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Multidrug-Resistant Methicillin-Resistant Staphylococcus aureus Associated with Bacteremia and Monocyte Evasion, Rio de Janeiro, Brazil

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We typed 600 methicillin-resistant Staphylococcus aureus (MRSA) isolates collected in 51 hospitals in the Rio de Janeiro, Brazil, metropolitan area during 2014–2017. We found that multiple new clonal complex (CC) 5 sequence types had replaced previously dominant MRSA lineages in hospitals. Whole-genome analysis of 208 isolates revealed an emerging sublineage of multidrug-resistant MRSA, sequence type 105, staphylococcal cassette chromosome mec II, spa t002, which we designated the Rio de Janeiro (RdJ) clone. Using molecular clock analysis, we hypothesized that this lineage began to expand in the Rio de Janeiro metropolitan area in 2009. Multivariate analysis supported an association between bloodstream infections and the CC5 lineage that includes the RdJ clone. Compared with other closely related isolates, representative isolates of the RdJ clone more effectively evaded immune function related to mononuclear cells, as evidenced by decreased phagocytosis rate and increased numbers of viable unphagocytosed (free) bacteria after in vitro exposure to monocytes.

Methicillin-resistant Staphylococcus aureus (MRSA) is characterized by the mainly clonal structure of bacterial populations and the worldwide spread of a few highly successful lineages, sequence types (STs), and clonal complexes (CCs) that cycle through waves of dominance (1,2). During the late 1990s, the Brazilian endemic clone (BEC), which belongs to the ST239(CC8)-staphylococcal cassette chromosome (SCC) mecIII lineage, comprised ≈80% of MRSA isolates in hospitals in Brazil (3). In the 2000s, isolates of the ST1(CC1)-SCCmecIV lineage supplanted BEC in ≥2 hospitals in the Rio de Janeiro metropolitan area of Brazil (4). More recent analyses have suggested that CC5 isolates might be increasing in prevalence in Brazil (5).

Most studies on the molecular epidemiology of MRSA in Brazil have analyzed a small number of isolates from a limited number of hospitals (5–9). We used molecular and genomic approaches to characterize 600 MRSA isolates collected from 51 hospitals in the Rio de Janeiro metropolitan area and identified a novel MRSA clone of ST105-SCCmecII spa t002 (ST105-SCCmecII-t002), which we termed the Rio de Janeiro (RdJ) clone, as a predominant cause of MRSA bloodstream infections (BSIs).

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Methods

Bacterial Isolates
We obtained the MRSA isolates from 600 patients at 51 hospitals in the Rio de Janeiro metropolitan area and confirmed MRSA using routine identification methods (Table 1; Appendix 1, https://wwwnc.cdc.gov/EID/article/27/11/21-0097-App1.xlsx). The sample comprised roughly equal numbers of isolates from blood samples from BSI patients, nonblood samples from patients with infections at another body site, and nasal swab samples; samples were collected during 2014–2017, most in 2015 and 2016. Patient age was available for 450 patients (Table 2). The research protocols were submitted to the Human Research Ethics Committee (CAAE submission no. 41614914.4.00005257) of the Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil); the study was considered non–human subject research.

Molecular Typing and Susceptibility Testing
We used restriction-modification (RM) tests to determine CC (10) and multiplex PCR to type SCCmec (11). We used PCR to screen for the lukSF-PV, agrII, SCCmecIII, and seh genes as previously described (12). We conducted antibiogram and susceptibility tests for glycopeptide drugs as recommended by Clinical and Laboratory Standards Institute guidelines (13).

Genome Sequencing and Analysis
We selected 208 isolates for whole-genome sequencing (WGS). Because of a strong predominance (179/208; 86.1%) of CC5 isolates, we focused our research on the CC5 lineage. We randomly selected isolates from blood (70/145; 48.3%), nonblood (52/114; 45.6%), and nasal swab (57/123; 46.3%) CC5 samples (Appendix 2, https://wwwnc.cdc.gov/EID/article/27/11/21-0097-App2.xlsx). The other 29 isolates used in WGS belonged to less abundant CCs. We prepared genomic DNA using the Wizard Genomic DNA Purification Kit (Promega Corporation, https://www.promega.com) and sequenced genome libraries by using Nextera XT DNA Library Prep Kit (Illumina, https://www.illumina.com) and the HiSeq 2500 system (Illumina) using paired-end reads of 125 bp. We trimmed reads using BBduk Trimmer version 1.0 (Geneious, https://www.geneious.com) and assembled genomes using Velvet Assembly version 7.0.4 (14) and SPAdes version 3.13.0 (15). We used RAST (https://rast.nmpdr.org) and manual inspection to annotate the isolates. We determined the genotypes of the sequenced strains using the MLST 2.0, SCCmecFinder 1.2, and spa Typ er 1.0 tools (https://cge.cbs.dtu.dk).

Phylogenetic Analysis and Divergence Times
We constructed a maximum-likelihood tree for 661 CC5 genomes: 179 genomes from the current investigation and 482 assembled genomes available on GenBank, chosen from the list provided by Challagundla et al. (8) (Appendix 3, https://wwwnc.cdc.gov/EID/article/27/11/21-0097-App3.pdf). We used a single-nucleotide polymorphism (SNP) alignment produced by Snippy to infer an initial phylogenetic tree in RAxML version 8.2.4 (16). To estimate when the ST105-SCCmecII-4002 lineage emerged in Rio de Janeiro, we used a Bayesian phylogenetic framework to analyze 73 genomes that passed our Mash Screen (17) quality cutoffs. We selected MRSA strain FCFHV36, the closest complete reference genome available in GenBank, using the WhatsGNU topgenome (-t) option (18). We used the SNP alignment to infer an initial phylogenetic tree in RAxML version 8.2.4 before using ClonalFrameML (19) to detect and mask areas of recombination. We used the SNP recombination-masked alignment to estimate divergence times in BEAST version 2.6.2 (20). We found a positive correlation between genetic divergence and isolation time using TempEst version 1.5.3 (21). We plotted the chronograms based on the maximum clade credibility tree using the TreeAnnotator program and visualized in FigTree version 1.4.3 (Appendix 3).

Genomic Island Characterization
We used Geneious Prime version 2020.1.2 to manually inspect the ΦSa3, vSa-α, vSa-β, vSa-β, and SaPI-1 genomic islands (22,23) and Swiss-Prot (Uniprot Consortium, https://www.uniprot.org) to determine the presence of potential genomic islands in the CC5 isolates.

Table 1. Sample types of methicillin-resistant Staphylococcus aureus isolates from colonized and infected patients, Rio de Janeiro, Brazil, 2014–2017

| Sample type                | No. (%) samples |
|----------------------------|-----------------|
| Blood                      | 197 (32.8)      |
| Nonblood                   | 216 (36.0)      |
| Anterior nasal swab        | 187 (31.2)      |
| Total                      | 600 (100.0)     |

Table 2. Age distribution of patients who had methicillin-resistant Staphylococcus aureus infections or colonizations, Rio de Janeiro, Brazil, 2014–2017

| Patient age range, y | No. (%) |
|----------------------|---------|
| <5                   | 46 (10.2) |
| 5–18                 | 16 (3.6)  |
| 19–59                | 180 (40.0) |
| >60                  | 208 (46.2) |
| Total                | 450 (100.0) |
annotate paralogues. To map the genetic context of genomic islands, we randomly selected representative genome sequences from different phylogenetic locations of the tree showing the most common CC5 lineages in the Rio de Janeiro metropolitan area (Figure 1). We determined gene presence or absence using BLAST analysis (https://blast.ncbi.nlm.nih.gov).

Phagocytosis Assays
We subjected the selected isolates to phagocytosis (Appendix 3 Table 1). In this assay, we considered the entire process of phagocytosis (i.e., binding and uptake) by detecting all cell-associated bacteria, whether internalized or externally attached, after washing. We cultured bacteria at 37°C for 18 h at 250 rpm in brain-heart infusion broth (Becton Dickinson, https://www.bd.com) before treating with 25 nmol SYTO 9 stain (Thermo Fisher Scientific, https://www.thermofisher.com) for 15 min and washing in phosphate-buffered saline (1× phosphate-buffered saline, pH 7.2). We incubated bacterial cells at 37°C for 30 min in 5% carbon dioxide with THP-1 monocytes in Roswell Park Memorial Institute 1640 medium for a multiplicity of infection of 10 (24). We did not use antimicrobial drugs at any time during these assays. We washed the infected monocytes with PBS once and then centrifuged them at 200 × g for 5 min. We

![Figure 1. Maximum-likelihood phylogenetic tree of 179 methicillin-resistant Staphylococcus aureus CC5 isolates from Rio de Janeiro, Brazil, 2014–2017 (red text) and 482 reference genomes (7). Red branches indicate the Rio de Janeiro clone of the lineage ST105(CC5)-SCCmecl-l002. Scale indicates substitutions per site. CC, clonal complex; SCC, staphylococcal chromosome cassette; ST, sequence type.](image-url)
resuspended THP-1 cells in PBS and analyzed them by flow cytometry (FACSCalibur; Becton Dickinson). We acquired 10,000 live THP-1 cells (as calculated by forward scatter and side scatter gating) and analyzed data using FlowJo10 software (https://www.flowjo.com). We calculated the number of bacteria-associated THP-1 cells as the frequency of fluorescent (i.e., SYTO 9–positive) THP-1 cells compared with total live THP-1 cells. In addition, we counted and compared the number of viable unphagocytosed bacterial cells in the culture supernatant of each assay at 0 and 30 min after incubation.

**Statistical Analyses**

We analyzed molecular typing, antimicrobial testing, and epidemiologic data using Pearson χ² tests. To assess the association of the CC5-SCC mec II group and the ST105-SCC mec II-t002 sublineage with BSI, we used Stata 16.0 (https://www.stata.com) to conduct a Mantel-Haenszel test stratified on a composite variable informed by participant age (>60 years vs. <60 years), year of specimen collection (2014, 2015, or 2016–2017), and hospital type (public vs. private). For the analysis of year of specimen collection, we combined data from 2016 and 2017 because few isolates were collected during 2017. We analyzed phagocytosis assays using a 1-way analysis of variance and Tukey multiple comparison test in GraphPad Prism 6 (GraphPad Software, Inc., https://www.graphpad.com).

**Results**

**Distribution of Genotypes (CC-SCC mec) and Antimicrobial Resistance**

Among the 600 isolates that underwent CC and SCC mec typing, most were categorized as CC5-SCC mec II (245/600; 40.8%) or CC5-SCC mec IV (137/600; 22.8%). The second most common lineage was CC30, comprised of lukSF-PV–positive CC30-SCC mec IV (109/600; 18.2%) and lukSF-PV–negative CC30-SCC mec II (8/600; 1.3%) isolates. The previously dominant CC1-SCC mec IV lineage (79/600; 13.2%) and BEC clone (7/600; 1.2%) were much less frequent. In addition, we observed low frequencies of STs related to other international lineages such as CC45-SCC mec II/IV (related to USA600), CC8-SCC mec IV (related to USA300), and CC22-SCC mec IV (related to EMRSA-15) (Figure 2, panel A).

Compared with isolates of other frequent clonal lineages, CC5-SCC mec II isolates were more likely to be multidrug-resistant, defined as having resistance to ≥4 non-β-lactam antimicrobial drugs (48.6% vs. 5.8%) (Table 3). In contrast, CC5-SCC mec IV strains showed more susceptibility to non-β-lactams; only 4.4% were multidrug-resistant. All 109 strains belonging to the CC30-SCC mec IV lineage, which is related to the community-acquired MRSA USA1100/Oceania South West Pacific clone, were susceptible to all non-β-lactams tested (Table 3).

**Distribution of Genotypes (CC-SCC mec) and Clinical Data**

In the univariate analysis, we found that the distribution of genotypes was associated with MRSA infection site (Figure 2, panel B). CC5-SCC mec II isolates were more common among blood (115/245; 46.9%) than nonblood (62/245; 25.3%) and nasal swab (68/245; 27.8%) samples, whereas CC5-SCC mec IV isolates were more common among nasal swab (55/137; 40.1%) and nonblood (52/137; 38.0%) than blood (30/137; 21.9%) samples. The third most frequent lineage, CC30-SCC mec IV, was more common among nonblood (60/109; 55.0%) than nasal swab (30/109; 27.5%) and blood (19/109; 17.4%) samples.

The distribution of MRSA lineages varied among age groups. CC5-SCC mec II was more common among patients ≥60 years of age (100/208; 48.1%). CC30-SCC mec IV prevalence was higher among younger populations and diminished with increasing age range; prevalence was 50.0% (8/16) among children 5–18 years of age, 21.1% (38/180) among adults 19–59 years of age, and 10.6% (22/208) among adults ≥60 years of age. Among children <5 years of age, the most prevalent lineage was CC5-SCC mec IV, which is sometimes known as the pediatric clone (21/46; 45.7%) (Figure 2, panel C). Adults 19–59 and >60 years of age had a similar prevalence of CC5-SCC mec IV isolates (23.9% among adults 19–59 years of age vs. 22.1% among adults >60 years of age). The proportion of CC5-SCC mec II isolates was also similar between adults 19–59 years of age (57/180; 31.7%) and adults >60 years of age (62/208; 29.8%). CC5-SCC mec II was associated with BSIs even after stratifying for the composite variable of age, hospital type, and year of isolation (p<0.01).

**Novel MRSA Clone**

To better characterize the circulating clones, especially those belonging to CC5, we used whole-genome sequencing on 208 isolates: 76 (36.5%) from blood samples, 69 (33.2%) from nasal swab samples, and 63 (30.3%) from nonblood samples. Most (179; 86.1%) isolates belonged to CC5, whereas 29 did not (Appendix 3 Table 2). Multilocus and spa-typing using WGS revealed 4 CC5 clones that constituted >75% of isolates (Table 4). The dominant genotype,
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Figure 2. Distribution of 600 MRSA isolates by lineage (A), sample type (B), and patient age (C), Rio de Janeiro Brazil, 2014–2017. A) MRSA isolates by lineage (CC-SCCmec type) among 600 isolates. Labels indicate proportions. B) MRSA isolates by sample type. Labels indicate number of isolates. C) MRSA isolates by patient age (data available for 450 patients). Labels indicate number of isolates. BEC, Brazilian endemic clone; CC, clonal complex; EMRSA, epidemic methicillin-resistant Staphylococcus aureus; MRSA, methicillin-resistant Staphylococcus aureus; SCC, staphylococcal cassette chromosome.

ST105(CC5)-SCCmecII-t002, the RdJ clone, comprised 41.9% (75/179) of the CC5 isolates. RdJ showed the second highest proportion of multidrug resistance (41/75; 54.7%), superseded only by ST5-SCCmecII-t539 (14/17; 82.4%). In contrast, only 1 (2.3%) strain of ST5-SCCmecIV-t002 was multidrug-resistant. MRSA lineages coexisting in the same hospital often displayed different resistance profiles.

In addition to being the most frequent MRSA clone, RdJ might be responsible for the higher frequency of CC5-SCCmecII isolates from blood samples. ST105-SCCmecII-t002 isolates were more common among...
blood (41/75; 54.7%) than nasal swab (20/75; 26.7%) and nonblood (14/75; 18.7%) samples; however, when adjusted for hospital type and year of isolation, this association became nonsignificant (p = 0.12).

Whole-Genome Phylogenetic Analysis of MRSA CC5 Isolates

The whole-genome phylogenetic analysis grouped CC5 isolates from this study into 3 of the 4 major phylogenetic groups corresponding mostly to the ST105(CC5)-SCC mec II-t002, ST5-SCC mec II-t539, and ST5-SCC mec IV-t002 genotypes and distributed widely throughout the CC5 tree (Figure 1). All SCC mec IV isolates cluster in the CC5-Basal clade. Isolates with the multidrug-resistant ST5(CC5)-SCC mec II-t539 genotype clustered with members of the paraphyletic group CC5-IIA described by Challagunda et al. (8). Most other CC5 isolates, including isolates of genotype ST105(CC5)-SCC mec II-t002, were grouped in clade CC5-IIIB. Although most of these isolates form the RdJ clade, which is found mostly in Rio de Janeiro, nearby outgroups to this clade are composed of previously sequenced isolates from São Paulo and Porto Alegre (25 and North America, as well as a few isolates from this study (Appendix 3). This pattern might indicate multiple introductions into Brazil. Our Bayesian analysis of the RdJ clade suggests a recent date of introduction, probably 2009 (95% highest posterior density 2007–2010) (Figure 3).

In comparison with other CC5 genomes, the clade that includes the ST105 genomes lacked key virulence genes. In addition to the apparent loss of the enterotoxin P gene (sep) noted by Challagunda et al. (8), isolates from this clade uniformly lacked the splD gene encoding serine protease D (Figure 1).

Monocytic Evasion

To better ascertain differences in pathogenicity of RdJ isolates, we assessed the in vitro phagocytosis rate and

| Lineages of methicillin-resistant *Staphylococcus aureus* clonal complex 5 isolates, Rio de Janeiro, Brazil, 2014–2017* |
|---------------------------------------------------------------|
| **Clones** | Blood | Anterior nasal swab | Nonblood | Total (%) |
| ST105-SCC mec II-t002 | 41 | 20 | 14 | 75 (41.9)% |
| ST5-SCC mec IV-t002 | 11 | 13 | 19 | 43 (24.0)% |
| ST5-SCC mec II-t539 | 5 | 4 | 8 | 17 (9.5)% |
| ST1635-SCC mec IV-t002 | 2 | 4 | 4 | 10 (5.6)% |
| ST5-SCC mec III-t007 | 2 | 3 | 1 | 6 (3.4)% |
| ST5-SCC mec II-t666 | 2 | 2 | 1 | 5 (2.8)% |
| ST105-SCC mec II-NT | 3 | 0 | 0 | 3 (1.7)% |
| ST105-SCC mec I-t010 | 1 | 0 | 1 | 2 (1.1)% |
| ST4876-SCC mec II-t002 | 1 | 0 | 1 | 2 (1.1)% |
| ST5-SCC mec IV-t1154 | 1 | 1 | 0 | 2 (1.1)% |
| ST5-SCC mec IV-NT | 0 | 2 | 0 | 2 (1.1)% |
| ST105-SCC mec II-NT | 1 | 0 | 0 | 1 (0.6)% |
| ST105-SCC mec II-t539 | 0 | 1 | 0 | 1 (0.6)% |
| ST1635-SCC mec IV-t062 | 0 | 0 | 1 | 1 (0.6)% |
| ST1635-SCC mec IV-t450 | 0 | 1 | 0 | 1 (0.6)% |
| ST1635-SCC mec IV-t769 | 0 | 0 | 1 | 1 (0.6)% |
| ST5-SCC mec II-t002 | 0 | 1 | 0 | 1 (0.6)% |
| ST5-SCC mec II-NT | 0 | 1 | 0 | 1 (0.6)% |
| ST5-SCC mec IV-t061 | 0 | 1 | 0 | 1 (0.6)% |
| ST5-SCC mec IV-t062 | 0 | 0 | 1 | 1 (0.6)% |
| ST5-SCC mec IV-t105 | 1 | 0 | 0 | 1 (0.6)% |
| ST5-SCC mec IV-t586 | 1 | 0 | 0 | 1 (0.6)% |
| ST5-SCC mec IV-t777 | 0 | 1 | 0 | 1 (0.6)% |

*SCC, staphylococcal chromosome cassette; NT, not typed by spa polymorphism; ST, sequence type.
†Clones defined by multilocus sequence type, SCC mec type, and spa polymorphism.
\( \chi \)-squared test.
\( \beta \)-lactam antimicrobial drugs.
\( p < 0.01 \) by Pearson \( \chi \)-squared test.
\( p = 0.12 \) when adjusted for private vs. public hospital and year of isolation.

Total isolates sequenced from total no. isolates collected.

Table 3. Antimicrobial resistance among 600 methicillin-resistant *Staphylococcus aureus* isolates, Rio de Janeiro, Brazil, 2014–2017*

| Lineage | Total | No. multidrug-resistant isolates, % |
|---------|-------|------------------------------------|
| CC5-SCC mec II | 245 | 119 (48.6)%† |
| CC5-SCC mec IV | 137 | 6 (4.4)% |
| CC30-SCC mec IV | 109 | 0 |
| CC1-SCC mec IV | 79 | 13 (16.5)% |
| CC30-SCC mec II | 8 | 3 (37.5)% |
| CC8-SCC mec III | 7 | 7 (100.0)% |
| CC45-SCC mec IV | 5 | 0 |
| CC45-SCC mec II | 4 | 2 (50.0)% |
| CC8-SCC mec IV | 4 | 0 |
| CC188-SCC mec IV | 1 | 0 |
| CC22-SCC mec IV | 1 | 0 |

†Multidrug-resistant defined as an isolate carrying ≥4 additional antimicrobial resistance traits to non-β-lactam antimicrobial drugs.
\( p < 0.01 \) by Pearson \( \chi \)-squared test.
viable counts of unphagocytosed (free) bacteria (Figures 4–6). Representative RdJ isolates showed very low rates of phagocytosis/host cell association (2.9%) compared with representatives of other CC5 lineages: 41.3% for ST5(CC5)-SCCmecII-t539 and 35.8% for ST5(CC5)-SCCmecIV-t002 strains (Figure 6, panel A). In addition, after a 30-minute interaction with THP-1 monocytes, the RdJ strains showed higher survival rates (5.58%) than other lineages: 0.88% for ST5(CC5)-SCCmecIV-t002 and 0.76% for ST5(CC5)-SCCmecII-t539 (Figure 6, panel B).

Discussion

Using molecular typing and phylogenetic analysis, we identified a third epidemic lineage of MRSA in Rio de Janeiro. CC5, and to a lesser extent CC30, have become the most prevalent MRSA lineages in Rio de Janeiro hospitals, replacing the previously dominant ST1(CC1)-SCCmecIV lineage, which had replaced the BEC lineage ST239(CC8)-SCCmecIII during 2004–2008 (4). At the time when the ST1(CC1)-SCCmecIV lineage replaced BEC, CC5 comprised only 10% of isolates (4); CC5 now constitutes >60% of isolates. Previously dominant clones, especially BEC, carried resistance to many non-β-lactam antimicrobial drugs, antiseptics, and heavy metals whereas the currently dominant strains are more susceptible (1).

Although CC5-SCCmecII was the predominant genotype in our sample, the proportions of the second and third most frequent genotypes, CC5-SCCmecIV and lukSF-PV–positive CC30-SCCmecIV, also had increased from prior studies (4). CC5-SCCmecIV (related to USA800), which was first isolated in children at a hospital in Portugal in 1992 (26), was overrepresented among patients <5 years of age in our sample. Some studies have suggested that this strain is more common among children (27), although the nature of this association remains unclear. The lukSF-PV–positive CC30-SCCmecIV genotype is related to the USA1100/Oceania South West Pacific clone (1), and is a distant relative of the historically epidemic and especially virulent phage type 80/81 lineage (28).

We previously showed that, in contrast to the 80/81 lineage, ST30(CC30)-SCCmecIV MRSA from Brazil displays a natural attenuation of the Agr and SaeRS virulence regulators (29), which might explain why this lineage was responsible for only 9.6% of BSIs in this study.

The large number of MRSA isolates genotyped in this study enabled us to assess the distribution of MRSA genotypes by patient age and sites of infection or colonization. We identified associations between the CC5-SCCmecII genotype, BSIs, and older age, possibly because of the increased virulence or invasiveness of this genotype. The CC5-SCCmecII genotype also is found in the USA1100 lineage ST5(CC5)-SCCmecII that was dominant among hospitals in the

Figure 3. Time-calibrated phylogenetic tree of methicillin-resistant Staphylococcus aureus ST105(CC5)-SCCmecII-t002 lineage, Rio de Janeiro, Brazil, 2014–2017. Chronogram constructed using Bayesian phylogenetic analysis of single-nucleotide polymorphisms from 73 genomes. Maximum clade credibility tree estimated using a strict clock rate of 1.1927 × 10^{-6} substitutions/site/year (95% highest posterior density 1.5054–2.3351 × 10^{-6}). Node labels indicate 95% highest posterior density values of major clades. Asterisks (*) indicate posterior values >0.98. Scale indicates substitutions per site per year. CC, clonal complex; SCC, staphylococcal cassette chromosome; ST, sequence type.
United States during the late 1990s (30), before the emergence of the USA300 clone (31). USA100 is still found in hospitals in the United States (32) and around the world (1).

In our sample, most (75/114; 65.8%) CC5-SCCmecII isolates belonged to ST105 and shared spa-type t002, suggesting the emergence of a new clone. ST105(CC5)-SCCmecII strains have previously infected humans and domestic animals (33), and 4 isolates from this lineage were reported in a hospital in São Paulo (7). Reports from other countries have occasionally shown a substantial prevalence of this lineage, including a study that showed colonization among 22.4% of patients admitted to a hospital in Pennsylvania, USA (34). Another study showed that ST105(CC5)-SCCmecII was the predominant lineage among patients who had MRSA BSI in Switzerland (35). In Portugal, ST105(CC5)-SCCmecII has been reported as the most abundant MRSA colonizing patients >60 years of age (33); a multicenter study identified this lineage as the second most common clone among patients who had BSIs (36). The first vancomycin-resistant S. aureus isolate in Portugal belonged to this lineage (33), a troubling finding because most vancomycin-resistant S. aureus isolates have belonged to the CC5 lineage (25).

Few studies exist on the molecular epidemiology of MRSA in Brazil and in other countries from South America; existing studies are based on a limited number of samples (5–7,9). As a result, the full extent of the dissemination of the ST105-SCCmecII-t002 genotype in Latin America is unknown. Since the late 2000s, ST105-SCCmecII-t002 has been reported as the second or third most frequent MRSA lineage in hospitals in the United States and some countries in Europe (33–35). For example, researchers documented an outbreak of ST105-SCCmecII-t002 MRSA among 18 neonates at Mount Sinai Hospital (New York, NY, USA) during 2014–15 (38). In addition, ST105 isolates comprised 87.5% of delafloxacin-resistant MRSA strains collected in 7 hospitals in New York (39). Altogether, these data show that ST105 is a major MRSA lineage not only in Rio de Janeiro but also in other countries. ST105-SCCmecII-t002 also might have spread in other regions of Brazil; therefore, more studies are needed to better track and investigate this lineage.

We used Bayesian molecular clock analysis to estimate the expansion of the RdJ clade in Rio de Janeiro
in 2009 (95% highest posterior density 2007–2010), which is consistent with previous estimates that date the origin of the ST105 lineage to the mid-1990s (8). The ST105 clade is characterized by a lack of virulence genes that are common among other CC5 strains. All ST105(CC5)-SCCmecII isolates lacked the sep gene encoding enterotoxin P, as noted by Challagundla et al. (8). In addition to its emetic properties, enterotoxin P is a superantigen that induces T-cell proliferation and production of proinflammatory cytokines (40). ST105(CC5)-SCCmecII-t002 strains showed resistance to fluoroquinolones, macrolides, and lincosamides. ST105(CC5)-SCCmecII isolates also lacked the serine protease encoding gene splD, despite the presence of the splABCF genes of the spl operon. Although the specific role of SplD in S. aureus pathogenesis is not known, some researchers have proposed that Spl serine proteases might use proteolysis to modulate host proteins critical to bacterial pathogenesis (41). Future work should address implications of the absence of SplD in the ST105(CC5)-SCCmecII lineage.

Compared with representatives of the ST5-SCCmecII-t539 and ST5-SCCmecIV-t002 lineages, representative isolates of the RdJ clade showed increased evasion of phagocytosis mechanisms upon exposure to monocyctic cells (i.e., THP-1). Multiple factors, including phagocytosis rate and the activity of toxic compounds released by monocytes, might affect the number of viable unphagocytosed bacterial cells (42). Moreover, we observed an increased number of viable RdJ free cells. The basis of this phenotype is unclear and deserves further study. Le Pabic et al. (43) implicated the small noncoding RNA, SprC, and its effect on regulation of the major autolysin Atl in S. aureus evasion of phagocytosis by human monocytes and macrophages. However, we did not find any differences in the sprC gene of the 6 representative strains tested, suggesting that the observed evasion might be multifactorial, probably linked to the production of several bacterial molecules (42).

One limitation of this study is the lack of more extensive clinical data such as the presence of indwelling catheters or lines and underlying conditions that might have affected our estimates. The association between ST105-SCCmecII-t002 and BSIs was attenuated when accounting for other variables such as hospital type and year of isolation, but still might be of clinical relevance. Access to more extensive clinical data would enable further exploration of this relationship. In addition, our reliance on samples from Rio de Janeiro might have affected our phylogenetic analysis; focused sampling in other geographic locations might show a more widespread epidemic.

In summary, we uncovered a new MRSA clone in hospitals in the Rio de Janeiro metropolitan area. Our findings emphasize the dynamic nature of the local rise and decline of various MRSA clones. In addition, these data indicate that MRSA clonal dynamics also might be associated with different manifestations of disease and host factors, such as age. This analysis revealed the emergence of a novel multidrug-resistant MRSA clone associated with BSIs. This association might be critical for assessing the clinical and epidemiologic risks associated with the spread of this clone and the biologic basis for its putative enhanced invasiveness.

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References
1. Figueiredo AMS, Ferreira FA. The multifaceted resources
and microevolution of the successful human and
animal pathogen methicillin-resistant Staphylococcus aureus.
Mem Inst Oswaldo Cruz. 2014;109:265–78. https://doi.org/
10.1590/0074-027640016
2. Planet PJ, Narechania A, Chen L, Mathema B, Boundy S,
Archer G, et al. Architecture of a species: phylogenomics of
Staphylococcus aureus. Trends Microbiol. 2017;25:153–66.
https://doi.org/10.1016/j.tim.2016.09.009
3. Teixeira LA, Resende CA, Ormonde LR, Rosenbaum R,
Figueiredo AM, de Lencastre H, et al. Geographic spread of
epidemic multiresistant Staphylococcus aureus clone in Brazil.
J Clin Microbiol. 1995;33:2400–4. https://doi.org/10.1128/
jcmm.33.9.2400-2404.1995
4. Silva-Cardvalho MC, Bonelli RR, Souza RR, Moreira S,
dos Santos LCG, de Souza Conceição M, et al. Emergence of
multiresistant variants of the community-acquired
methicillin-resistant Staphylococcus aureus lineages ST1-SCCmecIV in 2 hospitals in Rio de Janeiro, Brazil. Diagn Micr Org Infect Dis. 2009;65:300–5. https://doi.org/
10.1016/j.dmiro.2009.07.023
5. Chamon RC, Ribeiro SD, da Costa TM, Nouér SA,
Dos Santos KRN. Complete substitution of the Brazilian
endemic clone by other methicillin-resistant Staphylococcus
aureus lineages in two public hospitals in Rio de Janeiro,
Brazil. Braz J Infect Dis. 2017;21:185–9. https://doi.org/
10.1016/j.bjid.2016.09.015
6. Zuma AVP, Lima DF, Assaf APDC, Marques EA, Leão RS.
Molecular characterization of methicillin-resistant
Staphylococcus aureus isolated from blood in Rio de Janeiro
displaying susceptibility profiles to non-β-lactam antibiotics.
Braz J Microbiol. 2017;48:237–41. https://doi.org/10.1016/
jbmi.2016.09.016
7. Caiaffa-Filho HH, Trindade PA, Gabriela da Cunha P,
Alencar CS, Prado GVB, Rossi F, et al. Methicillin-resistant
Staphylococcus aureus carrying SCCmec type II was more
frequent than the Brazilian endemic clone as a cause of
nosocomial bacteremia. Diagn Microbiol Infect Dis. 2013;76:518–
20. https://doi.org/10.1016/j.diagmicrobio.2013.04.024
8. Challagundla L, Reyes J, Rafiqullah I, Sordelli DO,
Echaniz-Aviles G, Velazquez-Meza ME, et al. Phylogenomic
classification and the evolution of clonal complex 5 methi-
cillin-resistant Staphylococcus aureus in the Western Hemi-
sphere. Front Microbiol. 2018;9:1901. https://doi.org/
10.3389/fmicb.2018.01901
9. Arias CA, Reyes J, Carvajal LP, Rincon S, Diaz L, Panesso D,
et al. A prospective cohort multicenter study of molecular
epidemiology and phylogenomics of Staphylococcus aureus
bacteremia in nine Latin American countries [Erratum in:
Antimicrob Agents Chemother. 2017;61:e00095–18].
Antimicrob Agents Chemother. 2017;61:17. https://doi.org/10.1128/AAC.00816-17
10. Cockfield JD, Pathak S, Edgeworth JD, Lindsay JA. Rapid
determination of hospital-acquired meticillin-resistant
Staphylococcus aureus lineages. J Med Microbiol. 2007;56:614–
9. https://doi.org/10.1099/jmm.0.47074-0
11. Milheiroç G, Oliveira DC, de Lencastre H. Update to
the multiplex PCR strategy for assignment of mec element
types in Staphylococcus aureus [Erratum in: Antimicrob
Agents Chemother. 2007;51:4537-]. Antimicrob Agents
Chemother. 2007;51:3374–7. https://doi.org/10.1128/
AAC.00275-07
12. Beltrame CO, Botelho AMN, Silva-Cardvalho MC, Souza RR,
Bonelli RR, Ramundo MS, et al. Restriction modification
(RM) tests associated to additional molecular markers for
screening prevalent MRSA clones in Brazil. Eur J Clin
Microbiol Infect Dis. 2012;31:2011–6. https://doi.org/
10.1007/s10096-011-1534-1
13. Clinical and Laboratory Standards Institute. Performance
standards for antimicrobial susceptibility testing: 28th informa-
tional supplement (M100-S28). Wayne (PA): The Institute; 2018.
14. Zerbino DR, Birney E. Velvet: algorithms for de novo
short read assembly using de Brujin graphs. Genome Res.
2008;18:821–9. https://doi.org/10.1101/gr.074992.107
15. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M,
Kulikov AS, et al. SPAdes: a new genome assembly algo-
rithm and its applications to single-cell sequencing.
J Comput Biol. 2012;19:455–77. https://doi.org/10.1089/
cmb.2012.0021
16. Stamatakis A. RAxML version 8: a tool for phylogenetic
analysis and post-analysis of large phylogenies.
Bioinformatics. 2014;30:1312–3. https://doi.org/10.1093/
bioinformatics/btu033
17. Ondov BD, Starrett GJ, Sappington A, Kostic A, Koren S,
Buck CB, et al. Mash Screen: high-throughput sequence
containment estimation for genome discovery. Genome
Biol. 2019;20:232. https://doi.org/10.1186/s13059-019-1841-x
18. Mustafa AM, Planet PJ, WhatsGNU, a tool for identifying
proteomic novelty. Genome Biol. 2020;21:58. https://doi.org/
10.1186/s13059-020-01965-w
19. Didelot X, Wilson DJ. ClonalFrameML: efficient inference of
recombination in whole bacterial genomes. PLOS Comput
Biol. 2015;11:e1004041. https://doi.org/10.1371/journal.
pclb.1004041
20. Bouckaert R, Rambaut A, Drummond AJ, Suchard MA,
Xu X, et al. BEAST 2.5: an ad-
vanced software platform for Bayesian evolutionary analysis.
BMC Evol Biol. 2019;15:e100650. https://doi.org/10.1371/journal.
pclb.100650
21. Rambaut A, Lam TT, Max Carvalho L, Pybus OG. Exploring
the temporal structure of heterogeneous sequences using
TempEst (formerly Path-O-Gen). Virus Evol. 2016;2:vwe007.
https://doi.org/10.1093/viruses/vwe007
22. Aswani V, Najar F, Pantrangi M, Mau B, Schwan WR,
Shukla SK. Virulence factor landscape of a Staphylococcus
aureus sequence type 45 strain, MCRIF184. BMC Genomics.
2019;20:123. https://doi.org/10.1186/s12864-018-5394-2
23. Novick RP, Ram G. Staphylococcal pathogenicity
islands-movers and shakers in the genomic firmament.
Curr Opin Microbiol. 2017;38:197–204. https://doi.org/
10.1016/j.mib.2017.08.001
24. Melehan JH, James DBA, DuMont AL, Torres VJ, Duncan JA.
Staphylococcus aureus leukocidin A/B (LukAB) kills human
monocytes via host NLRP3 and ASC when extracellular, but not intracellular. PLoS Pathog. 2015;11:e1004970. https://doi.org/10.1371/journal.ppat.1004970

25. Panesso D, Planet PJ, Diaz L, Hugonnet JE, Tran TT, Narechania A, et al. Methicillin-susceptible, vancomycin-resistant Staphylococcus aureus, Brazil. Emerg Infect Dis. 2015;21:1844–8. https://doi.org/10.3201/eid2110.141914

26. Sá-Leão R, Santos Sanches I, Dias D, Peres J, Barros RM, de Lencastre H. Detection of an arscha clone of Staphylococcus aureus with low-level resistance to methicillin in a pediatric hospital in Portugal and in international samples: relics of a formerly widely disseminated strain? J Clin Microbiol. 1999;37:1913–20. https://doi.org/10.1128/JCM.37.6.1913-1920.1999

27. Rokney A, Baum M, Ben-Shimol S, Sagi O, Anuka E, Agmon V, et al. Dissemination of the methicillin-resistant Staphylococcus aureus pediatric clone (ST5-002-IV-PVL+) as a major cause of community-associated staphylococcal infections in Bedouin children, southern Israel. Pediatr Infect Dis J. 2019;38:230–5. https://doi.org/10.1097/INF.0000000000002126

28. DeLeo FR, Kennedy AD, Chen L, Bubeck Wardenburg J, Kobayashi SD, Mathema B, et al. Molecular differentiation of historic phage-type 80/81 and contemporary epidemic Staphylococcus aureus. Proc Natl Acad Sci U S A. 2011;108:18091–6. https://doi.org/10.1073/pnas.1111084108

29. Ramundo MS, Beltrame CO, Botelho AMN, Coelho LR, Silva-Cardvalho MC, Ferreira-Cardvalho BT, et al. A unique SaeS allele overrides cell-density dependent expression of saeR and lukSF-PV in the ST30-SCCmecIV lineage of CA-MRSA. Int J Med Microbiol. 2016;306:367–80. https://doi.org/10.1016/j.ijmm.2016.05.001

30. Roberts RB, Chung M, de Lencastre H, Hargrave J, Tomasz A, Nicoll DP, et al.; Tri-State MRSA Collaborative Study Group. Distribution of methicillin-resistant Staphylococcus aureus clones among health care facilities in Connecticut, New Jersey, and Pennsylvania. Microb Drug Resist. 2000;6:245–51. https://doi.org/10.1089/mdr.2000.6.245

31. Planet PJ. Life after USA300: the rise and fall of a superbug. J Infect Dis. 2017;215:571–7. https://doi.org/10.1093/infdis/jiw444

32. Carrel M, Perenechin EN, David MZ. USA300 methicillin-resistant Staphylococcus aureus, United States, 2000–2013. Emerg Infect Dis. 2015;21:1973–80. https://doi.org/10.3201/eid2111.150452

33. Almeida ST, Nunes S, Paulo ACS, Faria NA, de Lencastre H, Sá-Leão R. Prevalence, risk factors, and epidemiology of meticillin-resistant Staphylococcus aureus carried by adults over 60 years of age. Eur J Clin Microbiol Infect Dis. 2015;34:593–600. https://doi.org/10.1007/s10096-014-2267-8

34. Verghese B, Schwalm ND III, Dudley EG, Knabel SJ. A combined multi-virulence-focus sequence typing and staphylococcal cassette chromosome mec typing scheme possesses enhanced discriminatory power for genotyping MRSA. Infect Genet Evol. 2012;12:1816–21. https://doi.org/10.1016/j.meegid.2012.07.026

35. Blanc DS, Petignat C, Wenger A, Kuhn G, Vallet Y, Fracheboud D, et al. Changing molecular epidemiology of methicillin-resistant Staphylococcus aureus in a small geographic area over an eight-year period. J Clin Microbiol. 2007;45:3729–36. https://doi.org/10.1128/JCM.00511-07

36. Faria NA, Miragaia M, de Lencastre H; Multi Laboratory Project Collaborators. Massive dissemination of meticillin resistant Staphylococcus aureus in bloodstream infections in a high MRSA prevalence country: establishment and diversification of EMRSA-15. Microb Drug Resist. 2013;19:483–90. https://doi.org/10.1089/mdr.2013.0149

37. Melo-Cristino J, Resina C, Manuel V, Lito L, Ramirez M. First case of infection with vancomycin-resistant Staphylococcus aureus in Europe. Lancet. 2013;382:205. https://doi.org/10.1016/S0140-6736(13)61219-2

38. Sullivan MJ, Altman DR, Chacko KJ, Ciferri B, Webster E, Pak TR, et al. A complete genome screening program of clinical meticillin-resistant Staphylococcus aureus isolates identifies the origin and progression of a neonatal intensive care unit outbreak. J Clin Microbiol. 2019;57:e01261-19. https://doi.org/10.1128/JCM.01261-19

39. Iregui A, Khan Z, Malik S, Landman D, Quale J. Emergence of delafloxacin-resistant Staphylococcus aureus in Brooklyn, New York. Clin Infect Dis. 2020;70:1758–60. https://doi.org/10.1093/cid/ciz787

40. Omoe K, Imanishi K, Hu D-L, Kato H, Fugane Y, Abe Y, et al. Characterization of novel staphylococcal enterotoxin-like toxin type P. Infect Immun. 2005;73:5540–6. https://doi.org/10.1128/IAI.73.9.5540-5546.2005

41. Zdzalik M, Kalinska M, Wysocka M, Stec-Niemczyk J, Cichon P, Stach N, et al. Biochemical and structural characterization of SpID protease from Staphylococcus aureus. PLoS One. 2013;8:e76812. https://doi.org/10.1371/journal.pone.0076812

42. Flannagan RS, Heit B, Heinrichs DE. Antimicrobial mechanisms of macrophages and the immune evasion strategies of Staphylococcus aureus. Pathogens. 2015;4:826–68. https://doi.org/10.1093/cid/ciz783

43. Le Pabic H, Germain-Amiot N, Bordeau V, Felden B. A bacterial regulatory RNA attenuates virulence, spread and human host cell phagocytosis. Nucleic Acids Res. 2015;43:9232–48. https://doi.org/10.1093/nar/gkv783

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Multidrug-Resistant Methicillin-Resistant \textit{Staphylococcus aureus} Associated with Bacteremia and Monocyte Evasion, Rio de Janeiro, Brazil

Appendix 3

Appendix Methods

Phylogenetic Analysis and Divergence Times

A maximum-likelihood tree was constructed for 661 genomes; 179 clonal complex 5 (CC5) genomes from our collection and 482 CC5 assembled genomes available from GenBank (1), chosen from the list provided by Challagundla et al. (2). Read mapping to the N315 reference genome (GenBank accession no. BA000018.3) and variant calling were performed by using Snippy version 4.6.0 (https://github.com/tseemann/snippy). A single-nucleotide polymorphism (SNP) alignment produced by Snippy was used to infer an initial phylogenetic tree in RAxML version 8.2.4 (3) by using an ascertainment bias correction and general time-reversible substitution model (4) accounting for among-site rate heterogeneity using the gamma distribution and 4 rate categories (ASC_GTRGAMMA model) (5) for 100 individual searches with maximum parsimony random-addition starting trees. Node support was evaluated with 100 nonparametric bootstrap pseudoreplicates (6). For better visualization, the tree was edited by using Interactive Tree of Life version 4.2.3 (7).

To estimate the emergence time of the sequence type 105 isolates from Rio de Janeiro (RdJ clone), we used a Bayesian phylogenetic framework implemented in BEAST version 2.6.0 (8). For this analysis, genomes that failed our Mash Screen (9) cutoffs (i.e., a genome has a hit that is not \textit{S. aureus} and has Mash identity of $>0.85$ and shared-hashes value of $>100$) were excluded, leading to a total of 73/82 genomes included. To select the closest complete reference genome from GenBank that would capture all genomic regions in the 73 RdJ strains, we annotated genomes in Prokka 1.14.5 (10) by using WhatsGNU topgenome (-t) option (11).
approach identified FCFHV36 (GenBank accession no. CP011147.1) as the GenBank hit that was the closest to all 73 genomes and shared an average of 2,485 protein open reading frames with each genome. Read mapping to the complete reference genome FCFHV36 and variant calling were performed by using Snippy. A SNP alignment produced by Snippy was used to infer an initial phylogenetic tree in RAxML version 8.2.4 (3) by using an ascertainment bias correction and general time-reversible substitution model (4) to account for among-site rate heterogeneity using the gamma distribution and 4 rate categories (ASC_GTRGAMMA model) (5) for 100 individual searches with maximum parsimony random-addition starting trees. Node support was evaluated with 100 nonparametric bootstrap pseudoreplicates (6). The initial ML newick tree and the whole-genome alignment, including SNPs and invariant sites, were used as inputs for ClonalFrameML (12) to infer recombination using 100 pseudo-bootstrap replicates. A SNP alignment was then called from the resultant alignment using the SNP-sites tool (13).

The SNP alignment was then used to estimate divergence times in BEAST (8). To gauge the potential for temporal signal of our dataset, we used a regression of root-to-tip genetic distance versus isolation time as a diagnostic tool, as implemented in TempEst version 1.5.3 (14). A positive correlation between genetic divergence and isolation time was observed ($R^2 = 0.4177$), indicating suitability of this dataset for downstream molecular clock analysis in BEAST. The Hasegawa–Kishino–Yano nucleotide substitution model was used (15) with estimated base frequencies, and ascertainment bias for variable-only sites was corrected by editing the XML file and factoring in the number of invariable sites based on fully sequenced genomes (https://www.beast2.org/2019/07/18/ascertainment-correction.html). We implemented a strict clock model with a random starting tree, a coalescent constant population (16), and a uniform prior probability distribution of 10–3–10–7 substitutions/site/year. The chain length was set at 100 million Markov chain Monte Carlo steps with a 10,000-step thinning and was run 3 independent times. We also implemented an uncorrelated lognormal relaxed clock model in BEAST. The chain length was set at 200 million Markov chain Monte Carlo steps with a 20,000-step thinning. Since clock rate heterogeneity among branches was not large in the relaxed clock model runs (i.e., the 95% highest posterior density interval for the ucld.Stdev and rate.coefficientOfVariation parameters were 0–0.27), and the trees and divergence times produced by the strict (Figure 3) and relaxed clock techniques were largely indistinguishable. Sampling from the prior without sequence data also was used to assess whether the data are
informative on different parameters by looking for a departure of the posterior distribution from
the prior. Three independent strict clock runs were combined post convergence using
LogCombiner version 2.4.7 and resample posterior distribution of the tree files at a lower
frequency (30,000 steps), following inspections of the sampled parameters and their effective
sample size (i.e., >728) in Tracer version 1.7.1. The chronograms were plotted on the basis of the
maximum clade credibility tree using the TreeAnnotator program from the BEAST package after
removing 10% of the first posterior samples as a burn-in and were visualized in FigTree version
1.4.3. The tree file for Bayesian analysis RAxML is available in Mendeley data
(https://data.mendeley.com/datasets/3pz36bdb63/1).

References
1. Sayers EW, Cavanaugh M, Clark K, Ostell J, Pruitt KD, Karsch-Mizrachi I. GenBank. Nucleic Acids
   Res. 2019;47:D94–9. PubMed https://doi.org/10.1093/nar/gky989
2. Challagundla L, Reyes J, Rafiqullah I, Sordelli DO, Echaniz-Aviles G, Velazquez-Meza ME, et al.
   Phylogenomic classification and the evolution of clonal complex 5 methicillin-resistant
   Staphylococcus aureus in the Western Hemisphere. Front Microbiol. 2018;9:1901. PubMed
   https://doi.org/10.3389/fmicb.2018.01901
3. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large
   phylogenies. Bioinformatics. 2014;30:1312–3. PubMed
   https://doi.org/10.1093/bioinformatics/btu033
4. Lanave C, Preparata G, Sacone C, Serio G. A new method for calculating evolutionary substitution
   rates. J Mol Evol. 1984;20:86–93. PubMed https://doi.org/10.1007/BF02101990
5. Yang Z. A space-time process model for the evolution of DNA sequences. Genetics. 1995;139:993–
   1005. PubMed https://doi.org/10.1093/genetics/139.2.993
6. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution.
   1985;39:783–91. PubMed https://doi.org/10.1111/j.1558-5646.1985.tb00420.x
7. Letunic I, Bork P. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of
   phylogenetic and other trees. Nucleic Acids Res. 2016;44:W242–5. PubMed
   https://doi.org/10.1093/nar/gkw290
8. Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, et al. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLOS Comput Biol. 2019;15:e1006650. PubMed https://doi.org/10.1371/journal.pcbi.1006650

9. Ondov BD, Starrett GJ, Sappington A, Kostic A, Koren S, Buck CB, et al. Mash Screen: high-throughput sequence containment estimation for genome discovery. Genome Biol. 2019;20:232. PubMed https://doi.org/10.1186/s13059-019-1841-x

10. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30:2068–9. PubMed https://doi.org/10.1093/bioinformatics/btu153

11. Moustafa AM, Planet PJ. WhatsGNU: a tool for identifying proteomic novelty. Genome Biol. 2020;21:58. PubMed https://doi.org/10.1186/s13059-020-01965-w

12. Didelot X, Wilson DJ. ClonalFrameML: efficient inference of recombination in whole bacterial genomes. PLOS Comput Biol. 2015;11:e1004041. PubMed https://doi.org/10.1371/journal.pcbi.1004041

13. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, et al. SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. Microb Genom. 2016;2:e000056. PubMed https://doi.org/10.1099/mgen.0.000056

14. Rambaut A, Lam TT, Max Carvalho L, Pybus OG. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). Virus Evol. 2016;2:vew007. PubMed https://doi.org/10.1093/ve/vew007

15. Hasegawa M, Kishino H, Yano T. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol. 1985;22:160–74. PubMed https://doi.org/10.1007/BF02101694

16. Griffiths RC, Tavaré S. Sampling theory for neutral alleles in a varying environment. Philos Trans R Soc Lond B Biol Sci. 1994;344:403–10. PubMed https://doi.org/10.1098/rstb.1994.0079
### Appendix 3 Table 1. Phylogenetic groups of the methicillin-resistant *Staphylococcus aureus* CC5 strains randomly selected for phagocytosis and enumeration of viable unphagocytosed (free) bacterial cells, Rio de Janeiro, Brazil, 2014–2017*

| Strain* | Lineage | Clade position† |
|---------|---------|-----------------|
| CR14–026 | ST5(CC5)-SCCmecIV-t002 | CC5-Basal |
| CHU15–056 | ST5(CC5)-SCCmecIV-t002 | CC5-Basal |
| CR14–016 | ST5(CC5)-SCCmecII-t539 | CC5-IIA |
| CR15–071 | ST5(CC5)-SCCmecII-t539 | CC5-IIA |
| CD15–276 | ST105(CC5)-SCCmecII-t002 | CC5-IIB |
| CD16–016 | ST105(CC5)-SCCmecII-t002 | CC5-IIB |

*Two MRSA strains from Rio de Janeiro were selected by lottery from each of the 3 clades (i.e., CC5-Basal, CC5-IIA, and CC5-IIB) to which the CC5 MRSA were allocated. CC5, clonal complex 5.
†Clades were named according to the classification of Challagundla et al. (2).

### Appendix 3 Table 2. Molecular characterization of 29 methicillin-resistant *Staphylococcus aureus* non–clonal complex 5 strains, Rio de Janeiro, Brazil, 2014–2017*

| Multilocus sequence type | SCCmec type | Related clone | lukSF | No. isolates |
|--------------------------|-------------|---------------|-------|--------------|
| ST30(CC30)               | IV          | USA1100/OSPC  | +     | 12           |
| ST1(CC1)                 | IV          | USA400/MW2    | –     | 7            |
| ST8(CC8)                 | IV          | USA300        | +     | 3            |
| ST239(CC8)               | III         | BEC           | ND    | 3            |
| ST45(CC45)               | II          | USA600        | ND    | 2            |
| ST3603(CC45)             | IV          | USA600        | ND    | 1            |
| ST188(CC188)             | IV          | Sporadic (rare) MRSA | ND    | 1            |

*lukSF*, genes encoding Panton-Valentine leukocidin; ND, not done; ST, sequence type; +, positive; −, negative.