



A Prospective Cohort Multicenter Study of Molecular Epidemiology and Phylogenomics of *Staphylococcus aureus* Bacteremia in Nine Latin American Countries

Cesar A. Arias,^{a,b} Jinnethe Reyes,^b Lina Paola Carvajal,^b Sandra Rincon,^b Lorena Diaz,^b Diana Panesso,^{a,b} Gabriel Ibarra,^b Rafael Rios,^b Jose M. Munita,^c Mauro J. Salles,^d Carlos Alvarez-Moreno,^e Jaime Labarca,^f Coralith Garcia,^g Carlos M. Luna,^h Carlos Mejia-Villatoro,^{i†} Jeannete Zurita,^j Manuel Guzman-Blanco,^k Eduardo Rodriguez-Noriega,^l Apurva Narechania,^m Laura J. Rojas,ⁿ Paul J. Planet,^{m,o,p} George M. Weinstock,^q Eduardo Gotuzzo,^g Carlos Seas^g

Center for Antimicrobial Resistance and Microbial Genomics and Division of Infectious Diseases, University of Texas McGovern Medical School at Houston, Houston, Texas, USA^a; Molecular Genetics and Antimicrobial Resistance Unit, International Center for Microbial Genomics, Universidad El Bosque, Bogota, Colombia^b; Grupo de Genomica Microbiana, Instituto de Ciencias e Innovacion en Medicina, Clinica Alemana, Universidad del Desarrollo, Santiago, Chile^c; Division of Infectious Diseases, Department of Internal Medicine, Santa Casa de Sao Paulo School of Medicine, Sao Paulo, Brazil^d; Unidad Infectologia, Departamento de Medicina Interna, Facultad de Medicina, Universidad Nacional de Colombia, Clinica Universitaria Colombia, Colsanitas, Bogota, Colombia^e; Department of Infectious Diseases, School of Medicine, Pontificia Universidad Catolica de Chile, Santiago, Chile^f; Hospital Cayetano Heredia, Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru^g; Pulmonary Division, Department of Medicine, Jose de San Martin Hospital, University of Buenos Aires, Buenos Aires, Argentina^h; Clinica de Enfermedades Infecciosas, Hospital Roosevelt, Guatemala City, Guatemalaⁱ; Hospital Vozandes, Facultad de Medicina, Pontificia Universidad Catolica del Ecuador, Quito, Ecuador^j; Centro Medico de Caracas, Caracas, Venezuela^k; Hospital Civil de Guadalajara, Fray Antonio Alcalde, and Instituto de Patologia Infecciosa y Experimental, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico^l; Sackler Institute for Comparative Genomics, American Museum of Natural History, New York, New York, USA^m; Department of Molecular Biology and Microbiology, Case Western Reserve University, and Research Service, Louis Stokes Cleveland Department of Veterans Affairs, Cleveland, Ohio, USAⁿ; Department of Pediatrics, Division of Pediatric Infectious Diseases, Columbia University, College of Physicians and Surgeons, New York, New York, USA^o; Department of Pediatrics, Division of Infectious Diseases, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA^p; The Jackson Laboratory for Genomic Medicine, Farmington, Connecticut, USA^q

ABSTRACT *Staphylococcus aureus* is an important pathogen causing a spectrum of diseases ranging from mild skin and soft tissue infections to life-threatening conditions. Bloodstream infections are particularly important, and the treatment approach is complicated by the presence of methicillin-resistant *S. aureus* (MRSA) isolates. The emergence of new genetic lineages of MRSA has occurred in Latin America (LA) with the rise and dissemination of the community-associated USA300 Latin American variant (USA300-LV). Here, we prospectively characterized bloodstream MRSA recovered from selected hospitals in 9 Latin American countries. All isolates were typed by pulsed-field gel electrophoresis (PFGE) and subjected to antibiotic susceptibility testing. Whole-genome sequencing was performed on 96 MRSA representatives. MRSA represented 45% of all (1,185 *S. aureus*) isolates. The majority of MRSA isolates belonged to clonal cluster (CC) 5. In Colombia and Ecuador, most isolates ($\geq 72\%$) belonged to the USA300-LV lineage (CC8). Phylogenetic reconstructions indicated that MRSA isolates from participating hospitals belonged to three major clades. Clade A grouped isolates with sequence type 5 (ST5), ST105, and ST1011 (mostly staphylococcal chromosomal cassette *mec* [SCC*mec*] I and II). Clade B included ST8, ST88, ST97, and ST72 strains (SCC*mec* IV, subtypes a, b, and c/E), and clade C grouped

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Address correspondence to Cesar A. Arias, cesar.arias@uth.tmc.edu.

† Deceased.

mostly Argentinian MRSA belonging to ST30. In summary, CC5 MRSA was prevalent in bloodstream infections in LA with the exception of Colombia and Ecuador, where USA300-LV is now the dominant lineage. Clonal replacement appears to be a common phenomenon, and continuous surveillance is crucial to identify changes in the molecular epidemiology of MRSA.

KEYWORDS bacteremia, Latin America, *Staphylococcus aureus*

Staphylococcus aureus is a major human pathogen causing a wide range of infections, including bacteremia, infective endocarditis, and osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections (1). *S. aureus* is a leading cause of hospital-associated infections and is the most common cause of soft tissue infections requiring visits to emergency services in the United States (2). Bloodstream infection (BSI) is a potentially fatal complication of *S. aureus* disease, with an estimated incidence of 80 to 190 cases/100,000 inhabitants per year in developed countries (3–5). For example, in the United States and France, it is estimated that ca. 600,000 and 125,000 cases of *S. aureus* BSIs occur per year, respectively (3–5).

The emergence of methicillin-resistant *S. aureus* (MRSA) poses important therapeutic challenges (6). Although initially identified as an important nosocomial pathogen, MRSA is now endemic in the community (7). The Centers for Disease Control and Prevention (CDC) estimates that 80,461 invasive MRSA infections and 11,285 related deaths occurred in the United States in 2011 (8). Community-associated MRSA (CA-MRSA) strains were first described among healthy individuals with no health care contact or hospital-associated risk factors (8). In North America (NA), the CA-MRSA epidemic is widely attributed to the spread of a clone designated USA300-ST8 (9, 10). Interestingly, a USA300-related genetic lineage (designated USA-300 Latin American variant [USA300-LV]) has emerged in the northern part of South America (SA) and appears to have become endemic in hospitals in the region (Colombia, Venezuela, and Ecuador) (11–14). Recent phylogenetic analyses of USA300 and USA300-LV MRSA revealed that the two genetic lineages are closely related and that the NA and SA clades segregated geographically. Molecular clock analyses suggested that both clades had a common ancestor that may have emerged in the mid-1970s with individual segregation occurring by 1989 and 1985 for the NA and SA clades, respectively (14). Geographic segregation of these parallel epidemics coincided with the independent acquisition of the arginine catabolic mobile element (ACME) in NA isolates and a copper and mercury resistance (COMER) mobile element in SA isolates (14).

Apart from the introduction of the USA300-LV in the northern countries of SA, important changes in the population structure of MRSA have also occurred in Argentina and Brazil (15–17). However, a comprehensive molecular analysis of *S. aureus* bacteremia in Latin America has not been attempted before. Here, we sought to characterize the population structure of *S. aureus* bacteremia and the changing molecular epidemiology of MRSA in a prospective multicenter cohort study of *S. aureus* bacteremia spanning 3.5 years (2011 to 2014) in selected hospitals from nine Latin-American countries.

RESULTS

MRSA versus MSSA in bloodstream infections from 24 Latin American hospitals. A total of 1,346 isolates were collected from the bloodstreams of patients in 24 hospitals in Latin America. A total of 161 isolates were excluded due to protocol violations, which were commonly due to misidentification or contamination. The final analysis included 1,185 isolates from 1,010 patients. A total of 875, 102, 26, and 7 patients had one, two, three, and four isolates, respectively (see Table S2 in the supplemental material). Table 1 shows the distribution of bloodstream *S. aureus* isolates for each country. Overall, methicillin resistance was found in 45% of all isolates with important regional variations (Table 1). The hospitals with the highest rates of MRSA were those located in Brazil (62%), Venezuela (57%), Mexico (57%), Peru (54%), and

TABLE 1 Distribution of MRSA and MSSA in Latin America

Country	No. (%) of isolates		
	<i>S. aureus</i>	MRSA	MSSA
Argentina	149	60 (40)	89 (60)
Brazil	204	126 (62)	78 (38)
Chile	163	74 (45)	89 (13)
Colombia	188	41 (22)	147 (78)
Ecuador	101	29 (29)	72 (71)
Guatemala	138	74 (54)	64 (46)
Mexico	30	17 (57)	13 (43)
Peru	154	84 (54)	70 (46)
Venezuela	58	33 (57)	25 (43)
Total	1,185	538 (45)	647 (55)

Guatemala (54%). A total of 45% and 40% of *S. aureus* isolates recovered from hospitals in Chile and Argentina, respectively, exhibited methicillin resistance. Conversely, only a minority of *S. aureus* isolates obtained from Colombia and Ecuador were methicillin resistant (22% and 29%, respectively). Overall, the majority of MRSA isolates exhibited high rates of resistance to erythromycin (81%), clindamycin (74%), and ciprofloxacin (78%). Chloramphenicol (CHL), tetracycline (TET), rifampin (RIF), and trimethoprim-sulfamethoxazole (SXT) remained active against the majority of MRSA isolates with resistance rates of 15%, 5%, 3%, and 2%, respectively. All *S. aureus* isolates were susceptible to minocycline (MIN), linezolid (LZD), and vancomycin (VAN) (MIC_{90} , 1 μ g/ml) (see Tables S3 and S4). We did not detect any MRSA with reduced susceptibility to vancomycin. Methicillin-susceptible *S. aureus* (MSSA) isolates were highly susceptible to the majority of antibiotics tested (Table S4). The highest rates of resistance in MSSA were found for erythromycin and tetracycline (13% and 9%, respectively).

Genetic lineages of MRSA. Figure 1 shows the distribution of MRSA clones in Latin America. The most striking result was that the majority of MRSA isolates recovered from participating hospitals in Colombia and Ecuador (79% and 72%, respectively) belonged to the USA300-LV lineage (ST8) (11–14), consistent with the clonal replacement of MRSA described before in hospitals throughout these countries (11–14). All USA300-LV isolates harbored staphylococcal chromosomal cassette *mec* (*SCCmec*) IVc/E, and the majority (85% in Colombia and Ecuador) also carried *lukS-PV* and *lukF-PV* encoding Panton-Valentine leukocidin (PVL). Of note, isolates belonging to USA300-LV were fully susceptible to RIF, MIN, SXT, LZD, and VAN and only exhibited low resistance rates (3 to 16%) to erythromycin (ERY), clindamycin (CLI), ciprofloxacin (CIP), gentamicin (GEN), TET, and CHL. Multilocus sequence typing (MLST) of USA300-LV indicated that they belonged to sequence type ST8. The remaining MRSA recovered from participating hospitals in Colombia and Ecuador belonged to the Chilean/Cordobes (ST5, *SCCmec* I, previously prevalent in this region) (11–14), pulsed-field gel electrophoresis (PFGE) pattern USA100 (ST5, *SCCmec* II, previously designated New York/Japan clone), and Brazilian (ST239, *SCCmec* III) clones (Fig. 1). These clones exhibited higher rates of resistance than USA300-LV isolates. The clonal distribution of Venezuelan MRSA was more heterogeneous. Indeed, USA300-LV strains represented only 40% of MRSA but harbored more diverse *SCCmec* subtypes, including IVc/E (62%), IVb (23%), IVa (7.5%), and IVd (7.5%). Moreover, rates of resistance for Venezuelan USA300-LV isolates were higher than those observed in Colombian and Ecuadorian USA300-LV (ERY [23%], TET [15%], CIP [8%], and CLI [8%]). The Chilean/Cordobes clone was represented in a proportion similar to that of USA300-LV (42%).

The above findings contrast with those of the other participating hospitals in Latin American countries. In the participating tertiary care centers of Peru and Chile, the clonal distributions of MRSA were almost identical, with the overwhelming majority of isolates ($\geq 90\%$) belonging to the Chilean/Cordobes clone (Fig. 1). The included hospitals in Mexico, Guatemala, and Brazil had similar MRSA lineages, with the USA100

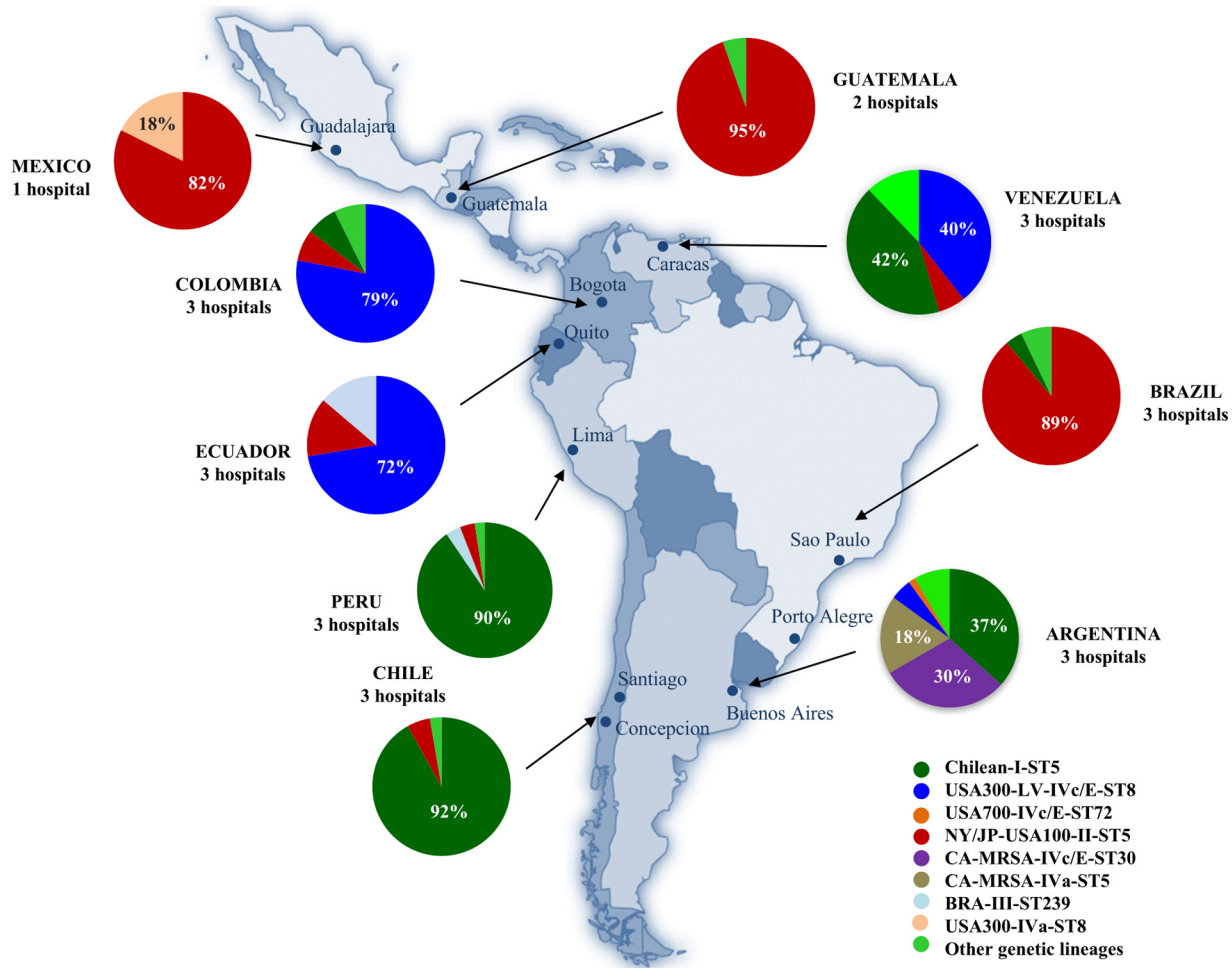


FIG 1 Clonal distribution of MRSA isolates in Latin American hospitals included in the study. Specific genetic lineages derived from pulsed-field gel electrophoresis, *SCCmec* typing, and antimicrobial susceptibility testing are indicated by colors in each corresponding country. ST, sequence type; LV, Latin American variant.

(ST5, *SCCmec* II) representing >80% of isolates recovered from bacteremia. Our results are consistent with previous reports of a major clonal replacement of MRSA in Brazil, where the Brazilian clone (ST239, *SCCmec* III) was the previous predominant clone (16, 17). Interestingly, MRSA from included hospitals in Argentina exhibited the most heterogeneous population structure compared with that from the rest of Latin America. Apart from the Chilean/Cordobes clone, we were able to characterize an important proportion of isolates belonging to three different community-associated genetic lineages, ST30 carrying *SCCmec* IVc/E (30%), ST5 harboring *SCCmec* IVa (18%), and USA300-LV (*SCCmec* IVc/E [7%]) (Fig. 1), consistent with previous local reports (15, 17, 18). In fact, Argentina was the only country in southern Latin America in which USA300-LV isolates were identified from bloodstream infections.

Phylogenetics of MRSA. To explore the genetic diversity of MRSA strains circulating in the hospitals included in this study, we constructed a phylogenetic tree that included 96 genomes from representative isolates of the major circulating MRSA clones in the region and isolates with unusual genotypes/phenotypes. Although the selection strategy was driven primarily by budget limitations, we opted to implement this approach (see Materials and Methods) to dissect the population structure of the major genetic lineages circulating in the region and to characterize emerging genotypes. We added 27 *S. aureus* genomes available on the NCBI database for comparison (see Table S1). The phylogenetic analysis revealed three major clades, denoted A, B, and C (Fig. 2 and 3).

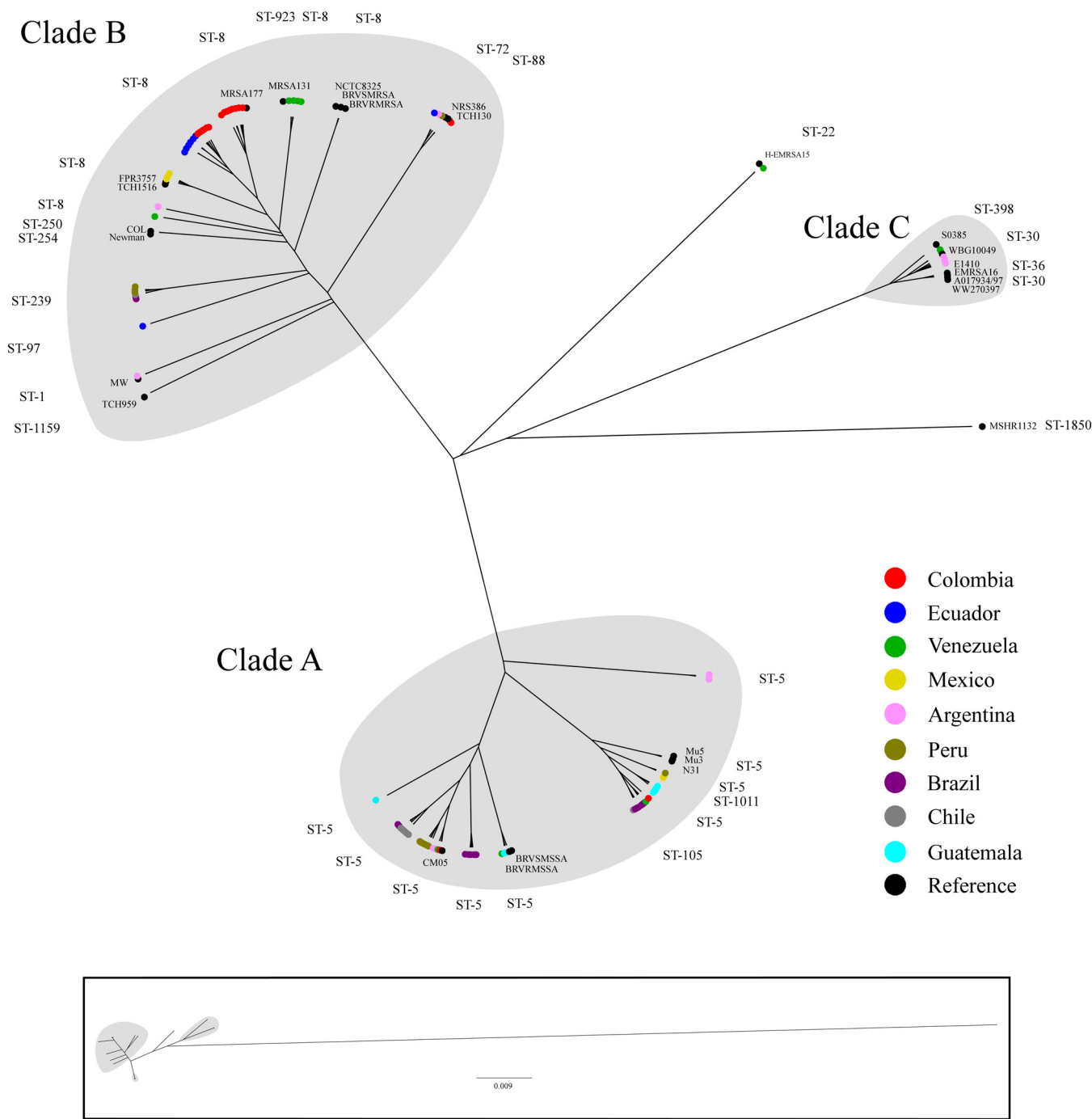


FIG 2 Whole-genome maximum likelihood phylogeny of MRSA isolates. The circular representations in the phylogenetic tree show the three main clades designated A, B, and C (highlighted in gray). Major clades (A, B, and C) are composed mainly of strains from CC5, CC8, and CC30, respectively (sequence types [ST] are indicated). Each branch tip is labeled by a colored circle according to the country of isolation or if it was considered a reference strain (black). The box at the bottom shows the representation of the true distance of the phylogenetic tree.

Clade A, which included *S. aureus* N315 (a hospital-associated MRSA strain) grouped isolates with ST5, ST105, and ST1011 harboring *SCCmec* types I and II, but also type IV (more related to community-associated strains) with subtypes IVa, IVc/E, and IVg (Fig. 2 and 3). Clade A included the most multidrug-resistant MRSA strains (Fig. 4), including strains with fluoroquinolone resistance harboring substitutions in *GyrA* and *GrlA* (coding for large subunits of DNA gyrase and topoisomerase IV, respectively) in most isolates. Of note, five genes encoding different aminoglycoside-modifying enzymes,

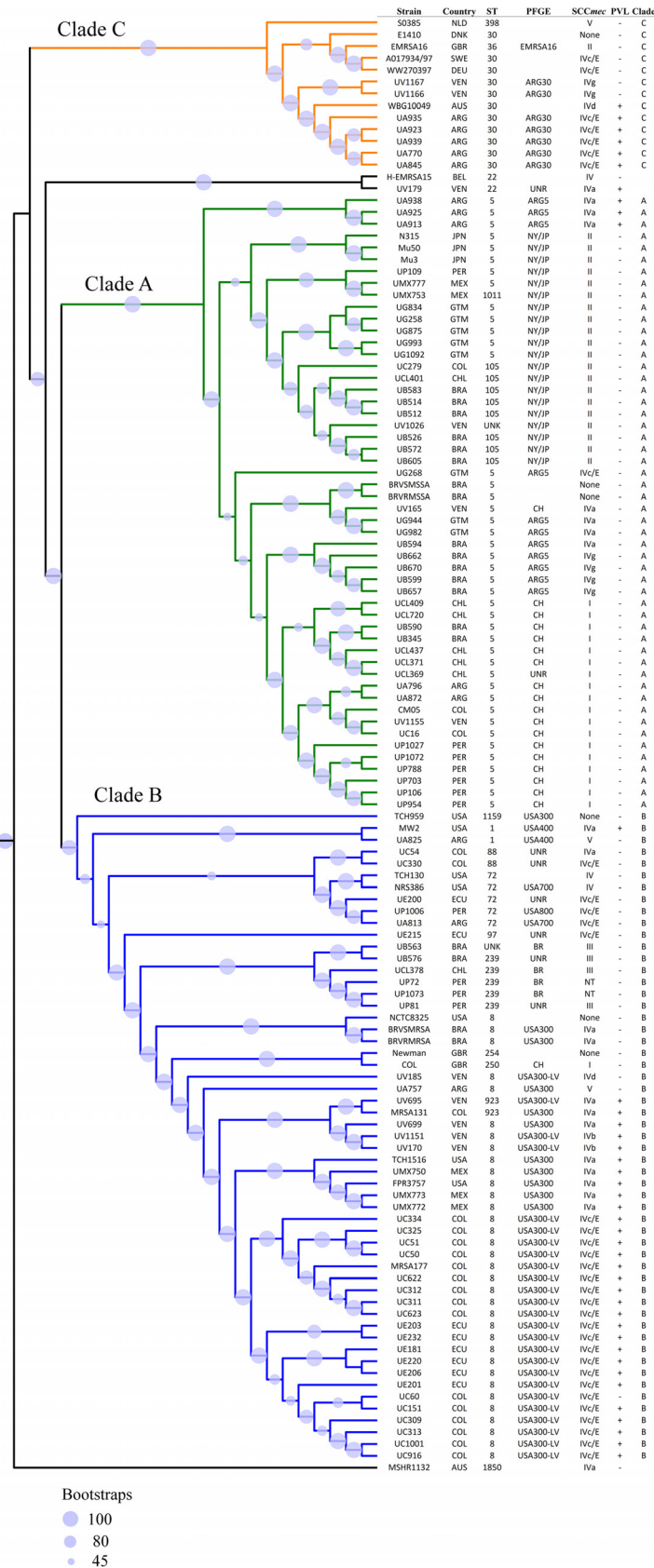


FIG 3 Site of isolation and molecular characteristics of MRSA lineages. The rectangular transformed representation shows the three main clades (A, B, and C) highlighted in green, blue, and orange, (Continued on next page)

including the gene encoding the bifunctional enzyme AAC(6′)-APH(2′) conferring high-level resistance to GEN, amikacin, and tobramycin, were identified (Fig. 4). Additionally, *ermA*, encoding a ribosomal methylase (macrolide-lincosamide-streptogramin B [MLS_B]-type resistance), was found in a high proportion of isolates of this clade. Interestingly, within the clade A, isolates that harbored SCC*mec* IV were fluoroquinolone susceptible and had fewer aminoglycoside and MLS_B-type resistance determinants (Fig. 4).

Clade B included ST8, ST88, ST97, and ST72 strains, mostly harboring SCC*mec* IV (subtypes a, b, c/E, and d) and were less enriched in antibiotic-resistance genes (Fig. 4). Within this clade, a small group (four strains) was ST239, which harbored SCC*mec* III and a high number of resistance genes, including those encoding aminoglycoside, tetracycline, MLS_B, and fluoroquinolone resistance (Fig. 4). Since the ST8 USA300-LV lineage (included within this clade) typically harbors the novel COMER mobile element and lacks the ACME island (typical of NA-USA300), we specifically analyzed the contents of both COMER and ACME within strains of clade B. As shown in Fig. 4, both islands were enriched in strains belonging to this clade and were almost absent in isolates belonging to clades A and C. Most of the COMER-containing strains were recovered in Ecuador (ECU) and Colombia (COL), in agreement with the geographic distribution of the USA300-LV genetic lineage. ACME was found only in Mexican strains with the profile typical of NA-USA300. Of interest, some isolates belonging to ST72, ST88, and ST97 harbored some fragments of the COMER island, supporting the notion that the COMER element was mobile and might represent remnants of this DNA fragment, reinforcing the genetic relationship of strains in clade B. Finally, clade C grouped mostly Argentinian MRSA isolates with ST30 and SCC*mec* IVc/E, a finding previously reported for this country (15, 17, 18).

DISCUSSION

In this work, we have assembled the largest cohort of patients with *S. aureus* bacteremia in Latin America to date. The study included patients enrolled in selected hospitals from nine countries, from Mexico to Argentina. Our main objective was to understand the clinical and molecular characteristics of *S. aureus* bacteremia on a large scale. Although we understand that the hospitals enrolled may not represent the situation of other hospitals in the countries included in this study, it is important to note that our findings are supported by many previous local studies (11–19). Thus, our results may actually reflect an accurate picture of circulating *S. aureus* lineages causing bloodstream infections in the region.

One of the most interesting findings of our study is that the proportion of MRSA strains remains high in the region but exhibits important regional variations. Indeed, rates of MRSA higher than 40% were found in the majority of countries, with the highest prevalence in the participating hospitals in Brazil (62%), a finding that is consistent with those of recently reported surveillance studies (11–19). A notable exception to the above appears to be in the included hospitals from both Colombia and Ecuador. In these centers, the rates of MRSA appear to have markedly decreased compared with those from previous studies. Indeed, in a multicenter surveillance carried out by our group in Colombia from 2006 to 2008 (which included the three hospitals of the current study), methicillin resistance in *S. aureus* was documented in ca. 50% of hospital-associated isolates (13). By contrast, in the present study, <30% of *S.*

FIG 3 Legend (Continued)

respectively. Molecular typing characteristics of the evaluated strains are shown, including multi locus sequence type (ST), pulse field gel electrophoresis type (PFGE), staphylococcal chromosomal cassette *mec* type (SCC*mec*), and presence (+) or absence (–) of the Panton-Valentine leukocidin (PVL). ARG, Argentina; AUS, Australia; BRA, Brazil; CHL, Chile; COL, Colombia; DEU, Germany; DNK, Denmark; ECU, Ecuador; GBR, Great Britain; GTM, Guatemala; JPN, Japan; MEX, Mexico; NDL, The Netherlands; PER, Peru; SWE, Sweden; USA, United States of America; VEN, Venezuela; ARG30, Argentinian clone ST30; ARG5, Argentinian clone ST5; CH, Chilean clone; NY/JJP, New York/Japan-USA100 clone; BR, Brazilian clone; NT, nontypeable; UNR, unrelated by PFGE.

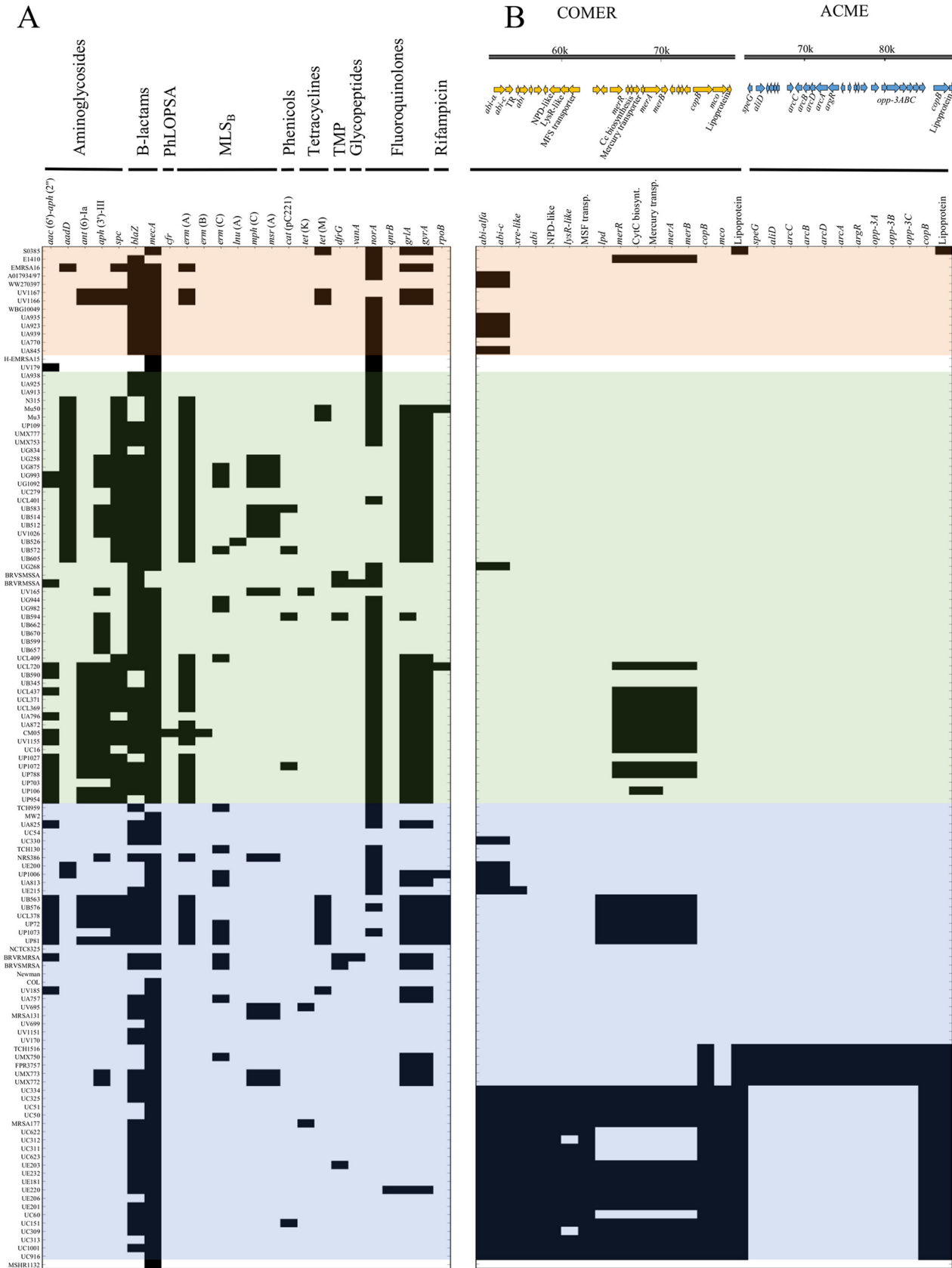


FIG 4 Genomic characteristics of Latin American MRSA strains. (A) Presence (dark) and absence (light) of 2,132 antibiotic resistance genes from the ResFinder 2.1 database, and (B) the presence (black) and absence (white) of the genes in the genetic locus “copper and mercury resistance” (COMER) (Continued on next page)

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aureus isolates from these hospitals were MRSA. Although the former study included isolates from clinical samples other than blood, the decrease in the rates of MRSA seems significant compared with previous reports of hospitals in Peru and Venezuela (19). Although we cannot exclude that such reductions in MRSA prevalence could be due to major changes in infection control practices in the participating hospitals, the lower rates of MRSA seem to be associated with the shift in the population structure.

In 2005, we characterized the first strains of CA-MRSA reported in Colombia and found that, in contrast to in other parts of the world, the majority of these infections were caused by MRSA isolates that belonged to a genetic lineage closely related to the USA300 strain prevalent in NA (we designated this lineage USA300-LV) (11–14). Both USA300 genetic lineages encompass ST8 isolates harboring *SCCmec* IV and genes encoding the PVL toxin. However, the most important genetic distinction between these two is the absence of the ACME island in USA300-LV, which is replaced by a gene cluster encoding proteins involved in the metabolism of copper and mercury (14). The results of our study, which are supported by several other local findings, indicate that in stark contrast with USA300, USA300-LV strains have been able to completely replace previously prevalent hospital-associated clones in Colombia and Ecuador (11–19). The reasons for such remarkable clonal switch in a relatively short time span are not known. Thus, the understanding of the pathogenic properties of this genetic lineage and the role of the COMER island in virulence and the ability to disseminate has become a priority of our future studies.

To dissect the population genetics of isolates from our studies, we selected isolates for whole-genome sequencing (WGS) based on our initial molecular characterization to offer a more detailed picture of the molecular epidemiology and population structure of MRSA. Our genomic analyses indicate that three major clades are likely to circulate in the Latin American centers included in this study, which in general, clustered in previous MLST analyses. The most multidrug-resistant of these clades is clade A, which encompasses the majority of MRSA strains of the region (except in Colombia and Ecuador). The strains are grouped within the CC5 (Chilean/Cordobes and NY/Japan-USA100 clones) and exhibit high rates of resistance to quinolones, *MLS_B*, and aminoglycosides associated with the presence of *SCCmec* I and II. An exception to the above characteristics is a cluster of ST5 isolates recovered in Argentina (ARG) and Brazil (BRA) that carry *SCCmec* IVa and IVg and have been associated with community infections in these countries (15, 16), suggesting that a novel ST5 CA-MRSA lineage is in its ascendancy in the southern parts of SA.

Clade B encompasses isolates from CC8 and CC239, including those belonging to USA300-LV and NA-USA300. In general, the CC8 isolates harbor the PVL genes, carry *SCCmec* IV, and are less enriched in antibiotic resistance genes than those of clade A, with the exception of isolates belonging to the Brazilian clone which harbor *SCCmec* III and harbor resistance profiles similar to those of clade A. Of note, 18% of Mexican MRSA isolates from a tertiary hospital (grouped in clade B) display the pattern typical of that of NA-USA300, suggesting that this strain is likely to be circulating in Mexico. Finally, clade C groups most of ST30 CA strains, which are found mainly in the ARG hospitals and are related to the Oceania-Pacific ST30 clone initially described in Australia. These strains also carry *SCCmec* IVc/E, with genes encoding PVL present in 61% of them. Of note, the Argentinian hospitals harbored the most highly diverse population of MRSA, and the reasons for such heterogeneity in the population genetics of MRSA are unclear.

FIG 4 Legend (Continued)

and “arginine catabolic mobile element” (ACME). The strains are organized according to the phylogenetic reconstruction of these strains, and the three main clades (A, B, and C) are highlighted in green, blue, and orange, respectively. (A) The genes are grouped according to the type antibiotic they confer resistance to: aminoglycosides, glycopeptides, macrolides-lincosamides-streptogramin B (*MLS_B*), trimethoprim (TMP), tetracyclines, beta-lactams, phenicols-lincosamides-oxazolidinones-pleuromutilins-streptogramin A (PhLOPSA), and fluoroquinolones. (B) The genetic organization of the loci COMER and ACME is shown; reference sequences were obtained from the strains CA12 (accession no. CP007672.1, regions 53520 to 77705) and USA300FPR3757 (accession no. CP000255.1, regions 63100 to 88681), respectively. TR, transcriptional regulator; Cc, cytochrome c.

As mentioned above, an important limitation of our study is that we only included a few participating centers in each country, and our results may not reflect the situation of the entire region. However, this is an inherent limitation of studies similar to ours, and a more comprehensive inclusion of hospitals with such diversity and different social and economic realities is challenging. Moreover, the results from many local studies seem to support our findings, making them more generalizable (11–19). Also, we did not have the support to perform WGS on the entire collection, which would have provided a deeper understanding of the population structure of the MRSA isolates. Nonetheless, our genomic selection strategy was robust (96 isolates), with the objective of including isolates that may accurately represent the phylogenetic picture of MRSA bacteremia in the participating centers. Indeed, our genomic findings are also supported by results from other studies and point to specific clonal changes that may contribute to the understanding of the dissemination and dynamics of bacteremic *S. aureus* infections in the region.

In summary, we presented a comprehensive characterization of *S. aureus* bacteremia in Latin America, providing strong evidence that clonal replacement is frequent and that the population structure is in continuous evolution. The identification of newly emerged genetic lineages would help in defining specific therapeutic approaches for MRSA infections in the region.

MATERIALS AND METHODS

Study design, inclusion criteria, and collection of isolates. We included patients in 24 tertiary care hospitals from ARG (3 hospitals in Buenos Aires), BRA (3 hospitals in Sao Paulo and Porto Alegre), Chile ([CHI] 3 hospitals in Santiago and Concepcion), COL (3 hospitals in Bogota), ECU (3 hospitals in Quito), Guatemala ([GTM] 2 hospitals in Guatemala City), Mexico ([MEX] 1 hospital in Guadalajara), Peru ([PER] 3 hospitals in Lima), and Venezuela ([VEN] 3 hospitals in Caracas) between January 2011 and July 2014. These hospitals were chosen because (i) they had infrastructures for clinical research that included personnel for data collection, (ii) the investigators were experienced in clinical studies, and (iii) they possessed microbiology laboratory infrastructures. In this prospective observational cohort study, we included patients older than 18 years who had an episode of *S. aureus* bacteremia (detailed inclusion and exclusion criteria are specified in the supplemental material). Isolates were identified by standard microbiological techniques at the local hospitals and were subsequently sent to a reference laboratory (Universidad El Bosque, Bogota, Colombia) for additional characterization (see the supplemental material). All participating hospitals had local Institutional Review Board (IRB) approval for the study.

Identification of bacterial isolates and antimicrobial susceptibility testing. The identification at the species level of all *S. aureus* isolates was performed by a multiplex PCR assay as described previously (20). Antimicrobial susceptibility testing was performed using the agar dilution method (see the supplemental material) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (21).

Molecular typing and detection of Pantone-Valentine leukocidin. SCCmec typing (I to V) and SCCmec IV subtyping were performed in all MRSA isolates by multiplex PCR according to previously reported methods (see the supplemental material) (22–25). PFGE was performed on all MRSA isolates with some modifications of a previously described method (26). Patterns were analyzed with the GelCompar II software (version 6.5; Applied Maths, Sint-Martens-Latem, Belgium) (see the supplemental material). The presence of *lukS-PV* and *lukF-PV* encoding PVL was investigated using PCR assays and primers described previously (27).

WGS and phylogenetic analysis. WGS was used to dissect the genetic diversity of MRSA. Due to funding limitations, we chose to sequence representatives of the most prevalent clones defined by PFGE (banding patterns), susceptibility profiles, and SCCmec typing. Additionally, representative isolates with unusual PFGE patterns and SCCmec typing were also selected for WGS to provide a broad view of the population structure of *S. aureus* in Latin America. For all assembled genomes, we performed MLST, detection of antibiotic resistance determinants, and detection of ACME and COMER (14, 28, 29) (see the supplemental material). All sequenced genomes from this study were included in the phylogenetic reconstruction and tree generation (see Table S1 in the supplemental material) (30–33).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00816-17>.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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