Phenotypic and Genotypic Characterization of Macrolide, Lincosamide and Streptogramin B Resistance among Clinical Methicillin-Resistant \textit{Staphylococcus aureus} Isolates in Chile

Mario Quezada-Aguiluz \textsuperscript{1,2,3,4}, Alejandro Aguyau-Reyes \textsuperscript{1,2,5,6}, Cinthia Carrasco \textsuperscript{1}, Daniela Mejías \textsuperscript{1}, Pamela Saavedra \textsuperscript{1}, Sergio Mella-Montecinos \textsuperscript{2,5,6}, Andrés Opazo-Capurro \textsuperscript{1,3,4}\textsuperscript{*}, Helia Bello-Toledo \textsuperscript{1,3}\textsuperscript{,4} and Gerardo González-Rocha \textsuperscript{1,3}\textsuperscript{*}

1. Introduction

Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is an important pathogen involved in both human and animal infections [1,2]. Although MRSA was initially described as producing healthcare-associated infections (HA-MRSA), the appearance of community-associated MRSA infections (CA-MRSA) has been documented since the 1990s [3]. MRSA...
has shown a remarkable ability to develop resistance to a myriad of antibiotics, as well as to different disinfectants and heavy metals [4]. Vancomycin (VAN), a member of the glycopeptides, has been used as an important option to treat MRSA infections [5]. However, the risk of dissemination of vancomycin-resistant or non-fully susceptible strains suggests that this antibiotic should be used sparingly [6]. For this reason, macrolides (erythromycin [ERY], lincosamides (clindamycin [CLI]), and streptogramins B (MLS\textsubscript{B}) have emerged as important therapeutic options to tackle CA-MRSA infections [7,8]. However, the increased use of these antimicrobials has favored the emergence of resistance to these drugs [9–11].

To date, there are three main MLS\textsubscript{B} resistance mechanisms described: i) changes in the ribosomal target site, which confers cross-resistance to the entire MLS\textsubscript{B} group [12]. This mechanism is conferred by ribosomal mutations or methylation of the 23S rRNA target site, which are mediated by the \textit{erm} genes (mainly \textit{ermA}, \textit{ermB}, and \textit{ermC}) [13,14]. Another mechanism corresponds to ii) an efflux-pump encoded by \textit{msrA}, which can drive out 14- and 15-membered macrolides and streptogramin B, producing the MS\textsubscript{B} phenotype [15]. Finally, another mechanism iii) relies on drug inactivation and it only confers resistance to lincosamides due to an enzyme encoded by the \textit{lnu} gene [11].

Significantly, the MLS\textsubscript{B} phenotype can be either constitutive (cMLS\textsubscript{B}) or inducible (iMLS\textsubscript{B}) [9]. Specifically, CLI, which is the MLS\textsubscript{B} agent used for the treatment of \textit{S. aureus} infections, is a weak MLS\textsubscript{B}-resistance inducer and may lead to treatment failure due to false susceptibility results displayed in in vitro antimicrobial susceptibility tests [16]. Therefore, it is necessary to perform the CLI susceptibility test in the presence of a strong inducer, such as ERY [12]. Another key point is that antibiotic resistance genes that mediate the MLS\textsubscript{B}-resistance phenotype are found in mobile-genetic elements (MGEs) and, in consequence, may be horizontally transferred to susceptible strains [17]. In Latin America, the resistance rates to MLS\textsubscript{B} antibiotics have been reported to be 74% and 81% to ERY and CLI, respectively, among HA-MRSA isolates [10].

In Chile, \textit{S. aureus} is one of the main etiological agents in health care-associated infections (HAIs) [18]. Specifically, it is the main cause of surgical wound infections (27%), and the second cause of pneumonia associated to invasive mechanical ventilation (21%). Likewise, it is involved in bloodstream infections (18%) and infections of the central nervous system (18%) [18]. Despite these data, the MLS\textsubscript{B}-resistance phenotype among HA- and CA-MRSA is still unknown among Chilean isolates. Therefore, the aim of our study was to detect and characterize the MLS\textsubscript{B}- and MS\textsubscript{B}-resistance phenotypes among HA-MRSA and CA-MRSA isolates collected between 2007 and 2017 from the \textit{S. aureus} surveillance program of the National Institute of Public Health of Chile (ISP).

### 2. Results

#### 2.1. Molecular Characterization of MRSA Isolates

All HA-MRSA (32) and CA-MRSA (36) isolates were resistant to FOX and \textit{mecA} positive. For HA-MRSA, the Staphylococcal Cassette Chromosome \textit{mec} (SSC\textit{mec}) analysis revealed the presence of the Type I and Type II elements in 27 (84.4%) and 5 (15.6%) isolates, respectively. In addition, in all isolates classified as HA-MRSA, the absence of the \textit{pvl} gene was confirmed. On the other hand, in all CA-MRSA (36), the \textit{pvl} gene and the type IV SSC\textit{mec} cassette were detected. Of these, 24 (66.7%) harbored the cassette subtype SSC\textit{mec} IVc, whereas 11 (30.5%), and 1 (2.8%) amplified for the subtypes IVa and IVb, respectively; therefore, they were confirmed as CA-MRSA.

The MLST analyses of HA-MRSA showed that 27 (84.4%) isolates belonged to ST5 and 5 (15.6%) to ST105, whereas most CA-MRSA isolates belonged to the ST8 (27/36) (Table 1).

|                      | ST 5 | ST 8 | ST 30 | ST 105 | ST 868 | ST 923 | ST 2802 | Total |
|----------------------|------|------|-------|--------|--------|--------|---------|-------|
| HA-MRSA             | 27   | 0    | 0     | 5      | 0      | 0      | 0       | 32    |
| CA-MRSA             | 1    | 28   | 4     | 0      | 1      | 1      | 1       | 36    |

Table 1. Sequence types (ST) of methicillin-resistant \textit{Staphylococcus aureus} strains isolated in Chile.
2.2. Antimicrobial Susceptibility Testing

The antibiotic resistance profiles were determined for both HA-MRSA and CA-MRSA isolates (Table 2). All isolates (32) of HA-MRSA were resistant to macrolides and to CLI. Moreover, 2 isolates (2/32) (6.3%) were also resistant to CHL and 1 isolate (1/32) (3.1%) to RIF. In the case of CA-MRSA, 9 isolates (9/36) (25%) were resistant to ERY, AZM and CLR, and one isolate was resistant to ERY and AZT (2.8%), but all were susceptible to CLI, CHL, and RIF (Table 2). All HA-MRSA, and CA-MRSA isolates were susceptible to LZD, VAN, DAP, and SXT (Table 3). Furthermore, the iMLS mechanism was detected in none of the two groups of MRSA isolates.

Table 2. Antibiotic resistance profiles among methicillin-resistant Staphylococcus aureus strains isolated in Chile.

| Resistance Profiles | HA-MRSA * | CA-MRSA * |
|---------------------|------------|------------|
| CLI ERY AZM CLR CHL | 2 (6.3)    | 0          |
| CLI ERY AZM CLR     | 29 (90.6)  | 0          |
| CLI ERY AZM CLR RIF | 1 (3.1)    | 0          |
| ERY AZM CLR         | 0          | 9 (25.0)   |
| ERY AZM             | 0          | 1 (2.8)    |
| All susceptible     | 0          | 26 (72.2)  |

*No. of isolates (percentage), CLI: clindamycin, ERY: erythromycin, AZM: azithromycin, CLR: clarithromycin, CHL: chloramphenicol, RIF: rifampicin; HA-MRSA: Hospital-acquired methicillin-resistant Staphylococcus aureus; CA-MRSA: Community-acquired methicillin-resistant Staphylococcus aureus.

Table 3. Minimum-inhibitory concentration (µg/mL) of some antimicrobials against methicillin-resistant Staphylococcus aureus strains isolated in Chile.

| Antimicrobials | MIC<sub>50</sub> | MIC<sub>90</sub> |
|----------------|------------------|------------------|
| Linezolid      | 2                | 2                |
| Vancomycin     | 1                | 1                |
| Daptomycin     | 0.25             | 0.25             |

The HA-MRSA group showed more extended resistance profiles than CA-MRSA. Among the HA-MRSA, the most prevalent resistance profile was CLI-ERY-AZM-CLR, with 90.6% of isolates. On the other hand, in the CA-MRSA group, the most prevalent antibiotic resistance profile was ERY, AZM, and CLR, with 25% of isolates.

2.3. Prevalence of msrA and erm Genes

The ermA gene was amplified in 28 (87.5%) HA-MRSA isolates compared with 6 (16.7%) in CA-MRSA (p < 0.001). Additionally, the ermC gene was found in 2 (6.3%) of HA-MRSA and in none of CA-MRSA isolates (p > 0.05), and the ermB gene was detected in none of the isolates. On the other hand, msrA was detected in 11 (30.6%) of the CA-MRSA isolates, but in none of the HA-MRSA (p < 0.005) (Table 4).

Table 4. Antibiotic resistance, and presence of resistance genes in methicillin-resistant Staphylococcus aureus strains isolated in Chile.

| Percentage of Resistant Isolates to: | Percentage of Resistance Genes: |
|-------------------------------------|---------------------------------|
| CLI ERY AZM CLR CHL RIF             | ermA ermB ermC msrA             |
| HA-MRSA 100 100 100 6.3 3.1 87.5 0 0 0 |
| CA-MRSA 0 27.8 27.8 25 0 0 16.7 0 6.3 30.6 |

CLI: clindamycin, ERY: erythromycin, AZM: azithromycin, CLR: clarithromycin, CHL: chloramphenicol, RIF: rifampicin; HA-MRSA: Hospital-acquired methicillin-resistant Staphylococcus aureus; CA-MRSA: Community-acquired methicillin-resistant Staphylococcus aureus.
3. Discussion

In recent years, we have observed an increased resistance to antibiotics, especially in those used for the treatment of serious infections associated with health care. MLS$_B$ group are antibiotics commonly used to treat skin and soft tissue infections caused by CA-MRSA [11]. The present study reports percentages of resistance to antibiotics in the MLS$_B$ group $\geq 90\%$ in HA-MRSA. This finding agrees with the results of previous studies carried out with strains collected in Chile [10,19]. Besides, 20\% of strains of CA-MRSA were resistant to MLS$_B$ group. These results show lower rates of resistance to these antibiotics in comparison to the official reports of the National Institute of Public Health of Chile (20\% v/s 29\%, respectively). On the other hand, our results showed higher values than previous reports that included strains isolated in Latin America, among both HA-MRSA (81\% for ERY and 74\% for CLI) and CA-MRSA [9,10,20–24].

Among the isolates included in this work, the predominant phenotype was the cMLS$_B$ phenotype. Molecular characterization of 68 MLSB-resistant MRSA revealed that among HA-MRSA, 87.5\% were positive for ermA. However, in the CA-MRSA strains, 16.7\% were positive for ermA, 6.3\% for ermC, and 30.6\% for msrA. The main mechanism of resistance to macrolides in CA-MRSA is mediated by the presence of the msrA gene, which results agree with previously published data [25].

Our results are in agreement with previous reports about the predominance of SCCmec type I-ST5 in HA-MRSA in Chile with classic resistance profiles of the Chilean/Cordobes clone that has a marked presence in hospitals of our country [10,26], and isolates of type IV-ST8 in CA-MRSA in Latin America, related to the USA-300 clone [10,19]. On the other hand, the dichotomy regarding the presence of MLS$_B$ or MS$_B$ resistance among HA-MRSA isolates highlights compared with CA-MRSA (97\% vs approximately 25\%, reaching statistical significance, $p < 0.005$). However, it is important to emphasize that these findings, which are consistent with the classic concept that hospital isolates of MRSA are multi-resistant and the community-based multi-susceptible and only resistant to β-lactams, should be monitored, since 20\% of the isolates of CA-MRSA were resistant to antibiotics in this group, that is, 1 over 5 isolates were not widely susceptible. Accordingly, it is important to perform the proper laboratory detection of these phenotypes to analyze these isolates, since if the criterion of resistance to methicillin and broad susceptibility is the method of choice, other families, including those of the MLS$_B$ group, could obtain biased results.

All the strains analyzed are susceptible to VAN, LZD, DAP, and SXT, keeping these antibiotics as an alternative treatment within the therapeutic arsenal available in Chile, which is consistent with previous reports [10,18].

In summary, despite the higher frequency of the cMLS$_B$ phenotype than iMLS$_B$ in this study, we recommend performing the D test to identify clindamycin-induced resistance and guide therapeutic procedures in both HA-MRSA and CA-MRSA. Likewise, it is not recommended ruling out the submission of suspected CA-MRSA strains in surveillance programs based exclusively on the criterion of resistance only to β-lactams.

4. Materials and Methods

4.1. MRSA Isolates

Thirty-two non-repetitive HA-MRSA isolates recovered from eight Chilean cities between 2007 and 2017 (Table 5), and thirty-six CA-MRSA isolates collected in ten Chilean cities between 2012 and 2017 (Table 6) were included in this study. All isolates were selected from the biorepository maintained by the National Institute of Public Health of Chile (ISP), Santiago, Chile. All isolates were cryo-preserved at −80 °C in glycerol (50\% v/v) and trypticase broth (2:1). The ISP criteria were used to define HA-MRSA and CA-MRSA [20].
Table 5. Hospital-acquired methicillin-resistant *Staphylococcus aureus* isolates from different Chilean cities.

| City           | Number of Isolates |
|----------------|--------------------|
| Santiago       | 15                 |
| Rancagua       | 2                  |
| Talca          | 1                  |
| Concepción     | 2                  |
| Los Ángeles    | 1                  |
| Temuco         | 3                  |
| Osorno         | 1                  |
| Puerto Montt   | 7                  |
| **Total**      | **32**             |

Table 6. Community-acquired methicillin-resistant *Staphylococcus aureus* isolates from various Chilean cities.

| City            | Number of Isolates |
|-----------------|--------------------|
| Valparaíso      | 1                  |
| Viña del Mar    | 1                  |
| Santiago        | 14                 |
| Rancagua        | 2                  |
| Talca           | 1                  |
| Concepción      | 5                  |
| Osorno          | 1                  |
| Los Ángeles     | 1                  |
| Temuco          | 3                  |
| Puerto Montt    | 7                  |
| **Total**       | **36**             |

4.2. Antimicrobial Susceptibility Testing

The cefoxitin test (FOX, 30 µg) for methicillin resistance detection, D-test, iMLS$_B$, cMLS$_B$, and MS phenotypes detection and antibiotics susceptibility determination, were performed by disk diffusion method on Mueller–Hinton agar following the CLSI recommendations and suggested breakpoints (2018) [27–29]. The antibiotics tested were erythromycin (ERY, 15 µg), clarithromycin (CLR, 15 µg), azithromycin (AZM, 15 µg), clindamycin (CLI, 2 µg), chloramphenicol (CHL, 30 µg), rifampicin (RIF, 5 µg), and trimethoprim/sulfamethoxazole (SXT, 25 µg).

The minimal inhibitory concentrations (MICs) of linezolid (LZD), vancomycin (VAN), and daptomycin (DAP) were determined using the broth microdilution method, according to CLSI guidelines and recommended breakpoints [28,29].

4.3. Characterization of MRSA Isolates

The presence of *mecA*, *pvl* in MRSA isolates, and the detection and characterization of the SCC$_{mec}$ element were performed by PCR-based protocols, as previously described [30–32]. Sequence types (ST) were obtained according to Opazo-Capurro et al. (2019), using the Pasteur’s scheme STs employing the bioinformatic tools available at the Center for Genomic Epidemiology (CGE) server (http://www.genomicepidemiology.org/, accessed on 13 March 2022) [33].

4.4. Molecular Detection of Antibiotic Resistance Genes

The detection of genes involved in the MLS$_B$ (*ermA*, *ermB* and *ermC*) and MS$_B$ (*msrA*) phenotypes were screened by conventional PCR according to protocols and primers previously described [34] (Supplementary Materials, Table S1).
4.5. Statistical Analyses

Pearson’s chi-squared test was used to determine associations between antibiotic resistance profiles, MLSB resistance genes, and MRSA types (CA or HA-MRSA). This was achieved utilizing the IBM SPSS Statistics version 23.0 software (SPSS Inc, Chicago, IL, USA), establishing statistical significance at \( p < 0.05 \) \[35\].

5. Conclusions

In Chile, in isolates of HA-MRSA, there is an evident predominance of ST5-SCCmecI, a Chilean/Cordobes clone, characteristically multiresistant, which includes resistance to antibiotics from the MLSB group; and susceptible to SXT and RIF. On the other hand, at the community level (CA-MRSA), there is an emergency of ST8-SCCmecIV, related to clone USA 300. Thus, microbiological surveillance of these isolates at the nosocomial level is required to verify whether the Chilean/Cordobes clone will be replaced by this community clone in Chile, and to monitor whether the latter will continue to increase its resistance to non-beta-lactam antibiotics, such as those of the MLSB group.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antibiotics11081000/s1. Table S1: Primers used in this study.

Author Contributions: Conceptualization: M.Q.-A., A.A.-R., S.M.-M., A.O.-C., H.B.-T., G.G.-R.; methodology; software: M.Q.-A., A.A.-R., C.C., D.M., P.S.; validation: formal analysis: M.Q.-A.; data curation, M.Q.-A., A.A.-R., A.O.-C.; writing—original draft preparation: M.Q.-A.; writing—review and editing: M.Q.-A., A.A.-R., A.O.-C., S.M.-M., H.B.-T., J.M.M., J.C.H., G.G.-R.; visualization: M.Q.-A., A.O.-C., G.G.-R.; supervision: G.G.-R.; project administration: A.A.-R., G.G.-R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Universidad de Concepción, Grant VRID No 218.085.040-1.01N (A.A.-R.; G.G.-R.; H.B.-T.; S.M.-M.), the National Agency for Research and Development (ANID)/Scholarship Program/DOCTORADO NACIONAL/2017 21171278 (M.Q.-A.) and by FONDECYT 1171805, the National Fund for Scientific and Technological Development (FONDECYT) of Chile (J.M.M.).

Acknowledgments: We want to thank the microbiologists of the Chilean Hospitals and the National Institute of Public Health of Chile (ISP), who kindly provided the isolates for this study.

Conflicts of Interest: The authors declare that there are no conflict of interest.

References

1. Foster, T.J. The Remarkably Multifunctional Fibronectin Binding Proteins of Staphylococcus aureus. Eur. J. Clin. Microbiol. Infect. Dis. 2016, 35, 1923–1931. [CrossRef]
2. Carfora, V.; Giacinti, G.; Sagrafoli, D.; Marri, N.; Giangolini, G.; Alba, P.; Feltrin, F.; Sorbara, L.; Amoruso, R.; Caprioli, A.; et al. Methicillin-Resistant and Methicillin-Susceptible Staphylococcus aureus in Dairy Sheep and in-Contact Humans: An Intra-Farm Study. J. Dairy Sci. 2016, 99, 4251–4258. [CrossRef] [PubMed]
3. Chambers, H.F.; Deleo, F.R. Waves of Resistance: Staphylococcus aureus in the Antibiotic Era. Nat. Rev. Microbiol. 2009, 7, 629–641. [CrossRef] [PubMed]
4. Malachowa, N.; Deleo, F.R. Mobile Genetic Elements of Staphylococcus aureus. Cell. Mol. Life Sci. 2010, 67, 3057–3071. [CrossRef] [PubMed]
5. Mermel, L.A.; Allon, M.; Bouza, E.; Craven, D.E.; Flynn, P.; O’Grady, N.P.; Raad, I.I.; Riijnders, B.J.; Sherertz, R.J.; Warren, D.K. Clinical Practice Guidelines for the Diagnosis and Management of Intravascular Catheter-Related Infection: 2009 Update by the Infectious Diseases Society of America. Clin. J. Infect. Chemother. 2010, 10, 81–84. [CrossRef]
6. Chang, S.; Sievert, D.M.; Hagerman, J.C.; Boulton, M.L.; Tenover, F.C.; Downes, E.P.; Shah, S.; Rudrik, J.T.; Pupp, G.R.; Brown, W.J.; et al. Infection with Vancomycin-Resistant Staphylococcus aureus Containing the vanA Resistance Gene. N. Engl. J. Med. 2003, 348, 1342–1347. [CrossRef] [PubMed]
7. Archer, N.K.; Mzaouit, M.J.; William Costerton, J.; Leid, J.G.; Powers, M.E.; Shirtliff, M.E. Staphylococcus aureus Biofilms: Properties, Regulation and Roles in Human Disease. Virulence 2011, 2, 445–459. [CrossRef] [PubMed]
8. Turner, N.A.; Sharma-Kuinkel, B.K.; Maskarinex, S.A.; Eichenberger, E.M.; Shah, P.P.; Carugati, M.; Holland, T.L.; Fowler, V.G. Methicillin-Resistant Staphylococcus aureus: An Overview of Basic and Clinical Research. Nat. Rev. Microbiol. 2019, 17, 203–218. [CrossRef] [PubMed]
32. Lina, G.; Piémont, Y.; Godail-Gamot, F.; Bes, M.; Peter, M.-O.; Gauduchon, V.; Vandenesch, F.; Etienne, J. Involvement of Panton-Valentine Leukocidin-Producing \textit{Staphylococcus aureus} in Primary Skin Infections and Pneumonia. \textit{Clin. Infect. Dis.} 1999, 29, 1128–1132. [CrossRef]

33. Opazo-Capurro, A.; Higgins, P.G.; Wille, J.; Seifert, H.; Cigarroa, C.; González-Muñoz, P.; Quezada-Aguiluz, M.; Domínguez-Yévenes, M.; Bello-Toledo, H.; Vergara, L.; et al. Genetic Features of Antarctic \textit{Acinetobacter radioresistens} Strain A154 Harboring Multiple Antibiotic-Resistance Genes. \textit{Front. Cell. Infect. Microbiol.} 2019, 9, 328. [CrossRef]

34. Dorneanu, O.S.; Lunca, C.; Nastase, E.V.; Tuchilus, C.G.; Vremera, T.; Iancu, L.S. Detection of Aminoglycoside and Macrolide Resistance Mechanisms in Methicillin-Resistant \textit{Staphylococcus aureus}. \textit{Rev. Med. Chir. Soc. Med. Nat. Iasi} 2016, 120, 886–891.

35. Jara, D.; Bello-Toledo, H.; Domínguez, M.; Cigarroa, C.; Fernández, P.; Vergara, L.; Quezada-Aguiluz, M.; Opazo-Capurro, A.; Lima, C.A.; González-Rocha, G. Antibiotic Resistance in Bacterial Isolates from Freshwater Samples in Fildes Peninsula, King George Island, Antarctica. \textit{Sci. Rep.} 2020, 10, 3145. [CrossRef]