Major Article

Resistance profile to antimicrobials agents in methicillin-resistant Staphylococcus aureus isolated from hospitals in South Brazil between 2014-2019

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Abstract

Introduction: Methicillin-resistant Staphylococcus aureus (MRSA) is a common pathogen causing healthcare-associated infections. Owing to the restricted use of beta-lactams in MRSA infections, non-beta-lactam antimicrobials are required for treatment. However, MRSA can develop resistance mechanisms to non-beta-lactam antimicrobials, which reduces viable treatment options. Here, we evaluated the antimicrobial susceptibility and resistance genes of MRSA isolated from hospitalized patients in South Brazil.

Methods: The antimicrobial susceptibilities of hospital MRSA (217) isolates were determined by disk diffusion or microdilution methods. Additionally, the presence of 14 resistance genes and SCCmec typing was performed by PCR. Results: Among the antimicrobials tested, we observed high erythromycin (74.2%), ciprofloxacin (64.5%), and clindamycin (46.1%) resistance rates and complete susceptibility to linezolid and vancomycin. Seventeen different patterns of MRSA antimicrobial resistance were observed, of which 42.9% represented multidrug resistance. Among erythromycin-resistant MRSA, 53.4%, 45.3%, 37.9%, 13.0%, and 6.8% carried ermA, msrA, msrB, ermC, and ermB genes, respectively. Among clindamycin-resistant MRSA, 83%, 17%, 10%, 4%, and 2% carried ermA, ermA, ermB, linA, and linB genes, respectively. Among gentamicin-resistant MRSA, 96.8%, 83.9%, and 9.7% carried aac(6’)/aph(2’’), aph(3’)-IIIa, and ant(4’)-Ia genes, respectively. Among tetracycline-resistant MRSA, 6.5% and 93.5% carried tetK and tetM genes, respectively. Lastly, among trimethoprim/sulfamethoxazole-resistant MRSA, 13.3% and 100% carried dfrA and dfrG genes, respectively. The SCCmec type IV isolates were detected more frequently, whereas the SCCmec type III isolates exhibited higher multidrug resistance.

Conclusions: The study data provides information regarding the MRSA resistance profile in South Brazil that is associated with the clinical conditions of patients and can contribute to clinical decision-making.

Keywords: Methicillin-resistant Staphylococcus aureus. Healthcare-associated infections. Antimicrobial susceptibility. Resistance genes.

INTRODUCTION

Antimicrobial resistance poses a significant challenge to modern medicine as well as to the possibility of effective treatment of infectious diseases1. Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most frequent causes of community- and healthcare-associated infections (CA-MRSA and HA-MRSA, respectively). A major concern remains owing to higher morbidity and mortality when compared with infections caused by methicillin-susceptible strains (MSSA), along with increased hospitalization and health care costs2. MRSA strains pose a threat to public health owing to their potential for genetic adaptation and remarkable ability to acquire resistance to multiple antimicrobials, along with the implications for the treatment of this pathogen3,4.

Methicillin resistance is mediated by the acquisition of genes (mecA or mecC) found in the mobile genetic element called staphylococcal cassette chromosome mec (SCCmec), which encodes an altered penicillin-binding protein (PBP2a or PBP2’) that confers low affinity for most beta-lactams5,6. The SCCmec elements are classified into thirteen different types (SCCmec I-XIII) based on structural organization and genetic content2. CA-MRSA strains generally harbor SCCmec type IV or V, and are susceptible to non-beta-lactam antimicrobials. HA-MRSA strains commonly harbor SCCmec types I, II, or III, which contain genes that confer resistance to non-beta-lactam antimicrobials7.

Owing to the restricted use of beta-lactams for treating infections caused by MRSA, non-beta-lactam antimicrobials, such as...
aminoglycosides, fluoroquinolones, folate inhibitors, glycopeptides, lincomamides, lipopeptide, macrolides, oxazolidinones, and tetracyclines, are required for the treatment of staphylococcal infections. However, these therapeutic options are reduced when MRSA isolates develop resistance mechanisms to survive in conditions with high concentrations of these antimicrobials.

Resistance is associated with different molecular mechanisms, as follows: 1) inactivation of antimicrobials by enzymes, such as inactivation of aminoglycosides by aminoglycoside-modifying enzymes (AMEs) (encoded by aac(6’)/aph(2’), aph(3’)-IIIa, and ant(4’)-Ia genes)\(^{10}\), trimethoprim by variants of dihydrofolic reductases (DHFRs) (dfrA and dfrG)\(^{31}\), or lincomamide by lincomamide nucleotidytransferases (linA and linB)\(^ {2,13}\); 2) alterations in ribosomal binding site (ermA, ermB, and ermC), which confers resistance to macrolides and lincomamides\(^ {9,12}\); 3) active efflux pumps, such as those encoded by msrA, msrB, and tetK\(^ {12}\), which impart resistance to macrolides, type B streptogramins, and tetracycline, respectively\(^ {14}\); and 4) ribosomal protection (tetM), that confers resistance to tetracycline\(^ {14}\). These mechanisms limit the therapeutic options available for the treatment and control of MRSA infections.

The latest data from the Centers for Disease Control and Prevention (CDC) show more than ten thousand deaths caused by MRSA, with high healthcare costs, in the US\(^ {15}\). In Latin America, the resources for monitoring the epidemiology of MRSA remain limited. Additionally, the true nature and extent of MRSA infections are inadequately known; this indicates that local data collection is limited. Additionally, the true nature and extent of MRSA infections are inadequately known; this indicates that local data collection is limited. Considering the importance of global surveillance studies on resistance profiles, along with the current challenges related to the treatment of MRSA infections, this study aimed to evaluate antimicrobial susceptibility and identify the resistance genes in MRSA obtained from hospitals in South Brazil.

### METHODS

#### Study design and clinical strains

This cross-sectional observational study was conducted using 217 MRSA isolates obtained between January 2014 and January 2019 (40 in 2014, 49 in 2015, 75 in 2016, 29 in 2017, and 24 in 2018) from hospitals in Porto Alegre in South Brazil. The study was registered under the Institutional Ethics Committee number 2.770.338. The strains were isolated from respiratory tract (75; 34.6%), blood (55; 25.3%), skin and soft tissue (42; 19.4%), bone and connective tissue (24; 11.1%), and sterile cavity liquid (12; 5.5%) samples, and from medical devices (9; 4.1%). The isolates were cryopreserved and stored at -20 °C until testing.

#### Identification of *S. aureus*

The isolates were identified as *S. aureus* using conventional microbiological methods, such as evaluation of colony morphology on sheep blood agar, Gram staining, catalase activity, production of coagulase, and growth on mannitol salt agar. Methicillin resistance was confirmed by the cefoxitin disk diffusion method and polymerase chain reaction (PCR) for the detection of mecA gene according to Clinical and Laboratory Standard Institute (CLSI) guidelines, 2019\(^ {17}\).

### Antimicrobial susceptibility tests

The susceptibility of isolates to ciprofloxacin (5 μg), clindamycin (2 μg), erythromycin (15 μg), gentamicin (10 μg), linezolid (30 μg), tetracycline (30 μg), and trimethoprim/sulfamethoxazole (1.25 μg/23.75 μg) was determined by the disk diffusion method on Mueller-Hinton agar (Oxoid, Basingstoke, England).

Clindamycin susceptibility was determined using a disk approximation test with erythromycin and clindamycin (D-test). The following resistant phenotypes were identified in the D-test: inducible phenotype (iMLS\(_B\)), when resistant to erythromycin and susceptible to clindamycin with formation of a D-shaped zone, constitutive resistance phenotype (cMLS\(_B\)) when resistant to both erythromycin and clindamycin, and MS phenotype when resistant to erythromycin and susceptible to clindamycin without formation of a D-shaped zone\(^ {18}\).

The minimal inhibitory concentration (MIC) of vancomycin was determined using the microdilution method in Mueller-Hinton broth (Oxoid, Basingstoke, England). The results of antimicrobial susceptibility were interpreted according to the CLSI guidelines\(^ {17}\). The strains obtained from the American Type Culture Collection (ATTC), *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213, were used as controls.

#### Detection of antimicrobial resistance genes

The detection of genes related to antimicrobial resistance, including (aac(6’)/aph(2’), ant(4’)-Ia, aph(3’)-IIIa, dfrA, dfrG, ermA, ermB, ermC, linA, linB, msrA, msrB, tetK, and tetM), in MRSA was confirmed by conventional PCR, as previously described, with certain modifications (Table 1\(^ {10,19,23}\).

Bacterial deoxyribonucleic acid was extracted by using Chelex\(^ {®} \) 100 (Bio-Rad, Richmond, CA, USA) and Proteinase K (Sigma-Aldrich, Poole, UK). The PCR reaction contained 0.2 mM of each deoxyribonucleotide triphosphate (10 mM), 2 mM of MgCl\(_2\) (50 mM), 1X PCR buffer (10 X), 0.5 μM of forward/reverse primers (10 μM), 1.5 U of Taq DNA polymerase (5 U/μL), and 1 μL of DNA template in a total volume of 25 μL. Amplifications were performed using a LifePro Thermal Cycler (Hangzhou Bioer Technology Co. Ltd., Hangzhou, China). The PCR amplicons were separated by electrophoresis in a 2.0% agarose gel (Sigma-Aldrich, USA) and stained with 0.1% ethidium bromide (0.4 μg/mL).

The PCR-positive controls, *S. aureus* JCSC 4469 (aac(6’)/aph(2’)), *S. aureus* N315 (ant(4’)-Ia), *S. aureus* JCSC 4488 (aph(3’)-IIIa and dfrG), *S. aureus* WIS (dfrA), *S. aureus* NCTC 10442 (ermA), *S. aureus* HDE 288 (ermB), *S. aureus* JCSC 4474 (ermC), *S. aureus* JCSC 6082 (linA), *S. aureus* JCSC 2172 (linB), *S. aureus* NCTC 8325 (msrA and msrB), *S. aureus* 85/2082 (tetK), and *S. aureus* JCSC 6943 (tetM) were included. A tube containing all components of the PCR mixture, except the template DNA, was used as the negative control.

### SCCmec typing

The SCCmec types I-X were identified by multiplex-PCR, as previously described\(^ {24}\). The *S. aureus* strains NCTC 10442, N315, 85/2082, JCSC 4474, WIS, HDE 288, JCSC 6082, JCSC 6943,
| Target gene | Primer sequence (5'-3') | Amplicon (bp) | Amplification conditions | Ref. |
|-------------|-------------------------|--------------|--------------------------|------|
| aac(6')/aph(2'') | F: CAG AGC CTT GGG AAG ATG AAG  
R: CCT CGT GTA ATT CAT GTT CTG GC | 348 | Pre cycle: 94 °C – 3 min | 11 |
| ant(4')-Ia | F: CAA ACT GCT AAA TCG GTA GAA GCC  
R: GGA AAG TTG ACC AGA CAT ACC GG | 294 | 35 cycles: 94 °C – 40 s,  
55 °C – 40 s, 72 °C – 40 s | |
| aph(3')-IIIa | F: GGC TAA AAT GAG AAT ATC ACC GG  
R: CTT TAA AAA ATC ATA CAG CTC GCC | 523 | Last cycle: 72 °C – 2 min | |
| dfrA | F: CAC TTG TAA TGG CAC GGA AA  
R: CGA ATG TGT ATG GTG GAA AG | 270 | Pre cycle: 94 °C – 4 min  
30 cycles: 94 °C – 1 min,  
52 °C – 30 s, 72 °C – 1 min | 19 |
| dfrG | F: TGC TGC GAT GGA TAA GAA  
R: TGG GCA AAT ACC TCA TTC C | 405 | Last cycle: 72 °C – 4 min | |
| ermA | F: TCT AAA AAG CAT GTA AAA GAA  
R: CTT CGA TAG TTT ATT AAAT AGT | 645 | Pre cycle: 93 °C – 3 min  
35 cycles: 93 °C – 1 min,  
52 °C – 1 min, 72 °C – 1 min | 20 |
| ermB | F: GAA AAG GTA CTC AAC CAA ATA  
R: AGT AAC GGT ACT TAA ATT GTT TAC | 639 | Last cycle: 72 °C – 5 min | |
| ermC | F: TCA AAA CAT AAT ATA GAT AAA  
R: GCT AAT ATT GTT TAA ATC GTC AAT | 642 | Pre cycle: 93 °C – 3 min  
35 cycles: 93 °C – 1 min,  
53 °C – 1 min, 72 °C – 1 min  
Last cycle: 72 °C – 5 min | 20 |
| linA | F: GTA TTA ACT GGA AAA CAG CAA AG  
R: GAG CTT CTT TTG AAA TAC ATG G | 323 | Pre cycle: 94 °C – 5 min  
35 cycles: 94 °C – 45 s,  | 21 |
| linB | F: CCT ACC TAT TGT TTG TGG AA  
R: ATA ACG TTA CTC TCC TAT TC | 925 | 48 °C 45 s, 72 °C – 1 min  
Last cycle: 72 °C – 5 min | |
| msrA | F: GGC ACA ATA AGA GTG TTT AAA GG  
R: AAG TTA TAT CAT GAA TAG ATT GTC CTG TT | 940 | Pre cycle: 94 °C – 5 min  
25 cycles: 94 °C – 1 min,  | 22 |
| msrB | F: TAT GAT ATC CAT AAT AAT TAT CCA ATC  
R: AAG TTA TAT CAT GAA TAG ATT GTC CTG TT | 595 | 50 °C 1 min, 72 °C – 1 min  
Last cycle: 72 °C – 10 min | |

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and JCSC 6945 were used as the positive controls for the SCCmeC types I, II, III, IV, V, VI, VII, IX, X, and X respectively. The PCR mixture components without the DNA template were used as negative control.

**Statistical analysis**

Statistical analysis was performed using SPSS version 20.0 software (SPSS, Chicago, IL, USA). Chi-square test or Fisher’s exact test was performed to analyze the results. p value < 0.05 was considered statistically significant.

**RESULTS**

**Antimicrobial susceptibility**

In the antimicrobial susceptibility tests of the MRSA isolates, the highest resistance rates were observed for erythromycin (74.2%; 161/217), ciprofloxacin (64.5%; 140/217), and clindamycin (46.1%; 100/217). Furthermore, 2.3% (5/217) of the isolates exhibited intermediate resistance to erythromycin and 1.4% (3/217) to clindamycin. The overall prevalence of iMLS$_{B'}$, cMLS$_{B'}$, and MS$_{B'}$ phenotypes was 7.4% (16/217), 46.1% (100/217), and 26.3% (57/217), respectively. Conversely, lower resistance rates were observed against gentamicin (28.6%; 62/217), tetracycline (14.3%; 31/217), and trimethoprim-sulfamethoxazole (13.8%; 30/217). Additionally, 1.8% (4/217) of the isolates exhibited intermediate resistance to trimethoprim-sulfamethoxazole. All isolates were susceptible to linezolid and vancomycin, with MIC values to vancomycin of 0.25 μg/mL (41.0%; 89/217), 0.5 μg/mL (26.3%; 57/217), 0.75 μg/mL (16.6%; 36/217), 1 μg/mL (13.4%; 29/217), and 1.5 μg/mL (2.8%; 6/217) (Figure 1).

Among the 177 out of the 217 MRSA isolates that exhibited resistance to non-beta-lactam antimicrobials, we observed 17 distinct patterns (P) of antimicrobial resistance (Figure 2), of which 12 were grouped and 5 were singular patterns. The dominant resistance pattern (P1)—erythromycin and ciprofloxacin resistance—was observed in 37 isolates. The fifth pattern of antimicrobial resistance (P5) was identified in 18 isolates that were resistant to six antimicrobials. Furthermore, resistance patterns to five (P6) and four (P2) antimicrobials were observed in 10 and 31 isolates, respectively (Figure 2).

Upon analyzing the prevalence of MRSA resistance among isolates collected in different years, we observed that 93 isolates (42.9%) exhibited multidrug resistance, i.e., they were resistant to three or more classes of antimicrobial agents, excluding isolates with intermediate resistance. Among them, 32 (34.4%) exhibited resistance against at least three different classes of antimicrobials, 28 (30.1%) against four classes, 15 (16.1%) against five classes, and 18 (19.4%) against six classes.

**Detection of antimicrobial resistance genes**

The detection of the resistance genes showed that, among erythromycin-resistant MRSA representing the macrolides class, the most frequently encountered gene was ermD (86; 53.4%), followed by msrA (73; 45.3%), msrB (61; 37.9%), ermC (21; 13.0%), and ermB (11; 6.8%); 3 (1.9%) isolates tested negative for these genes. In the lincosamides class, among 100 clindamycin-resistant isolates, 83 (83%) harbored ermA, 17 (17%) harbored ermC, 10 (10%) harbored ermB, 4 (4%) harbored linA, and 2 (2%) harbored linB; 4(4%) isolates tested negative for these genes. Among gentamicin-resistant MRSA, which represented the aminoglycosides class, out of 62 isolates, 60 (96.8%) harbored aac(6')-aph(2'')-I, 52 (83.9%) harbored aph(3')-IIIa, and 6 (9.7%) harbored ant(4')-Ia genes. In the tetracyclines class, of the 31 tetracycline-resistant MRSA isolates, 2 (6.5%) harbored tetK and 29 (93.5%) harbored tetM genes. Furthermore, in the folate inhibitors class, of the 30 trimethoprim/sulfamethoxazole-resistant MRSA isolates, 4 (13.3%) harbored dfrA and 30 (100%) harbored dfrG genes.

Among macrolide-resistant MRSA, the most common gene combination was msrA + msrB (27.3%), followed by ermA +

| Target gene | Primer sequence (5’-3’) | Amplicon (bp) | Amplification conditions | Ref. |
|-------------|------------------------|--------------|-------------------------|-----|
| tetK        | F: CAG CAG ATC CTA CTC CTT | 168          | Pre cycle: 93 °C – 5 min | 21  |
|             | R: TCG ATA GGA ACA GCA GTA |              | 35 cycles: 93 °C – 1 min, |      |
|             |                         |              | 54 °C 1 min, 72 °C – 1 min |      |
|             |                         |              | Last cycle: 72 °C – 10 min |      |
| tetM        | F: GTG GAC AAA GGT ACA ACG AG | 405          | Pre cycle: 93 °C – 5 min | 23  |
|             | R: CGG TAA AGT TCG TCA CAC AC |              | 35 cycles: 93 °C – 1 min, |      |
|             |                         |              | 52 °C 1 min, 72 °C – 1 min |      |
|             |                         |              | Last cycle: 72 °C – 10 min |      |

**TABLE 1:** Continuation.

| Target gene | Primer sequence (5’-3’) | Amplicon (bp) | Amplification conditions | Ref. |
|-------------|------------------------|--------------|-------------------------|-----|
|             |                        |              | Pre cycle: 93 °C – 5 min | 21  |
|             |                        |              | 35 cycles: 93 °C – 1 min, |      |
|             |                        |              | 54 °C 1 min, 72 °C – 1 min |      |
|             |                        |              | Last cycle: 72 °C – 10 min |      |

- **aac(6’)/aph(2’’):** gene that encodes aminoglycoside-6’-N-acetyltransferase/2’’-O-phosphoryltransferase; **ant(4’)-Ia:** gene that encodes aminoglycoside-4’-O-phosphoryltransferase I; **aph(3’)-IIIa:** gene that encodes aminoglycoside-3’-O-phosphoryltransferase III; **dfrA:** gene that encodes dihydrofolate reductase A; **dfrG:** gene that encodes dihydrofolate reductase B; **ermA:** gene that encodes erythromycin ribosomal methylase A; **ermC:** gene that encodes erythromycin ribosomal methylase C; **linA:** gene that encodes lincosamide nucleotidyltransferases A; **linB:** gene that encodes lincosamide nucleotidyltransferases B; **msrA:** gene that encodes macrolides streptogramins resistance A; **msrB:** gene that encodes macrolides streptogramins resistance B; **tetK:** tetracycline resistance protein K; **tetM:** tetracycline resistance protein M.
msrA + msrB (5%), and ermA + ermB + msrA + msrB (2.5%). In the aminoglycosides class, the gene combination aac(6')/aph(2'') + aph(3')-IIIa (82.2%) was more common, followed by aac(6')/aph(2'') + ant(4')-Ia (4.8%), and aac(6')/aph(2'') + aph(3')-IIIa + ant(4')-Ia (1.6%). Finally, among the fluoroquinolones, lincosamides, and folate inhibitors, the gyrA + grlA (80.7%), ermA + ermB (7.0%), and dfra + dfrg (13.3%) combinations, respectively, were observed more frequently. The pattern of antimicrobial-resistance gene distribution among resistant MRSA is outlined in Table 2.

SCCmec typing

The SCCmec type IV (57.1%) was the most frequent SCCmec type among the MRSA isolates, followed by type III (17.1%), type I (13.4%), type II (9.2%), and type V (1.4%). Four MRSA isolates were nontypable. Isolates of the MRSA SCCmec types VI, VII, IX, and X were not detected. The antimicrobial resistance distribution pattern with respect to the MRSA SCCmec types is presented in Table 3.

In general, the MRSA SCCmec type III strains exhibited higher multidrug resistance (p < 0.001). In contrast, the MRSA SCCmec type IV strains were more multidrug-susceptible compared to the other SCCmec types (p < 0.001). The MRSA SCCmec type I, type II, and type III strains were more resistant to ciprofloxacin, clindamycin, and erythromycin than the MRSA SCCmec type IV strains, which were significantly susceptible to the same antimicrobials (p < 0.001). Similarly, the MRSA SCCmec type I and type II strains were more resistant to these antimicrobials than MRSA SCCmec type V strains, which were significantly susceptible (p < 0.001). In addition, the MRSA SCCmec type I and type III strains were more resistant to gentamycin than the MRSA SCCmec type IV and type V strains, that were susceptible to the same antimicrobial (p < 0.001). Lastly, the MRSA SCCmec type III strains were more resistant to trimethoprim-sulfamethoxazole and tetracycline than most MRSA SCCmec type I, type II, and type IV strains, which were susceptible to the same antimicrobials (p < 0.001).
### TABLE 2: Distribution of antimicrobial resistance genes.

| Antimicrobial classes     | Number of resistant MRSA isolates (%) | Resistance genes (%) |
|---------------------------|---------------------------------------|----------------------|
| **Macrolides**            |                                       |                      |
| Erythromycin              | 161 (74.2)                            | ermA 64 (39.7)       |
|                           |                                       | ermC 14 (8.7)        |
|                           |                                       | msrA 10 (6.2)        |
|                           |                                       | msrB 2 (1.2)         |
|                           |                                       | ermA + ermB 2 (1.2)  |
|                           |                                       | ermA + emrC 1 (0.6)  |
|                           |                                       | ermA + msrA 2 (1.2)  |
|                           |                                       | msrA + msrB 4 (27.3) |
|                           |                                       | emrC + msrA + msrB 2 (1.2) |
|                           |                                       | emrA + ermB + msrA 1 (0.6) |
|                           |                                       | emrA + msrA + msrB 8 (5.0) |
|                           |                                       | emrC + msrA + msrB 2 (1.2) |
|                           |                                       | emrA + ermB + emrC + msrA 1 (0.6) |
|                           |                                       | emrA + ermB + emrC + msrB 4 (2.5) |
|                           |                                       | msrA + emrC + msrA + msrB 1 (0.6) |
|                           |                                       | Unknown 3 (1.9)      |
| **Lincosamides**          |                                       |                      |
| Clindamycin               | 100 (46.1)                            | ermA 68 (68.0)       |
|                           |                                       | ermC 13 (13.0)       |
|                           |                                       | ermA + ermB 7 (7.0)  |
|                           |                                       | ermA + ermC 1 (1.0)  |
|                           |                                       | ermA + ermB + emrC 3 (3.0) |
|                           |                                       | emrA + linA 2 (2.0)  |
|                           |                                       | emrA + linA + linB 2 (2.0) |
|                           |                                       | Unknown 4 (4.0)      |
| **Aminoglycosides**       |                                       |                      |
| Gentamycin                | 62 (28.6)                             | ant(4')-Ia 2 (3.2)   |
|                           |                                       | aac(6')/aph(2'') 5 (8.1) |
|                           |                                       | aac(6')/aph(2'') + ant(4')-Ia 3 (4.8) |
|                           |                                       | aac(6')/aph(2'') + aph(3')-IIIa 51 (82.2) |
|                           |                                       | aac(6')/aph(2'') + aph(3')-IIIa + ant(4')-Ia 1 (1.6) |
| **Folate inhibitors**     |                                       |                      |
| Trimethoprim-sulfamethoxazole | 30 (13.8)                         | dfrG 26 (86.7)       |
|                           |                                       | dfrA + dfrG 4 (13.3) |
| **Tetracyclines**         |                                       |                      |
| Tetracycline              | 31 (14.3)                             | tetK 2 (6.5)         |
|                           |                                       | tetM 29 (93.5)       |

*aac(6’)/aph(2’’):* gene that encodes aminoglycoside-6’-N-acetyltransferase/2’’-O-phosphoryltransferase; *ant(4’)-Ia:* gene that encodes aminoglycoside-4’-O-phosphoryltransferase I; *aph(3’)-IIIa:* gene that encodes aminoglycoside-3’-O-phosphoryltransferase III; *dfrA:* gene that encodes dihydrofolate reductase A; *dfrG:* gene that encodes dihydrofolate reductase B; *ermA:* gene that encodes erythromycin ribosomal methylase A; *ermB:* gene that encodes erythromycin ribosomal methylase B; *ermC:* gene that encodes erythromycin ribosomal methylase C; *linA:* gene that encodes lincosamide nucleotidyltransferases A; *linB:* gene that encodes lincosamide nucleotidyltransferases B; *msrA:* gene that encodes macrolides streptogramins resistance A; *msrB:* gene that encodes macrolides streptogramins resistance B; *tetK:* tetracycline resistance protein K; *tetM:* tetracycline resistance protein M.

### TABLE 3: Antimicrobial resistance distribution between the methicillin-resistant Staphylococcus aureus SCCmec types.

| Antimicrobials                  | SCCmec types | I (n = 29) | II (n = 20) | III (n = 37) | IV (n = 124) | V (n = 3) | NT (n = 4) |
|---------------------------------|--------------|-----------|------------|-------------|-------------|----------|----------|
| Erythromycin                    | SCCmec type III | 28 (96.6%) | 20 (100%) | 33 (89.2%) | 75 (60.5%) | 1 (33.3%) | 4 (100%) |
| Ciprofloxacin                   | SCCmec type II | 28 (96.6%) | 20 (100%) | 33 (89.2%) | 55 (44.0%) | -        | 4 (100%) |
| Clindamycin                     | SCCmec type II | 28 (96.6%) | 19 (95.0%) | 32 (86.5%) | 18 (14.5%) | 1 (33.3%) | 2 (50.0%) |
| Gentamycin                      | SCCmec type III | 24 (82.8%) | 2 (10.0%) | 29 (78.4%) | 3 (2.4%)  | -        | 4 (100%) |
| Tetracycline                    | SCCmec type IV | -         | 2 (10.0%) | 26 (70.3%) | 2 (1.6%)  | 1 (33.3%) | -        |
| Trimethoprim-sulfamethoxazole   | SCCmec type IV | -         | 1 (5.3%)  | 20 (54.1%) | 5 (4.0%)  | -        | 4 (100%) |

NT: non-typable. Data are indicated by the number of isolates (%).

* MRSA SCCmec type III was more multidrug-resistant (p < 0.001).
* MRSA SCCmec type IV was more multidrug-susceptible (p < 0.001).
* MRSA SCCmec type I, II, and III were more resistant to erythromycin, ciprofloxacin, and clindamycin, than MRSA SCCmec type IV (p < 0.001).
* MRSA SCCmec types I and II were more resistant to erythromycin, ciprofloxacin, and clindamycin, than MRSA SCCmec type V (p < 0.001).
* MRSA SCCmec types I and III were more resistant to gentamycin than MRSA SCCmec types IV and V (p < 0.001).
* MRSA SCCmec type III was more resistant to trimethoprim-sulfamethoxazole and tetracycline than most MRSA SCCmec types I, II, and IV (p < 0.001).
DISCUSSION

In the last two decades, the proportion of MRSA has increased worldwide. At present, MRSA may be considered the first class of multidrug-resistant (MDR) pathogens, based on the emergence of the concomitant resistance of MRSA to multiple commonly used non-beta-lactam antimicrobials (for e.g., aminoglycosides, macrolides, fluoroquinolones, and tetracycline).

In the present study, 177 MRSA (81.6%) isolates exhibited resistance to at least one of the non-beta-lactam antimicrobials tested, which is indicative of the high resistance rates for erythromycin, ciprofloxacin, and clindamycin antimicrobials. These resistance rates are in accordance with findings from other studies in southern and other regions of Brazil.

Erythromycin and clindamycin are members of the macrolide-lincosamide-streptogramin B (MLSb) family, which exhibit excellent potential in MRSA infections and are frequently used to treat staphylococcal skin and soft tissue infections (SSTIs). erm gene-mediated resistance to MLSb can be expressed in constitutive (cMLSb phenotype) or inducible (iMLSb phenotype) forms. In this study, the prevalence of cMLSb was 38.7%, whereas other studies conducted in Brazil reported cMLSb resistance of approximately 14.3% and 68.2%.

Besides, an important issue in the application of clindamycin is the inducible resistance owing to the presence of methylase synthesis inducers, such as erythromycin, which leads to increased failure in clinical therapeutic applications. In this study, a prevalence of the iMLSb phenotype was observed among 7.4% of the MRSA isolates tested, which is consistent with that reported by Bottega et al. (7.9%) and higher than that reported by Pereira et al. (4.5%), with both studies conducted in Brazil.

The distribution of resistance genes detected in this analysis demonstrates that ermA (39.6%) was the predominant gene compared to ermC (9.7%) and ermB (5.1%). In contrast, in another Brazilian study, it was shown that ermC (38.6%; 17/44) was identified more frequently than ermB (9.1%; 4/44). In this study, one MRSA isolate carried both ermA and ermC, which encode proteins for erythromycin and clindamycin resistance. The coexistence of these genes in MRSA isolates was also observed in other studies.

Among the msr genes, msrA, which confers resistance to macrolides and type B streptogramins, had the highest prevalence (33.6%), followed by msrB (28.1%). In contrast to the data from this study, resistance via efflux pumps (associated with msrAmserB) was not detected in MRSA in a study by Khobadaneh et al., whereas Sarrou et al. detected only msrA in MRSA isolates. The msrA + msrB gene combination of resistance was more prevalent in this study. Additionally, the ermA + ermB + msrA + msrB gene combination was detected in four isolates. These findings are consistent with those of a study on the development of genotype prevalence in Serbia performed by Misić et al., in which similar results of genetic combinations were reported.

The predominance of MRSA in SSTIs and their treatment using ciprofloxacin consequently led to an increase in fluoroquinolone resistance, and thereby limited the therapeutic use of this class of antimicrobials. In this study, 64.5% of MRSA isolates were resistant to ciprofloxacin. In two studies with MRSA isolated from seven hospitals in Rio de Janeiro, Brazil, resistance to fluoroquinolones varied between 60.6% and 93%. While in another study that used isolates collected from three cities in a southern Brazilian state, 79% of the MRSA isolates exhibited fluoroquinolone resistance.

In this study, the rates for trimethoprim-sulfamethoxazole, tetracycline, and gentamicin resistance were observed to be low, which was consistent with recent reports from other studies conducted in Brazil.

Trimethoprim-sulfamethoxazole (folate inhibitors class) is an alternative choice for the treatment of mild to moderate SSTIs caused by MRSA, based on the results of susceptibility tests. The presence of the dfrG gene was confirmed in 86.7% of MRSA isolates, and the association between dfrA and dfrG was confirmed in 13.3%. Moreover, Coelho et al. compared S. aureus isolates collected from Portuguese-speaking African countries with a Brazilian MRSA clone (ST239-III), and observed 78% prevalence of the dfrG gene, 19% of the dfrA gene, or 3% of both.

Aminoglycosides constitute an important class of antimicrobials, especially for the treatment of complicated staphylococcal infections synergistically with glycopeptides or beta-lactams. In an attempt to confirm the resistance to aminoglycosides in MRSA, the presence of genetic elements that encode AMEs was evaluated, with 82.2% of MRSA exhibiting the aac(6')-aph(2'')/aph(3')-IIa gene association. Previous reports showed the presence of this association among 9% and 55.5% isolates. Tetracycline has exhibited clinical efficacy in cases of community-associated MRSA SSTIs. In this study, the presence of tetK and tetM genes was observed in 6.5% and 93.5% of isolates, respectively.

In contrast, all the MRSA isolates tested were susceptible to linezolid and vancomycin, as observed in other Brazilian studies. Currently, resistance to oxazolidinones (including linezolid) among S. aureus is rare, whereas prolonged exposure to vancomycin leads to the emergence of MRSA with reduced vancomycin susceptibility, and the strains are categorized as vancomycin-intermediate S. aureus (VISA) and heterogeneous VISA (hVISA), as reported in other studies conducted in Brazil. Nevertheless, vancomycin remains the first-line therapeutic choice for the treatment of invasive MRSA infections, such as bacteremia, pneumonia, and osteoarticular infection; linezolid is an alternative for the treatment of invasive hVISA and VISA infections.

SCCmec typing provides useful information regarding resistance to antimicrobials and the origin of S. aureus strains. In our study, SCCmec IV and III were the most common SCCmec types, which is consistent with findings reported earlier. In addition, the MRSA SCCmec type III strains exhibited higher multidrug resistance, whereas the MRSA SCCmec type IV were more multidrug-susceptible compared to other SCCmec types. Previous studies have shown that HA-MRSA isolates generally contain SCCmec types I, II, or III, which confer resistance to non-beta-lactam antimicrobials and tend to lead to multidrug-resistance. Furthermore, SCCmec type IV was most commonly detected among the MRSA isolates,
and this characteristic is often observed in CA-MRSA strains, which are generally susceptible to non-beta-lactam antimicrobials and harbor SCCmec types IV or V.

Our study has certain limitations. First, we were unable to test other therapeutic options, such as ceftaroline, daptomycin, and tigecycline. Second, we could not determine the MICs for all the antimicrobials tested. Despite these limitations, the HA-MRSA isolates in our setting were confirmed to be MDR, which limits the therapeutic options available for the treatment of infections caused by such MRSA isolates. Third, the isolates included in this study were not genotyped to assess the clonality. In this study, high erythromycin, ciprofloxacin, and clindamycin resistance rates were observed, and the isolates exhibited considerable diversity of genes related to non-beta-lactam resistance mechanisms in MRSA strains. This indicates the urgency for the development of alternative therapeutic options. Despite the fact that multidrug resistance is increasing in the study setting, linezolid and vancomycin appear to be effective therapeutic options for MDR-MRSA strains. The study data provide information regarding the resistance profile of MRSA isolates from South Brazil, and along with data on the clinical conditions of the patients, it can contribute to the clinical decision-making process.

ACKNOWLEDGMENTS

We thank the Postgraduate Program in Health Sciences of Porto Alegre (PPGCS) of the Federal University of Health Sciences of Porto Alegre (UFCSPA).

FINANCIAL SUPPORT

We received financial support from the Research Foundation of the State of Rio Grande do Sul (FAPERGS - 02/2017) and National Council for Scientific and Technological Development (CNPq - 14/2014).

AUTHORS’ CONTRIBUTIONS

AMR: conception and planning of the study; obtaining, analyzing and interpreting data; statistical analysis; elaboration and writing of the manuscript; effective participation in research orientation; critical review of the literature and the manuscript; approval of the final version of the manuscript. MPB: elaboration and writing of the manuscript; critical review of the literature and the manuscript; approval of the final version of the manuscript. LLR: planning of the study; approval of the final version of the manuscript. KCR: critical review of the literature and the manuscript; approval of the final version of the manuscript. CAGD: conception and planning of the study; effective participation in research orientation; critical review of the literature and the manuscript; approval of the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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