

Antimicrobial resistance of *Staphylococcus aureus* isolated from the intensive care unit of a general hospital in southern Brazil

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Abstract

Background: *Staphylococcus aureus* is recognized as a major cause of nosocomial infections worldwide. The aim of this study was to evaluate the antimicrobial resistance of *S. aureus* isolates and the possible changes of the resistance patterns after the implementation of norms of hospital infection control.

Methodology: During the period of 2001 to 2004, antimicrobial resistance of *S. aureus* (n = 104) isolated from patients in the intensive care unit of the Hospital Divina Providência (Porto Alegre, Brazil) was determined. The progress of *S. aureus* control in this hospital through 2002, after the implementation of norms for infection control, was also evaluated.

Results: The glycopeptides presented larger *in vitro* activity against the isolates of *S. aureus* (100% of susceptibility for vancomycin and teicoplanin). The percentage of samples showing resistance to at least one drug was 96%, 97% and 100% for the years 2001, 2003 and 2004, respectively. Except for ampicillin and penicillin, antimicrobial resistance decreased from 2001 to 2004. A total of 26 phenotypic profiles were identified; among them a single profile (phenotype B) was identified in all three years of investigation, corresponding to 50% of the isolates. The number of isolated *S. aureus* decreased the following years, totaling 50 in 2001, 34 in 2003, and 20 in 2004.

Conclusions: A reduction in the number of isolates and antimicrobial resistance of *S. aureus* from the intensive care unit was observed after the implementation of norms for infection control.

Keywords: antimicrobial; hospital infection; resistance; staphylococci

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Introduction

The increasing prevalence of bacterial resistance to antibiotics, mostly associated with the extensive use of antimicrobial agents, may result in an insufficient array of substances to combat some bacterial infections. Increased resistance among microorganisms such as *Pseudomonas* spp. and *Enterococcus* spp. have been described [1]. The prevalence of *Staphylococcus aureus* resistant to conventional antibiotics has been increasing at high levels in some hospitals [2,3].

S. aureus is a bacterium that colonizes both the community and hospital settings [4]. The wide spectrum of antibiotics in the hospital environment leads to the development of increased resistance to these antimicrobial agents. Among hospitalized patients, 5-10% acquire infection during the internment. Globally, *S. aureus* is one of the principal microorganisms involved in nosocomial infections, and strains resistant to methicillin (MRSA) represent

15-45% of all *S. aureus* isolated [3,5,6]. Infections caused by these bacteria are treated mainly with vancomycin. Emergencies with MRSA isolates with reduced susceptibility to vancomycin reinforced the importance of growing research in this area [7,8].

The intensive care unit (ICU) of the Hospital Divina Providência, located in Porto Alegre, Southern Brazil, frequently enrolls patients infected by *S. aureus* with variable antimicrobial resistance degree. With the intention of determining the prevalence of these isolates, *S. aureus* samples submitted to the Laboratory of Clinical Analyses by the Center of Intensive Therapy of this Hospital during the year of 2001 were analysed.

In 2002, the Commission for Control of Hospital Infection (CCIH) of the Hospital Divina Providência started to implement the rules set by the Brazilian Ministry of Health, adapting the procedures of that health institution to the norms of this law. Certain procedures were established in the hospital routine

as follows: (1) Implementation of the Program for Control of Hospital Infection (PCIH), with views to the possible maximum reduction of the incidence and of the severity of the hospital infections; (2) Epidemiological surveillance of hospital infections; (3) Washing of the hands, which is recognized as the most important independent action for the prevention and control of hospital infections; (4) Strict practice of the general recommendations defined by the Ministry of Health concerning (a) the use of the antiseptic, disinfected and sterile materials; (b) the norms for cleaning, disinfecting and sterilization; (c) the norms for procedures in the area of Microbiology; (d) the norms for laundry; and (e) the orientations for Hospital Pharmacy [9].

Seeking to monitor the progress of the bacterial control of this hospital and to compare the resistance profile of the isolated *S. aureus* in the following years, the materials directed by the Intensive Care Unit to the Laboratory of Clinical Analyses were investigated during the years of 2003 and 2004.

Materials and methods

Delineation

A non-controlled transverse study was conducted to determine the prevalence of antibiograms of the isolates of *S. aureus*. These isolates were typed in agreement with their susceptibility to the antimicrobial agents.

Samples

A total of 104 samples from materials originating from patients in the ICU of the Hospital Divina Providencia (Porto Alegre, Brazil) were used. Samples were transported to the Laboratory of Clinical Analyses for strain isolation and characterization. Of these samples, fifty were collected in the period of January to December of 2001; thirty-four in the period of January to December of 2003; and twenty in the period of January to December of 2004. The samples consisted of blood culture, corporal fluids, secretions, materials from procedures (stems, catheters, drains), spittle and swabs from surgery wounds. The strain isolation was accomplished in blood agar media. Bacteria were identified by morphological and biochemical tests following standard protocols [10]. Samples originating from a same patient were excluded, as well as those that had origin in the same location or that had origin in a different place, but presented the same bacteria and antimicrobial sensibility profile, received within a time frame of three or less days.

Antibiotic Susceptibility Test (AST)

The 104 selected isolates were cultured in Mueller-Hinton agar to perform the AST. The method used for the susceptibility test to the antimicrobial agents was the disk diffusion assay described by Kirby-Bauer, following the norms set by the Clinical Laboratory Standards Institute [11]. Commercial antibiotic disks were from Laborclin (São Paulo, Brazil). The following antibiotics were used: Ampicillin 10 µg, Cefalothin 30 µg, Cefoxitin 30 µg, Gentamicin 10 µg, Amikacin 30 µg, Chloranphenicol 30 µg, Tetracycline 30 µg, Sulfamethoxazole 25 µg, Rifampicin 5 µg, Ampicillin/Sulbactam 10/10 µg, Oxacillin 1 µg, Penicillin 10 U, Erythromycin 15 µg, Clindamycin 2 µg, Vancomycin 30 µg, Teicoplanin 30 µg.

After evaluating the size of the specific inhibition zones for each antibiotic, the following designations were assigned: S for sensitive, I for intermediate resistance, and R for resistant.

For the purposes of phenotypical typing, a numerical code profile was created based on the resistance to the antimicrobial of each isolate. Resistance was classified as 1, intermediate resistance as 2, and sensibility as 3 [12].

Ethical aspects

The samples used in this study were from routine clinical materials from the laboratory. Because acquiring the samples did not involve the conduct or treatments of the patients, and also did not require any inquiry of the patients, the consent was not needed. However, the completion of a term of commitment was required on the part of the researchers to ensure the correct use of the clinical findings in characterizing the handling of the materials and data supplied by the laboratory, for research and scientific ends. This research was approved by the Ethics Committee of the Universidade Federal do Rio Grande do Sul, Brazil (register no. 2001/39).

Statistical method

Statistical analysis was performed by means of the χ^2 test [13].

Results

Antimicrobial susceptibility

The antimicrobial susceptibility of the *S. aureus* isolated from patients in the ICU was determined. In 2001, fifty isolates originated predominantly from

Table 1. Antimicrobial resistance in *S. aureus* isolated from patients at ICU.

Samples	n	Ampicilin	Cefalothin	Cefoxitin	Gentamicin	Amikacin	Chloranphenicol	Tetracycline	Sulfametoxazol	Rifampicin	Amp/Sulbactan	Oxacillin	Penicillin	Erythromycin	Clindamycin	Vancomycin	Teicoplanin
2001																	
Spittle	22	20	18	18	18	18	17	18	18	16	18	18	18	18	18	0	0
Nasal secretion	10	10	10	10	8	8	8	8	8	8	8	10	10	10	8	0	0
Catheter tip	6	6	4	4	4	4	2	4	4	4	4	6	6	4	4	0	0
Other*	12	8	6	6	6	6	6	6	6	6	6	6	10	6	6	0	0
Total	50	44	38	38	36	36	33	36	36	34	36	40	44	38	26	0	0
2003																	
Spittle	14	14	9	8	8	8	10	8	8	8	14	9	14	9	9	0	0
Surgery wound	6	5	3	3	2	3	2	3	3	2	4	3	4	3	3	0	0
Tracheal aspirate	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	0
Other**	10	8	4	4	4	4	7	5	4	4	8	4	8	4	4	0	0
Total	34	31	20	19	18	19	23	20	19	18	30	20	30	20	20	0	0
2004																	
Tracheal aspirate	8	8	3	3	5	5	4	5	4	4	8	5	8	5	3	0	0
Spittle	7	7	1	1	1	1	2	2	1	0	7	1	7	1	1	0	0
Other†	5	5	1	1	1	1	1	1	1	1	5	1	5	1	1	0	0
Total	20	20	5	5	7	7	7	8	6	5	20	7	20	7	5	0	0

* Samples from cervical secretion, hemoculture, torax drainage, tracheal aspirate, jejunostomy and surgery wound.

** Samples from hemoculture, bronchial wash, peritoneal fluid, and abdominal, cervical and pulmonary secretions.

† Samples from hemoculture, catheter tip and abdominal secretion.

Table 2. Antimicrobial resistance profile of *S. aureus* isolated from ICU

Antimicrobial	2001		2003		2004		P values*
	n	%	n	%	n	%	
Ampicillin	44	88	31	91	20	100	0.653
Cefalothin	38	76	20	59	5	25	<0.001
Cefoxitin	38	76	19	56	5	25	<0.001
Gentamicin	36	72	18	53	7	35	<0.001
Amikacin	36	72	19	56	7	35	<0.001
Chloranphenicol	33	66	23	68	7	35	<0.001
Tetracycline	36	72	21	62	8	40	0.002
Sulfametoxazole	37	74	19	56	6	30	<0.001
Rifampicin	34	68	18	53	5	25	<0.001
Amp/Sulbactan	37	74	30	88	20	100	0.189
Oxacillin	38	76	20	59	7	35	<0.001
Penicillin	44	88	31	91	20	100	0.649
Erythromycin	38	76	20	59	7	35	<0.001
Clindamycin	37	74	20	59	7	35	<0.001
Vancomycin	0	0	0	0	0	0	n.d.
Teicoplanin	0	0	0	0	0	0	n.d.

* P values were determined by the χ^2 test; n.d., not determined.

spittle (44%), nasal secretion (20%), and catheter tips (12%). The remaining samples corresponded to 4% of each of the following: cervical secretion, blood culture, thorax drain, aspirated of windpipe, jejunostomy, and surgical wound. The origin of the samples and the respective resistance to the antibiotics tested is presented in the Table 1. A great proportion of resistant isolates was observed: 96% of the samples presented resistance to at least one drug. Only two isolates (1 and 22), both coming from spittle, were susceptible to all the antimicrobials tested. In 2003, 34 ICU isolates were from spit (41%), surgery wound (18%), aspirate of trachea (12%), blood cultures (9%), bronchial fluid (9%), and secretions and peritoneal fluid (11%). The distribution of the samples and the respective resistance to the tested antibiotics are shown in the Table 1. A great proportion of resistant isolates was observed, since 97% of the samples presented resistance to at least one drug. A single isolate (25) originating from hemoculture was susceptible to all the antimicrobials tested. In 2004, twenty isolates arose from aspirate from the windpipe (40%), spittle (35%), blood culture (10%), catheter tips (10%) and abdominal secretion (5%). The origin of the samples and the respective resistance to the antibiotics tested are shown in Table 1. In this last year, however, 100% of the isolates presented resistance to at least one drug.

Among the *S. aureus* isolated in 2001, high resistance to β -lactams was observed. The resistance to ampicillin and penicillin was 88% and the resistance to the other antimicrobials tested remained in the range from 72-76% of the isolates, except for the rifampicin (68%) and chloramphenicol (66%). A single isolate presented intermediate resistance to teicoplanin and all isolates were susceptible to vancomycin (Table 2). In 2003, the resistance to β -lactams remained high. The resistance to ampicillin and penicillin was 91% and the resistance to other antimicrobials was within 53-68%, except for ampicillin/sulbactam (88%). All the isolates were susceptible to teicoplanin and vancomycin (Table 2). Among the isolates studied in 2004, the resistance to the β -lactams ampicillin and penicillin increased to 100%, and in ampicillin/sulbactam and in the other isolates the resistance was between 25% and 40%. All the isolates were susceptible to vancomycin and teicoplanin (Table 2).

Most of *S. aureus* isolated in 2001 presented resistance to two or more antimicrobials. Among these, samples of different origins demonstrated

resistance to 14 of the 16 antimicrobial agents tested [supplementary data]. The smallest resistance indexes were observed in samples from cervical secretion, blood culture, and jejunostomy (classified as Other). In this group, 50% of the isolates were susceptible or they presented resistance to only one or two antimicrobial agents.

The *S. aureus* isolated in 2003 also presented a multi-resistance pattern. Among these, mostly isolated samples from different origins showed resistance to 14 of the 16 antimicrobial agents tested. The isolates of 2004 presented a different profile, with most of the samples resistant to three antimicrobial agents [supplementary data].

Typing of isolates by AST

For typing the isolates, numeric codes were attributed in agreement with the susceptibility to each antimicrobial tested, and then the isolates were grouped in agreement with the generated characteristic profile. The results of the antimicrobial susceptibility test of the 50 isolates of 2001 resulted in eleven different phenotypic profiles (Table 3). The phenotypic profile B, corresponding to multi-resistant lineages, was predominant, representing 62% of the samples. This phenotypic profile was found in all the sample groups (spittle, nasal secretion, catheter tip, and Other). The profiles J and K were only found in blood culture samples and jejunostomy, respectively. The result of AST for the 34 isolates of 2003 showed eight novel phenotypic profiles (Table 3), with only three profiles corresponding to those observed in the year of 2001. The phenotypic profile B stayed predominant, representing 50% of the samples. In 2004 the result of AST revealed seven new phenotypic profiles (Table 3), and only two corresponded to the previous years: profile B with 20% of the samples and profile M with 45% of the samples.

Associating the susceptibility profiles with the different sources of the isolates, ten profiles were related to the spittle samples, while three profiles were associated with nasal secretion. The isolates from catheter tips, tracheal aspirate and of other origins corresponded to four, seven and twelve different profiles, respectively [supplementary data].

Discussion

The infections caused by multi-resistant strains of *S. aureus* represent an important problem that affects many health institutions. Due to the large number of procedures and to the diverse possibilities

Table 3. Typing of *S. aureus* isolates by antimicrobial susceptibility testing.*

Isolate	AST profile	Phenotypical profile
Year 2001		
1,22	3333333333333333	A
2-5,9-14, 17-21, 24-27, 29-33, 37, 43-46, 49-50	1111111111111133	B
6	1111111111111132	C
7,16	1111131121111133	D
8,15	133333333323333	E
23	1113333331111233	F
28	111333333111233	G
34, 38, 39, 40	133333333313333	H
35,36	1111131111111133	I
41,42	233333333313333	J
47,48	33333333333233	K
Year 2003		
25	333333333333333	A
2-5, 7-9, 12, 19, 21-24, 26, 28, 29, 33	1111111111111133	B
15	133333333313333	H
1	1133333331111133	L
6, 10, 11, 20, 31	1333333331313333	M
13, 14, 27, 34	1333313331313333	N
16	33332333332233	O
17	1113111111111133	P
18	1111131131111133	Q
30	1333331331313333	R
32	333331333333333	S
Year 2004		
7, 8, 15, 20	1111111111111133	B
4, 9, 10, 12, 14, 16-19	1333333331313333	M
1	1333211331112233	T
2	1111121111111133	U
3	1331111131111333	V
5	1333333311313333	W
6	1331133331311333	X
11	1333331331313333	Y
13	1333313331313333	Z

* Susceptibility profiles are given as a numeric code based on the resistance (1), intermediate resistance (2), and susceptibility (3) to the antibiotic tested.

of contamination for existent *S. aureus* in these places, it is necessary to review the evolution of antimicrobial resistance and the therapeutic response of these bacterial strains.

The decrease in the number of *S. aureus* isolates that occurred in the subsequent years to the implementation of the norms of infection control for CCIH of the Hospital Divina Providencia is extremely positive. This reduction demonstrates the importance of the implementation of programs of infection control and it evokes the need for their maintenance.

When analyzing the antimicrobial resistance of the *S. aureus* samples from the Center of Intensive Therapy of the Hospital Divina Providencia, particularly for the year of 2001, high resistance levels were observed in comparison with data

described by the SENTRY Antimicrobial Surveillance Program, for Brazil [14] and Latin America [15]. Although multiple-drug resistance was observed for MRSA isolates (prevalence 23%) from both the hospital and community in Jamaica, methicillin-susceptible isolates were susceptible to nearly all antimicrobial agents tested [16].

A low proportion of isolates presented susceptibility to penicillin and ampicillin. It was expected, since, currently, it has been recognized that only a small percentage of the *S. aureus* lineages from hospital origins does not produce β -lactamases. The high resistance to penicillin and the total susceptibility to vancomycin are commonly noted for *S. aureus* isolated at different hospitals worldwide [17-20]. Among the lineages tested in this study, the resistance to drugs such as clindamycin and

rifampicin was much higher than that described for *S. aureus* isolates (n = 390) in the hospitals in Malaysia [18]. The low resistance to clindamycin is supported by the lack of exposure of the lineages of *S. aureus* to this drug, since it is not available in the hospitals of Malaysia.

Urassa *et al.* [21] compared the resistance profile of *S. aureus* isolated from neonates, children, and adults hospitalized at a general hospital of Tanzania (n = 260). Those authors did not observe significant differences among the groups, and the resistance values to tetracycline and penicillin were comparable to those observed in the present study. The resistance values to erythromycin and tetracycline obtained for isolates of CTI from the Hospital Divina Providencia were still comparable with those described for isolates of *S. aureus* from respiratory origin at a hospital of Turkey, in the period of 1999-2002 [19].

Comparing the response of the isolates of *S. aureus* to the antibiotics, particularly to β -lactams, an increase in the resistance is observed with time. In 2001, for instance, the *S. aureus* isolates presented a resistance rate to ampicillin and penicillin of 88%, against 91% in 2003 and 100% in 2004. This fact is not isolated for this institution, having already been observed in other hospitals [6,18,20].

In spite of the considerable progress in antimicrobial therapy, resistance in Gram-positive pathogens continues to increase, mainly in relation to the drugs commonly used in medical practice. The predominant profile observed in this study just indicated sensibility to vancomycin and teicoplanin (phenotypic profile B), corroborating the fact that these glycopeptides remain as the reference therapy for serious infections caused by multi-resistant Gram-positive strains [22]. Lineages with increased resistance to teicoplanin have been reported in Europe and United States [23]. Resistance to vancomycin is uncommon, but *S. aureus* with decreased susceptibility have been reported in the United States and Japan [24,25]. However, considering only the isolates of 2004, phenotype B proved to not be the predominant profile, signalling an important change in the resistance profile of the isolates. Except for ampicillin and penicillin, the resistance to the other antimicrobials decreased significantly. The values of resistance to drugs such as gentamicin, tetracycline and oxacillin were similar to those described for hospitals in Brazil [14] and in Latin America [15].

In this study, only three isolates presented sensitivity to all the antimicrobials tested,

representing 3% of the total (phenotypic profile A), and 52 isolates demonstrated sensitivity only to vancomycin and teicoplanin, representing 50% of the total (profile B). In spite of the predominance of profile B, the other isolates alternate resistance and susceptibility to the tested drugs, resulting in a great phenotypic diversity, as can be observed in Table 5 (profiles D to Z). Although molecular typing (such as PFGE, RAPD, RFLP) should be performed to show the prevalence of particular strain(s) in the ICU, the characterization based on phenotypical aspects can contribute to the discrimination among different isolates [12,26]. This information is an important tool for the characterization and control of infectious microorganisms.

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Conflict of interest

No conflict of interest is declared.

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