strains.

Our data are highly relevant not only for surveillance systems, but to map on a wider scale the dynamics of circulation of such microorganism and raise discussions on containment strategies and rational use of empiric chemotherapy.

Conclusion

This study showed relevant data on the levels of MRSA in critical areas and associated specimens respiratory tract secretions and blood. The high levels of antimicrobial resistance suggest the extent of

the phenomenon and confirm data from the recent literature on the nature of the multidrug-resistant strains of *S. aureus*. Retrospective studies on antimicrobial susceptibility patterns should improve strategies to limit the emergence of multi-drug-resistant bacteria. Our data are highly relevant for surveillance systems and to map on a wider scale the dynamics of circulation of MRSA and raise discussions on containment strategies and rational use of empiric chemotherapy

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Competing and conflicting interests

The authors declare that they have no conflict of interest.

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