ORIGINAL RESEARCH

Antimicrobial resistance trends in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates obtained from patients admitted to intensive care units. 2010-2015

Tendencias de resistencia antimicrobiana en Staphylococcus aureus y Staphylococcus epidermidis resistentes y susceptibles a meticilina aislados, obtenidos en unidades de cuidados intensivos, 2010-2015

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| Absctract |

Introduction: The emergence and spread of antimicrobial-resistant strains in hospitals, mainly in intensive care units (ICU), has become a serious public health problem.

Objective: To analyze the temporal trends of bacterial resistance phenotypes of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates obtained from ICU patients of a tertiary hospital in Cartagena, Colombia, between 2010 and 2015.

Methods: A cross-sectional study was carried out between January 2010 and December 2015. Methicillin-resistant and Methicillin-susceptible *S. aureus* and *S. epidermidis* isolates (MRSA, MSSA, MRSE and MSSE) were used. Culture medium microdilution technique was used to detect minimal inhibitory concentration (MIC).

Results: 313 *Staphylococcus spp.* isolates were identified, and most of them were methicillin-resistant (63.6%). Methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE) strains represented 13.7% and 27.8%, respectively, of the total sample. The highest antibiotic resistance values in MRSA and MRSE isolates were observed for the following antibiotics: erythromycin (57.6% and 81.2%, respectively), clindamycin (54.6% and 71.0%), ciprofloxacin (48.4% and 36.4%) and trimethoprim-sulfametoxazole (36.4% and 51.4%).

Conclusions: The results reported here suggest the need to rethink the control strategies designed to minimize antibiotic resistance in the hospital in which the study was conducted.

Keywords: Staphylococcus Aureus; Staphylococcus Epidermidis; Staphylococcal Infections; Methicillin Resistance; Methicillin-Resistant Staphylococcus Aureus; Intensive Care Units (MeSH).

| Resumen |

Introducción. La aparición y la diseminación de cepas resistentes en hospitales, principalmente en unidades de cuidado intensivo (UCI), se han convertido en un serio problema de salud pública.

Objetivo. Analizar la tendencia de los fenotipos de resistencia de *Staphylococcus aureus* y *Staphylococcus epidermidis* resistentes y susceptibles a meticilina aislados en pacientes atendidos en UCI de un hospital de alta complejidad de Cartagena, Colombia, del 2010 al 2015.

Materiales y métodos. Estudio analítico transversal realizado entre enero de 2010 y diciembre de 2015. Se utilizaron aislamientos de *S. aureus* y *S. epidermidis* meticilino-susceptibles y meticilino-resistentes (SARM, SASR, SERM y SESM). La técnica de susceptibilidad empleada fue el método microdilución en caldo para la detección de la concentración mínima inhibitoria.

Resultados. Se identificaron 313 aislamientos de *Staphylococcus spp.*, la mayoría resistentes a meticilina (63.6%). Las cepas SARM y SERM correspondieron al 13.7% y al 27.8% del total de aislamientos, respectivamente. Los mayores porcentajes de resistencia en SARM y SERM correspondieron a eritromicina (57.6% y 81.2%, respectivamente), clindamicina (54.6% y 71.0%), ciprofloxacina (48.4% y 36.4%) y trimetoprima-sulfametoxazol (36.4% y 51.4%).

Conclusión. Los resultados encontrados sugieren el replanteamiento de las estrategias de control de la resistencia antimicrobiana en el hospital objeto de estudio.

Palabras clave: Staphylococcus aureus; Staphylococcus epidermidis; Infecciones estafilocócicas; Resistencia a la meticilina; Staphylococcus aureus resistente a meticilina; Farmacorresistencia microbiana; Unidades de cuidados intensivos (DeCS).

Introducción

The last 20 years have been characterized by the emergence and spread of antibiotic-resistant bacteria, which in turn have become a determining factor for the failure of antibiotic therapies, mainly in patients admitted to intensive care units (ICU). (1,2) This public health problem has serious implications on morbidity and mortality rates, and on public health systems stability, provided that both direct and indirect economic costs increase heavily due long-term hospitalizations and greater drug consumption. (3)

To date, different strategies have been developed to minimize the impact this public health problem can have on health systems, including appropriate antibiotics prescription, cleaning and disinfection programs, antimicrobial stewardship programs, and epidemiological surveillance of antimicrobial resistance. (2,4)

It has been estimated that in patients admitted to a hospital the risk of contracting a nosocomial infection is ~10%. In addition, said risk almost quadruples when the individual is admitted to the ICU, since antimicrobial therapy is a common practice in ICU patients. (5,6) Moreover, multidrug-resistant bacteria infection susceptibility may be higher in these critically ill patients (7-9), which limits treatment options and makes mandatory to determine antimicrobial resistance profiles among clinical isolates retrieved from said patients.

Given how antimicrobial resistance works, any modification to be made in antimicrobial management schemes should be based on both antimicrobial resistance surveillance data and bacterial etiology. (10)

Changes made to the antimicrobial management schemes are not generally applicable to other hospitals, for they are based on the intrinsic characteristics of each hospital and depend on several aspects such as the type of hospital (facilities), antibiotics availability, and specific local data obtained from monitoring antimicrobial resistance. (11)

Staphylococcus spp. are frequently cultured from hospitalized patients and are characterized by being resistant to multiple antimicrobials. Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE) can be used as markers to evaluate antimicrobial resistance time trends in critically ill patients. (12-19)

Espinosa *et al.* (20), in a systematic review on antimicrobial resistance in gram-positive cocci in Colombian hospitals, reported a higher isolation frequency of methicillin-resistant *staphylococci* in ICU isolates in comparison to non-ICU isolates. (20) This finding is consistent with those of Villalobos *et al.* (16) and Villalobos *et al.* (21), who reported MRSA antibiotic resistance phenotypes and profiles in tertiary hospitals in Colombia. In this context, the importance of an active *in vitro* antimicrobial resistance surveillance program should be noted.

Effective surveillance of antimicrobial resistant organisms allows generating reports that provide necessary information for analyzing and monitoring resistance patterns; likewise, these surveillance data can be used for making decisions regarding the treatment. (22)

The objective of the present study was to evaluate the temporal trends of MRSA and MRSE antibiotic resistance phenotypes from isolates obtained between 2010 and 2015 from ICU patients in a tertiary hospital located in Cartagena, Colombia.

Materials and methods

Study population

All *S. aureus* and *S. epidermidis* isolates were obtained from patients admitted between January 2010 and December 2015 to three different ICUs (11 adult ICU beds, 11 pediatric ICU beds, and 6 neonatal ICU beds) in a tertiary hospital in Cartagena, Colombia. Samples collection was performed before chemotherapy was carried out.

Study design

A cross-sectional study was carried out to evaluate antimicrobial resistance trends of methicillin-resistant and methicillin-susceptible *S. aureus* (MRSA and MSSA, respectively) and methicillin-resistant and methicillin-susceptible *S. epidermidis* (MRSE and MSSE, respectively) by using isolates obtained from ICU patients as resistant markers.

Bacterial identification

A MicroScan® Microbiology System (Siemens Healthcare Diagnostics) was used to perform an automated identification process of the staphylococcal species.

Antimicrobial susceptibility tests

A MicroScan® automated system (Dade Behring, Sacramento, USA) was used to determine antibiotic resistance/susceptibility by using the minimal inhibitory concentration (MIC) technique.

Antibiotics

Antibiotic susceptibility was determined for the following antibiotics: clindamycin (CLI, R≥4 µg/mL), ciprofloxacin (CIP, R≥4 µg/mL), daptomycin (DAP, S ≤1 µg/mL), erythromycin (ERI, R≥8 µg/mL), gentamicin (GEN, R≥16 µg/mL), linezolid (LZD, R≥8 µg/mL), moxifloxacin (MXF, R≥4 µg/mL), nitrofurantoin (NIT, R≥128 µg/mL), oxacillin (OXA, R≥4 µg/mL for *S. aureus*, R≥0.5 µg/mL for coagulase-negative *staphylococci*), penicillin (PEN, R≥0.25 µg/mL), rifampin (RIF, R≥4 µg/mL), trimethoprim-sulfamethoxazole (SXT, R≥ 4/76 µg/mL), and vancomycin (VAN, R≥16 µg/mL for *S. aureus*, R≥32 µg/mL for coagulase-negative *staphylococci*).

MIC interpretive standards for all antimicrobials tested were defined according to the standard breakpoints established by the Clinical and Laboratory Standards Institute (CLSI). (23) Isolates were classified as sensitive (S) or resistant (R); besides, strains with intermediate sensitivity (I) were included in the latter category.

Statistical Analysis

IBM SPSS Statistics for Windows, version 23.0, was used for statistical analysis. Annual absolute and relative resistance frequencies were analyzed. For dichotomous outcomes effect size in terms of odds ratio (24) and associated 95% confidence intervals, 95%CI were

used. Fisher's exact test was performed in order to compare antibiotic resistance percentages between *S. aureus* and *S. epidermidis* isolates, and Chi-square test for linear trends was used to analyze the 6-years resistance trend.

All tests of significance were calculated with a two-tailed alpha of 5%, and multiple antibiotic resistance (MAR) index was determined using the procedure described by Krumperman (25).

Ethical considerations

The study was approved by the Bioethics Committee of Universidad de San Buenaventura (Affidavit 005/August-19-2014), which classified it as a risk-free research according to the provisions of article 11 of Resolution 8430/1993, issued by the Colombian Ministry of Health (26), provided that the data used here were obtained from bacterial strains collected from patients admitted to ICUs. Likewise, the ethical principles for medical research involving human subjects outlined by the Declaration of Helsinki were followed. (27)

Results

A total of 313 staphylococcal strains were isolated and identified, of which 63.6% (199/313; 95%CI 58.1- 68.7) were methicillin-resistant staphylococcal isolates (Table 1: MRSA + MRSE + other MR-CoNS). Said isolates were obtained from patients admitted to the adult ICU (AICU) (57.1%; 93/163; 95%CI: 49.4- 64.4), the neonatal ICU (NICU) (78.8%; 67/85; 95%CI: 69.0-86.2), and the pediatric ICU (PICU) (60.0%; 39/65; 95%CI: 47.9-71.0) (Table 1).

Table 1. Distribution of methicillin-resistant and methicillin-susceptible *Staphylococcus* species isolates according by type of intensive care unit service. 2010-2015.

	AICU		PICU		NICU	
	n (%)	95%CI	n (%)	95%CI	n (%)	95%CI
MSSA	34 (20.9)	15.3 to 27.7%	14 (21.5)	13.3 to 33.0%	3 (3.5)	1.2 to 9.9%
MRSA	27 (16.6)	1.6 to 23.0%	11 (16.9)	9.7 to 27.8%	5 (5.9)	2.5 to 13.0%
MSSE	17 (10.4)	6.6 to 16.1%	7 (10.8)	5.3 to 20.6%	5 (5.9)	2.5 to 13.0%
MRSE	28 (17.2)	12.2 to 23.7%	14 (21.5)	13.3 to 33.0%	45 (52.9)	42.4 to 63.2%
Other MS-CoNS	19 (11.6)	7.6 to 17.5%	5 (7.7)	3.3 to 16.8%	10 (11.8)	6.5 to 20.3%
Other MR-CoNS	38 (23.3)	17.5 to 30.4%	14 (21.5)	13.3 to 33.0%	17 (20.0)	12.9 to 29.7%
Total	163		65		85	

AICU: adult intensive care unit; PICU: pediatric intensive care unit; NICU: neonatal intensive care unit; MSSA: methicillin-susceptible *S. aureus*; MRSA: methicillin-resistant *S. aureus*; MSSE: methicillin-susceptible *S. epidermidis*; MRSE: methicillin-resistant *S. epidermidis*; MS-CoNS: methicillin-susceptible coagulase-negative *staphylococci*; MR-CoNS: methicillin-resistant coagulase-negative *staphylococci*.
Source: Own elaboration.

According to the six years susceptibility test results , methicillin resistance rates of *S. aureus* and *S. epidermidis* for the whole period were 45.7% (43/94; 95%CI: 36.0-55.8) and 75.0% (87/116; 95%CI: 66.4-82.0), respectively.

Regarding species identification, by using the automated identification system it was found that 53.0% (116/219; 95%CI: 43.4-59.5) of the coagulase-negative *Staphylococcus* (CoNS) that were tested belonged to the *S. epidermidis* species, while 15.5% (34/219; 95%CI: 11.3-20.9), to *S. hominis subsp hominis*, and 11.4% (25/219; 95%CI: 7.9-16.3) to *S. haemolyticus*. The remaining 20.1% of isolates belonged to other coagulase-negative *staphylococci* (CoNS) species,

including *S. auricularis, S. hominis subsp novobiosepticum, S. warneri, S. lugdunensis, S. simulans, S. xylosus, S. intermedius, S. sciuri, S. capitis subsp capitis,* and *S. capitis subsp urealyticus*.

Resistance to methicillin was detected in 71.2% (156/219; 95%CI: 64.9-76.8) of the CoNS isolates. In addition, 13.7% (43/313; 95%CI: 10.4-18.0) and 27.8% (87/313; 95%CI: 23.1-33.0) isolates were MRSA and MRSE, respectively (Table 1).

Sample-based MRSA, MSSA, MRSE, and MSSE isolates distribution is shown in Table 2. Most of these strains (52.9 to 96.6%) were isolated from blood cultures, while the remaining ones were obtained from orotracheal and ocular secretions (<30%), and other body fluids (<10%).

Table 2. Distribution of methicillin-resistant and methicillin-susceptible *Staphylococcus* species isolates based on the sample type they were retrieved from. 2010-2015.

	MSSA		MRSA		MSSE		MRSE	
	n (%)	95%CI	n (%)	95%CI	n (%)	95%CI	n (%)	95%CI
Blood	27 (52.9)	39.5 to 66.0%	24 (55.8)	41.1 to 69.6%	28 (96.6)	82.8 to 99.4%	74 (85.1)	76.1 to 91.1%
Secretions *	15 (29.4)	18.7 to 43.0%	12 (27.9)	16.8 to 42.7%	0 (0)	0.0 to 11.7%	6 (6.9)	3.2 to 14.2%
Fluids †	4 (7.8)	3.1 to 18.5%	4 (9.3)	3.7 to 21.6%	1 (1)	0.6 to 17.2%	1 (1.1)	0.2 to 6.2%
Bronquial lavage	3 (5.9)	2.0 to 15.9%	1 (2.3)	0.4 to 12.2%	0 (0)	0.0 to 11.7%	1 (1.1)	0.2 to 6.2%
Urine	2 (3.9)	1.1 to 13.2%	0 (0)	0.0 to 8.2%	0 (0)	0.0 to 11.7%	3 (3.5)	1.2 to 9.7%
Catheter tips	0 (0)	0.0 to 7.0%	2 (4.7)	1.3 to 15.5%	0 (0)	0.0 to 11.7%	2 (2.3)	0.6 to 8.0%
Total	51		43		29		87	

MSSA: methicillin-susceptible *S. aureus*; MRSA: methicillin-resistant *S. aureus*; MSSE: methicillin-susceptible *S. epidermidis*; MRSE: methicillin-resistant *S. epidermidis*.
* Secretions: orotracheal, ocular and other secretions.
† Fluids: pleural, peritoneal and other fluids.
Source: Own elaboration.

Antimicrobial resistance profiles are shown in Table 3. All staphylococcal isolates were identified as sensitive to daptomycin (DAP). Regarding MRSA and MRSE isolates, the highest resistance rates were found for erythromycin (ERI) (57.6% and 81.2%, respectively), clindamycin (CLI) (54.6% and 71.0%, respectively), ciprofloxacin (CIP) (48.4% and 36.4%, respectively) and trimethoprim-sulfamethoxazole (SXT) (36.4% and 51.4%, respectively).

In addition, resistance percentages to ERI, CLI, gentamicin (GEN), CIP, and SXT were far higher in in MRSA than in MSSA isolates. A similar behavior was only observed between MRSE and MSSE isolates (Table 4).

Table 3. Antimicrobial resistance profiles of the methicillin-resistant and methicillin-susceptible *Staphylococcus* species isolates. 2010-2015.

	MSSA		MRSA		MSSE		MRSE	
	n (%)	95%CI	n (%)	95%CI	n (%)	95%CI	n (%)	95%CI
CLI	8 (15.7)	8.2 to 28.0%	18 (54.6)	38.9 to 67.5%	3 (14.3)	19.9 to 52.7%	44 (71.0)	51.6 to 71.6%
CIP	1 (3.1)	1.1 to 13.2%	15 (48.4)	26.4 to 54.4%	3 (16.7)	9.9 to 38.4%	20 (36.4)	18.3 to 36.6%
DAP	0 (0)	0.0 to 7.0%	0 (0)	0.0 to 8.2%	0 (0)	0.0 to 11.7%	0 (0)	0.0 to 4.2%
ERI	6 (15.8)	12.5 to 34.6%	19 (57.6)	43.3 to 71.6%	8 (38.1)	31.4 to 65.6%	56 (81.2)	65.9 to 83.6%
GEN	1 (2.5)	0.4 to 10.3%	12 (36.4)	18.6 to 45.1%	4 (19.1)	7.6 to 34.6%	51 (70.8)	58.6 to 77.7%
LZD	0 (0)	0.0 to 7.0%	4 (12.5)	3.7 to 21.6%	0 (0)	0.0 to 11.7%	0 (0)	0.0 to 4.2%
MXF	0 (0)	0.4 to 10.3%	11 (33.3)	14.9 to 40.2%	1 (4.8)	5.5 to 30.6%	7 (11.5)	5.5 to 18.5%
NIT	2 (12.5)	1.1 to 13.2%	3 (23.1)	2.4 to 18.6%	0 (0)	0.0 to 11.7%	0 (0)	0.0 to 4.24%
RIF	1 (2.5)	0.4 to 10.3%	5 (15.2)	5.1 to 24.5%	1 (4.8)	1.9 to 22.0%	22 (30.6)	18.3 to 36.6%
SXT	2 (5)	1.1 to 13.2%	12 (36.4)	16.8 to 42.7%	5 (25)	7.6 to 34.6%	37 (51.4)	32.7 to 53.0%
VAN	0 (0)	0.0 to 7.0%	2 (6.1)	1.3 to 15.5%	1 (4.8)	0.6 to 17.2%	1 (1.4)	0.2 to 6.2%

MSSA: methicillin-susceptible *S. aureus*; MRSA: methicillin-resistant *S. aureus*; MSSE: methicillin-susceptible *S. epidermidis*; MRSE: methicillin-resistant *S. epidermidis*; CLI: clindamycin; CIP: ciprofloxacin; DAP: daptomycin; ERI: erythromycin; GEN: gentamicin; LZD: linezolid; MXF: moxifloxacin; NIT: nitrofurantoin; RIF: rifampin; SXT: trimethoprim-sulfamethoxazole; VAN: vancomycin.
Source: Own elaboration.

Table 4. Comparison of antibiotic resistance phenotypes of methicillin-resistant and methicillin-susceptible *Staphylococcus* species isolates. 2010-2015.

MRSA vs. MSSA	Antibiotic resistance phenotypes	p-value*	Effect size (OR)	95%CI
	CLI-R	0.0001	6.2	2.4 to 15.9
	CIP-R	≤0.00001	16.0	3.4 to 74.7
	ERI-R	0.0003	5.1	2.1 to 12.3
	GEN-R	0.0001	21.7	2.7 to 174.1
	SXT-R	0.0011	9.5	2.0 to 45.3
MRSE vs. MSSE	Antibiotic resistance phenotypes	p-value*	Effect size (OR)	95%CI
	CLI-R	0.0097	3.1	1.3 to 7.4
	CIP-R	0.536	1.4	0.5 to 3.7
	ERI-R	0.0054	3.4	1.4 to 8.0
	GEN-R	≤0.00001	10.7	3.8 to 29.9
	SXT-R	0.014	3.6	1.3 to 9.8

MSSA: methicillin-susceptible *S. aureus*; MRSA: methicillin-resistant *S. aureus*; MSSE: methicillin-susceptible *S. epidermidis*; MRSE: methicillin-resistant *S. epidermidis*; CLI-R: clindamycin-resistant; CIP-R: ciprofloxacin-resistant; ERI-R: erythromycin-resistant; GEN-R: gentamicin-resistant; SXT-R: trimethoprim-sulfamethoxazole-resistant.
* p-values were estimated by means of Fisher's exact test.
Source: Own elaboration.

Concerning MSSA strains, all resistance percentage values were <20%; on the contrary, resistance percentages to ERI and SXT in MSSE isolates were comparable with the high percentage values observed in MRSA isolates (p=0.41; X^2=0.68 and p=0.296; X^2=1.1, respectively).

Vancomycin MIC values for 2 MRSA isolates (4.7%; 95%CI: 1.3-15.5) were 8 μg/mL, vancomycin-intermediate *S. aureus* (VISA), and 16 μg/mL, vancomycin-resistant *S. aureus* (VRSA), while in 2 MRSE isolates (2.3%; 95%CI: 0.6-8.0) vancomycin MIC values were ≥16 μg/mL.

Resistance phenotypes trends in MRSA and MRSE isolates were higher in the last year of the study (January-December 2015), with the exception of resistance to SXT, since SXT-resistant isolates were not identified during this period. On the other hand, for the whole six years of the study, the lowest resistance percentages in MRSA and MRSE isolates were found for SXT (27.9% and 42.5%, respectively) (Table 5).

Table 5. Trends of antimicrobial resistance phenotypes of methicillin-resistant and methicillin-susceptible *Staphylococcus* species isolates. 2010-2015.

Resistance phenotypes	2010		2011		2012		2013		2014		2015	
	MRSA	MRSE	MRSA	MRSE	MRSA	MRSE	MRSA	MRSE	MRSA	MRSE	MRSA	MRSE
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
CLI-R	3 (100)	6 (66.7)	0 (0)	7 (70)	1 (16.7)	7 (63.6)	1 (11.1)	13 (68.4)	12 (92.3)	11 (84.6)	6 (60)	10 (66.7)
ERI-R	3 (100)	16 (100)	0 (0)	8 (80)	3 (50)	8 (72.7)	1 (11.1)	13 (68.4)	12 (92.3)	10 (76.9)	6 (60)	10 (66.7)
CIP-R	1 (100)	6 (66.7)	0 (0)	7 (70)	3 (50)	7 (63.6)	2 (22.2)	13 (68.4)	9 (69.2)	11 (84.6)	2 (20)	3 (20)
GEN-R	3 (100)	11 (68.8)	0 (0)	6 (54.5)	3 (50)	11 (84.6)	0 (0)	13 (68.4)	6 (46.2)	10 (76.9)	1 (10)	9 (60)
SXT-R	2 (66.7)	11 (68.8)	0 (0)	6 (54.5)	5 (83.3)	9 (69.2)	0 (0)	11 (57.9)	5 (38.5)	0 (0)	0 (0)	0 (0)

MSSA: methicillin-susceptible *S. aureus*; MRSA: methicillin-resistant *S. aureus;* MSSE: methicillin-susceptible *S. epidermidis*; MRSE: methicillin-resistant *S. epidermidis*; CLI-R: clindamycin-resistant; CIP-R: ciprofloxacin-resistant; ERI-R: erythromycin-resistant; GEN-R: gentamicin-resistant; SXT-R: trimethoprim-sulfamethoxazole-resistant. Note: Multiresistant strains were included individually for each antibiotic evaluated.
Source: Own elaboration.

Furthermore, in the case of methicillin-resistant isolates, a high frequency of multi-drug resistant strains was observed (MRSA: 26/43; 60.5%; 95%CI: 45.6-73.6; MRSE: 68/87; 78.2%; 95%CI: 68.4-85.6). In this regard, 28 multiresistant phenotypes (MR-ANT) were identified in 26 MRSA (12 MR-ANT) and 68 MRSE (16 MR-ANT) strains. The MR-Ant frequency order was as follows: CLI-CIP-ERI-GEN-SXT (MRSA: 19.2%; 5/26; 95%CI: 8.5-37.9; MRSE: 23.5%; 16/68; 95%CI: 15.0- 34.9), CLI-CIP-ERI (MRSA: 23.1%; 6/26; 95%CI: 11.0-42.1), CLI-ERI-GEN (MRSE: 14.7%; 10/68; 95%CI: 8.2-25.0), and CLI-ERI-GEN-SXT (MRSE: 11.8%; 8/68; 95%CI: 6.1-21.5).

Multiple antibiotic resistances were also observed: 9 isolates (MRSA: 2/43; 4.7%; 95%CI: 1.3-15.5; MRSE: 7/87; 8.1%; 95%CI: 4.0-15.7) were resistant to a combination of seven antibiotics; 22 (MRSA: 6/43; 14.0%; 95%CI: 6.6-27.3; MRSE: 16/87; 18.4%; 95%CI: 11.7- 27.8), to a six antibiotics combination, and 13 (MRSA: 1/43; 2.3%; 95%CI: 0.4-12.1; MRSE: 12/87; 13.8%; 95%CI: 8.1-22.6), to a five antibiotics combination.

The highest MAR index values observed in the methicillin-resistant *Staphylococcus* species isolates ranged from 0.8 to 0.9, while in methicillin-susceptible strains these ranged from 0.2 and 0.4. In addition, in most methicillin-resistant isolates MAR index was higher than 0.2 (MRSE: 79.3%; 69/87; 95CI 69.7 to 86.5%; MRSA: 60.5%; 26/43; 95%CI: 45.6-73.6), in contrast with methicillin-susceptible isolates, where most of them had a MAR index value lower than 0.2 (MSSE: 24.1%; 7/29; 95%CI: 12.2-42.1; MSSA: 3.9%; 2/51; 95%CI: 1.1-13.2).

On the other hand, the analysis of the methicillin-resistance trend of *S. aureus* isolates allowed finding a significant increase (p=0.031; X^2 lineal trend=4.673; 1 df; slope=1.72) during the six years study period, somehow this epidemiological characteristic was not observed in other bacterial strains for the same period (Chi-square for lineal trend, MSSA: p=0.732; MRSE: p=0.082; MSSE: p=0.323) (Figure 1).
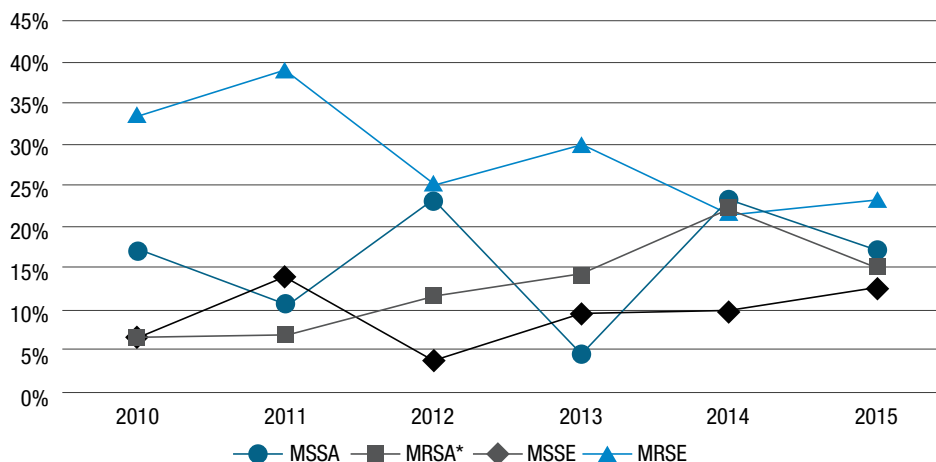

Figure 1. Frequency of methicillin-resistant and methicillin-susceptible *Staphylococcus* species isolates. 2010-2015.
MSSA: methicillin-susceptible *S. aureus*; MRSA: methicillin-resistant *S. aureus*; MSSE: methicillin-susceptible *S. epidermidis*; MRSE: methicillin-resistant *S. epidermidis*.
* p=0.031; X^2 lineal trend=4.673; 1df; m=1.72.
Source: Own elaboration.

Discussion

Staphylococcus species have emerged as significant pathogens in both hospital-acquired and community-onset infections. (28-31) Hospital isolates of bacterial strains tend to have higher rates of resistance to antimicrobial agents, especially those collected from ICU patients. (20)

In this study, a higher frequency of methicillin-resistant *staphylococci* clinical isolates was reported in comparison with methicillin-susceptible isolates, which is consistent with the findings of national (16,32-38) and

international studies. (12,39-44) In part, this could be explained by the relationship between antibiotic selective pressure and the emergence of antimicrobial-resistant organisms in hospitals. (45,46)

Most methicillin-resistant strains, mainly MRSE, were isolated from patients who were admitted to the NICU. This finding is consistent with several works reporting that *S. epidermidis* is one of the most common bacterial pathogens in this population. (32,39,41,43,47-51) Major risk factors for presenting these staphylococcal infections include prematurity, invasive procedures, prolonged hospitalization, and immune immaturity. (52,53)

On the other hand, the percentage values of MRSA and MRSE strains isolated from blood cultures are similar to those reported by Cortes *et al.* (34) in bacteremic patients admitted to the ICUs of 33 Colombian hospitals.

All these similarities suggest three possible scenarios at a national level: (i) failure of empirical antibacterial therapy (34,54), (ii) increased hospital stay lengths for clinically ill patients (55), and increased risk of mortality in ICU patients. (34,56)

Also, it was found that MRSE isolates showed a higher resistance frequency to antistaphylococcal antibiotics such as ERI, CLI and SXT that MRSA strains did. Somehow, in the case of ciprofloxacin, this pattern was not observed. Similar results were described by Álvarez *et al.* (57) In an attempt to explain these findings, the authors of the present study suggest two key factors: selective pressure caused by antibiotic use and cross-transmission of resistant bacteria.

Significant differences between antimicrobial resistance rates of methicillin-resistant and methicillin-susceptible *Staphylococcus* isolates have also been reported by other authors, although they have done so when comparing coagulase-negative *Staphylococcus* strains isolated from septicemic patients. (31)

About a third of the methicillin-resistant isolates were also resistant to other antibiotics including macrolides, lincosamides, quinolones, aminoglycosides, rifamycins, oxazolidinones, nitrofurans and antifolates. In addition, if a MAR index value >0.2 can be considered a multiple antimicrobial resistance characteristic (58), the presence of these multidrug-resistant strains could support the emergence of the three hypothetical scenarios described above.

Out of 43 MRSA isolates, 2 vancomycin-intermediate and vancomycin-resistant strains were identified in adult bacteremia cases. To date, there are no reports of VISA or VRSA isolates in Colombian hospitals. (21,33,35-37,49,57) However, one study conducted in Caldas, Colombia, in 1996 (38), reported the existence of VRSA strains isolated from patients admitted to the ICU, but these isolates were identified using the disc diffusion method. Despite the fact that VRSA isolates presence has not been confirmed by a reference laboratory, this finding could be interpreted as an early warning of the occurrence of clinically relevant pathogens for which only limited therapeutic options are available.

A final aspect that should be noted in this context is that in a recent study Rossi *et al.* (59) reported vancomycin resistance transmission between MRSA strains isolated from blood cultures.

Conclusion

The most effective antibiotics in MRSA and MRSE isolates were daptomycin, linezolid and vancomycin. The significant and rising trend in the number of MRSA isolates may be an indication of the need to redefine existing strategies to minimize antimicrobial resistance. It is necessary to periodically conduct surveillance studies in all hospitals in the country to achieve effective control strategies of multi-drug-resistant infections and to reduce antimicrobial resistance rates.

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