Genomic diversity of prevalent *Staphylococcus epidermidis* multidrug-resistant strains isolated from a Children´s Hospital in México City in an eight-years survey

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Staphylococcus epidermidis is a human commensal and pathogen worldwide distributed. In this work, we surveyed for multi-resistant S. epidermidis strains in eight years at a children’s health-care unit in México City. Multidrug-resistant S. epidermidis were present in all years of the study, including resistance to methicillin, beta-lactams, fluoroquinolones, and macrolides. To understand the genetic basis of antibiotic resistance and its association with virulence and gene exchange, we sequenced the genomes of 17 S. epidermidis isolates. Whole-genome nucleotide identities between all the pairs of S. epidermidis strains were about 97% to 99%. We inferred a clonal structure and eight Multilocus Sequence Types (MLST’s) in the S. epidermidis sequenced collection. The profile of virulence includes genes involved in biofilm formation and phenol-soluble modulins (PSMs). Half of the S. epidermidis analyzed lacked the ica operon for biofilm formation. Likely, they are commensal S. epidermidis strains but multi-antibiotic resistant. Uneven distribution of insertion sequences, phages, and CRISPR-Cas immunity phage systems suggest frequent horizontal gene transfer. Rates of recombination between S. epidermidis strains were more prevalent than the mutation rate and affected the whole genome. Therefore, the multidrug resistance, independently of the pathogenic traits, might explain the persistence of specific highly adapted S. epidermidis clonal lineages in nosocomial settings.
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Abstract

Staphylococcus epidermidis is a human commensal and pathogen worldwide distributed. In this work, we surveyed for multi-resistant S. epidermidis strains in eight years at a children’s healthcare unit in México City. Multidrug-resistant S. epidermidis were present in all years of the study, including resistance to methicillin, beta-lactams, fluoroquinolones, and macrolides. To understand the genetic basis of antibiotic resistance and its association with virulence and gene exchange, we sequenced the genomes of 17 S. epidermidis isolates. Whole-genome nucleotide identities between all the pairs of S. epidermidis strains were about 97% to 99%. We inferred a clonal structure and eight Multilocus Sequence Types (MLST’s) in the S. epidermidis sequenced collection. The profile of virulence includes genes involved in biofilm formation and phenol-soluble modulins (PSMs). Half of the S. epidermidis analyzed lacked the ica operon for biofilm formation. Likely, they are commensal S. epidermidis strains but multi-antibiotic resistant. Uneven distribution of insertion sequences, phages, and CRISPR-Cas immunity phage systems suggest frequent horizontal gene transfer. Rates of recombination between S. epidermidis strains were more prevalent than the mutation rate and affected the whole genome. Therefore, the multidrug resistance, independently of the pathogenic traits, might explain the persistence of specific highly adapted S. epidermidis clonal lineages in nosocomial settings.

Introduction

Staphylococcus epidermidis (SE) is a typical commensal bacterium of the human skin microbiome (Byrd et al. 2018). However, some SE strains behave as pathogens colonizing surgery wounds, medical devices, and in some circumstances, they reach the human bloodstream causing severe bacteremia and potential mortality (Chessa et al. 2015; Otto 2009). Children are especially prone to acquire methicillin-resistant SE strains in perinatal hospitals (Marchant et al. 2013; Villari et al. 2000). The genetic basis for pathogenicity of SE strains includes genes related to biofilm formation (adhesion), phenol soluble modulins (PSMs), and diverse Mobile Genetic Elements (MGEs) like phages, insertion sequences (ISs), and pathogenicity islands, that may be associated with the transfer of antibiotic and virulence traits (Bouchami et al. 2016; Miragaia et al. 2009; Rolo et al. 2017) (Conlan et al. 2012; Kozitskaya et al. 2005; Meric et al. 2015). There is no clear genetic distinction between pathogenic and commensal non-pathogenic SE strains, even though nosocomial strains are enriched in virulence and antibiotic resistance genes (Conlan et al. 2012; Kozitskaya et al. 2005; Meric et al. 2018). It has been proposed that these genes are within the pool of accessory genome mobilized within and between species (Meric et al. 2015; Rolo et al. 2017). In this sense, the emergence of methicillin-resistant staphylococci has been linked to MGEs such as the Staphylococcal Chromosomal Cassette (SCCmec), and the Arginine Catabolic Mobile Element (ACME) that play a role in adaptation to the skin and other human surfaces (Miragaia et al. 2009; Planet et al. 2013; Rolo et al. 2012). Recent phylogenetic studies indicate that ACME having originated from SE and transferred horizontally to S. aureus (Barbier et al. 2011; Onishi et al. 2013; Planet et al. 2013), whereas SCCmec may have originated and diversified in different staphylococci species independently (Miragaia 2018). Other factors, including, genomic rearrangements mediated by IS256 in pathogenic SE strains (Gu et al. 2005; Ziebuhr et al. 1999a), conjugative transfer of antibiotic resistance (Forbes & Schaberg 1983) and the metabolic state of the staphylococci cell, may also be linked to pathogenicity (Bosi et al. 2016). Thus, pathogenicity in staphylococci may be viewed as a set of evolving genetic traits for adaptation to specific niches (Ben Zakour et al. 2008; Ziebuhr et al. 1999b).
The population structure of *SE* is essentially clonal as determined by Multilocus Sequence Typing (MLST) (Miragaia et al. 2002; Miragaia et al. 2007; Thomas et al. 2007). Although, nosocomial *SE* strains show high genetic diversity among isolates from distant geographic locations (Miragaia et al. 2007) several clonal complexes are disseminated globally including the ST2, ST5, and ST23 which indeed are the most frequently found in clinical environments (Lee et al. 2018; Miragaia et al. 2007). Besides the clonal structure, recombination has been estimated to occur two times more frequently than mutation (Miragaia et al. 2007). Genomic analysis had calculated that about 40% of the core genes of *SE* had undergone recombination (Meric et al. 2015). It suggests that recombination might be a general property of *SE* for rapid evolution in clinical settings but conserving high linkage disequilibrium between alleles. In this case, recombination might act as a cohesive force, maintaining the clonal population structure but allowing clones to diverge.

Multidrug resistance is a significant concern in most health care settings worldwide because of the difficulties associated with clinical treatment and the potential of dissemination. It has been reported that the prevalent *SE* clonal lineages ST5, ST12, and ST23 often display high resistance toward most of the antibiotic classes (Martinez-Melendez et al. 2016; Widerstrom et al. 2012). Furthermore, there is evidence of increasing of rifampicin resistance over time in clinical *SE* isolates that belong to ST2 and ST23 from the USA, Australia, and some European countries (Lee et al. 2018). Mutations in the *rpoB* gene conferring rifampicin resistance have emerged independently, suggesting the view that a limited number, well adapted multi-resistant clonal *SE* lineages, prevail in clinical settings (Lee et al. 2018; Martinez-Melendez et al. 2016). Reports of the profiles of antibiotic multi-resistance changes over time are scarce (Lee et al. 2018). Surveillance of rifampicin resistance in a study involving 24 countries and 96 institutions suggests annual local variations for this antibiotic (Lee et al. 2018). Therefore, the correct identification of pathogenic *SE* strains and its drug resistance profile will contribute to prevent and treat these bacterial infections in the clinic.

In this work, we evaluated the prevalence of *SE* in comparison with other staphylococci species isolated in a single hospital in México City during eight years period. Then, we aimed to assess the antibiotic resistance profiles of *SE* isolates, and through genomics, to know the genome structure of a selected set of antibiotic multi-resistant *SE* strains. In this context, we provide a genome-wide measure of recombination, to shed light on the mechanisms of *SE* genetic diversification and the evolution of multidrug-resistance in local sites.

**Materials & Methods**

**Ethical considerations.** This study was carried out following the recommendations of the ethics review committee of the Facultad de Medicina-UNAM. The *SE* strains used in this work were obtained by a donation from the microbiology collection of the Instituto Nacional de Perinatología “Isidro Espinosa de los Reyes” (INPer). A consent form was not required. Original identification keys and clinical data concerning the isolates are maintained under the control of INPer. In the present work, new strain identifiers were assigned to the INPer strains. Authors do not have access in any form to the specific clinical information of strains and patients.

**Clinical isolation of staphylococci and characterization.** Staphylococci strains were recovered from primus isolates taken from patients at INPer. They were conserved frozen (-80 °C in an
Characterization of staphylococci was carried out by streaking the cultures on tryptic soy agar plates (BD Diagnostic Systems, Germany). Plates were incubated for 18-24 hours at 37 °C to obtain isolated colonies. Individual colonies were used to inoculate tryptic soy broth and grown overnight after which genomic DNA was extracted (see below). Identification of *Staphylococcus* species was carried out using the VITEK®2 equipment (bioMérieux SA, 376 Chemin de l’Ome. France). Briefly, staphylococci colonies were resuspended in 0.45% saline solution and adjusted to a turbidity of 0.5 in a McFarland nephelometer. The bacterial suspension was transferred into the VITEK®2 GP ID card testing 64 biochemical properties for gram-positive bacteria, and eight specific tests for *SE* species (phosphatase and urease production, growth in 6% NaCl, resistance to novobiocin and polymyxin B, arginine hydrolysis, catalase, D-mannitol fermentation). Additional growth characteristics, biochemical (D-mannitol fermentation, coagulase, and catalase), and molecular tests (PCR detection of *coa* and *mecA*; see below) were performed at the arrival of the staphylococci collection to the Pathogenicity Laboratory at Faculty of Medicine, UNAM.

**Molecular tests.** To confirm the presence/absence of the coagulase gene (*coa*), and the methicillin-coding gene (*mecA*) in *SE* and *S. aureus* strains, we did a duplex PCR according to Hookey (Hookey et al. 1999). In brief, a PCR amplification of a 177 bp fragment of the *coa* gene, with primers coa-forward 5´-AACTTGAAAT-AAAACCACAAGG-3´, and coa-reverse 5´-TACCTGTACCAGCATCTCTA-3´. In the same reaction, a 458 bp fragment of the *mecA* gene was amplified using primers mecA-forward 5´-ATGGCAAAGATATTCAACTAAC-3´ and mecA-reverse 5´-GAGTGCTACTCTAGCAAAGA-3´. PCR reactions were performed on a BIO-RAD C1000 Thermal Cycler (BIO-RAD, USA). The amplification conditions were as follows: 94 °C for 10 minutes and 30 cycles at 94 ° C for 30 seconds, 56.2 ° C for 30 seconds and 72 ° C for 30 seconds and a final amplification at 72 ° C for 10 min; PCR products were separated by electrophoresis in a 1% agarose gel stained with ethidium bromide and visualized with UV light.

**Antibiotic resistance.** The antibiotype for 17 antibiotics were determined using automatic VITEK®2 equipment with panel card AST-GP577 (VITEK®2 Biomerieux, Francia). Resistance and susceptibility patterns to 17 antimicrobials were determined according to the M100S Standards Institute (2019). The following antibiotics were tested using the respective reference MIC: Amoxicillin/Clavulanic Acid: ≤4/2 µg/mL, Ampicillin: >8 µg/mL, Cefazolin: ≤2 µg/mL, Ciprofloxacin: ≥4 µg/mL, Clindamycin: >4 µg/mL, Erythromycin: >8 µg/mL, Gentamicin: >16 µg/mL, Imipenem: 8 µg/mL, Levofloxacin: ≥4 µg/mL, Linezolid: ≥8 µg/mL, Oxacillin: ≥0.5 µg/mL, Penicillin: ≥0.25 µg/mL, Rifaxmicin: >4 µg/mL, Synercid: > µg/mL, Tetracycline: ≥16 µg/mL, Trimethoprim/Sulfamethoxazole: >4/76 µg/mL, and Vancomycin: ≥32 µg/mL.

Methicillin resistance was evaluated by the disc diffusion method for cefoxitin on Mueller-Hinton agar plates (OXOID Ltd, Basingstoke, Hampshire, England), and with the plate method in Mueller-Hinton agar plates supplemented with 6 µg/mL of oxacillin, according to the standards from the Center for Disease Control and Prevention, USA (Centers for Disease Control and Prevention 2019).
Genome sequencing. Genomic DNA was isolated by using Lysostaphin-Lysozyme modified method of Hookey (Hookey et al. 1999), only modifying the lysis step: Briefly, bacterial cells were resuspended in 250 μl of lysis solution (lysozyme 50mg/ml; lysostaphyn 1 mg/ml in 25 mM Tris-HCl- 10 mM EDTA; all reagents from SIGMA-ALDRICH, St. Louis MO USA), and incubated at 37°C by 90 minutes. The sample was then warmed up at 95°C for 10 minutes and centrifuged at 8 000 x g, after which the sample was processed as indicated in the method.

DNA integrity and purity were assessed by agarose gel electrophoresis and spectrophotometry (NanoDrop 2000, ThermoFisher, Waltam, MA, USA); final concentrations were assessed using the Qubit high sensitivity dsDNA assay (ThermoFisher). Library’s were generated with Nextera XT DNA library preparation kit (Illumina, San Diego, CA, USA), according to the manufacturer's protocol, using 1 ng of DNA. Libraries quality were evaluated on an Agilent 4200 TapeStation system (Santa Clara, CA, USA) and sequenced using a NextSeq 500/550 mid output kit (2x150bp) (Illumina), at an estimated depth of coverage of 100x.

Genome assembly and annotation. Assemblies were made with Spades 3.6.0 in genomes sequenced in high coverage (50x to 100x). Genome annotations were obtained from the PATRIC server (Wattam et al. 2018) (https://www.patricbrc.org/), through the automated bioinformatic method RAST (Rapid Annotation Subsystem Technologies) (Antonopoulos et al. 2017). Gene annotation of antibiotic resistance and virulence-related genes were obtained from the section of Special Genes of PATRIC. Annotations for virulome were supported with other sources of information such as PATRIC VF, VFDB, and Victors. For the prediction of antibiotic resistance genes, the PATRIC server was used to search specialty genes using the databases CARD (Comprehensive Antibiotic Resistance Database) and NDARO (National Database of Antibiotic Resistant Organism) (Davis et al. 2016).

Genome comparisons and pangenome modeling. The Average Nucleotide Identity by MUMmer (ANIm) and the Genomic Coverage (G_{cov}) was calculated with the JSpecies program, with MUMmer used as a pairwise comparison tool for pairs of SE genomes (Richter et al. 2016). The pangenome was modeled using the GET_HOMOLOGUES package and its default values (Contreras-Moreira & Vinuesa 2013). Briefly, groups of orthologous proteins were computed using the Ortho-MCL integrated into GET_HOMOLOGUES. Accessory genes (unique genes and genes present in at least in two genomes but not in all genomes), were obtained from the cloud, shell, and soft-core pangenome components according to GET_HOMOLOGUES (Contreras-Moreira & Vinuesa 2013). The distribution of accessory genes in SE genomes was illustrated with the heatmap.2 function of the R’s ggplot2 package.

Phylogeny. A consensus core genome calculated from the pangenome model of 29 SE genomes was obtained with GET_HOMOLOGUES (Contreras-Moreira & Vinuesa 2013). The resulting 1575 core protein clusters were subject to multiple alignments with MUSCLE (Edgar 2004), gaps removed with TrimAl v2.1, and were concatenated using homemade Perl scripts (Capella-Gutierrez & Gabaldon 2013). Phylogenetic trees were constructed by the maximum likelihood (ML) method based on the substitution matrix of JTT (Jones, Taylor, Thornton), with 1000 bootstrap replicates, using RaxML program (Stamatakis 2015). To draw and edit the phylogenetic trees, we used the iTool program (Letunic & Bork 2019).
Recombination. The inference of recombination was performed with ClonalFrameML (Didelot & Wilson 2015). First, we ran GET-HOMOLOGUES to obtain the common protein clusters encoded by the genomes of each set of SE strains (Contreras-Moreira & Vinuesa 2013). Second, they were converted to nucleotide sequences and concatenated using homemade Perl scripts. Multiple alignments were made as described above for phylogeny construction. Third, a RAxML (Stamatakis 2015) tree was done to obtain the Newick format and the transition/transversion parameter \( \kappa \) for running ClonalFrameML under default parameters (Didelot & Wilson 2015). Z-score statistics were obtained for all the sets of SE genomes and p-values using the web application Z Score Calculator (https://www.socscistatistics.com/tests/ztest/zscorecalculator.aspx). BoxPlots were performed with the R ggplot2 system.

Mobile elements identification. Prophages were identified with PHAST (Arndt et al. 2017). Only predictions ranked as “intact prophages” were considered for analysis. IS, CRISPR-Cas elements and spacer sequences were obtained from PATRIC annotations (Antonopoulos et al. 2017). Then, IS were classified into families by BLASTx comparison with the ISfinder (Siguier et al. 2006). The identity of the spacers within the CRISPR-Cas elements, was determined by BLASTn comparisons with the NCBI virus database. Only identical matches with a phage sequence in the database were recorded (Paez-Espino et al. 2016).

Identification and classification of SCCmec and ACME proteins. All the predicted proteins that compose the 12 types of SCCmec and the six ACME types were downloaded from GenBank (Table S1) and compiled in a local protein database (SE-17db). Then, all encoded proteins of the 17 SE genomes were used as a query in BlastP similarity searches with the SE-17db. Best-blast hits with a minimum similarity of 80% and 60% of coverage over the length of the smaller protein were taken as evidence of homology with the respective SCCmec or ACME type. To classify the SCCmec types, we used the phylogenies of the recombinases ccrA, B, and C, performed according to the method described above. ACME types were classified by the presence/absence of the genes of the operons arg, opp, ars, and kpd according to a reference table (Table S2).

GenBank accession numbers. S. epidermidis of the INPer collection used in this work were uploaded in GenBank with the following Biosample identifiers: SAMN11086744, SAMN11086745, SAMN11086746, SAMN11086747, SAMN11086748, SAMN11086749, SAMN11086750, SAMN11086751, SAMN11086752, SAMN11086753, SAMN11086754, SAMN11086755, SAMN11086756, SAMN11086757, SAMN11086758, SAMN11086759, SAMN11086760. The accession numbers for the genomes of reference S. epidermidis strains are listed in Table S3.

Results

Survey of antibiotic multidrug-resistant intrahospital staphylococci in eight years period. We studied the incidence of staphylococci species and strains multi-resistant to antibiotics in the INPer children hospital from 2006 to 2013. A total of 822 staphylococci strains were recovered from distinct infection sites of newborns and adults. Staphylococcus species were identified using the VITEK®2 system and standard clinical methods, including tests for biofilm formation, coagulase reaction, presence of coa and mecA gene, and resistance to 17 antibiotics (see
methods). The analysis showed that SE strains were the most abundant (573 strains), followed by S. aureus (146 strains), and other coagulase-negative Staphylococcus (CoNS) species in low proportion (81 strains) (Fig. S1). The SE group was composed mainly of strains recovered from blood samples and catheters (Fig. S1).

The number of antibiotic multi-resistant SE strains per year showed similar profiles through the studied period (Fig. 1, A-H). Methicillin resistance was found in about 80% of the SE isolates. SE strains multi-resistant to nine antibiotics were the most frequently found throughout the eight years. In contrast, S. aureus strains showed resistance to about four antibiotics as the most common profile (Fig. S2). Similarly, the resistance to each antibiotic remained without change, during the eight-year study (Fig. 1, I-P). These results suggest the persistence of a multidrug-resistant clone or few clones of SE at the hospital during the eight years, or frequent horizontal exchange of genes encoding antibiotic resistance between SE clones. To study these alternatives, we choose one or more SE strains per year to totalize 17 clinical SE coagulase-negative (CoNS) strains (Fig. 1). These particular strains came from nosocomial infections of 14 newborns and three adults, isolated from three different infection sites: blood (8 strains), catheters (7 strains), cerebrospinal fluid (1 strain), and soft tissue (1 strain) (Table 1).

**Broad gene catalog from draft genomes.** We obtained the whole genome sequence of 17 nosocomial SE strains (see Material & Methods). After testing different parameters with the assembler programs Velvet and Spades (Bankevich et al. 2012; Zerbino 2010), we got draft genomes assemblies consisting of about 92 up to 432 contigs with a 60-70x average sequence covverture per genome (Table S4). To assert that the 17 assemblies represent a substantial part of the SE genomes, we compared the total genome length, and the number and length of the predicted ORFs, with 11 complete genomes of SE downloaded from GenBank (Table S5). There were no differences between the genome length of the SE INPer genomes and the complete genomes from the GenBank (unpaired t-test = 2.33; p-value = 0.022), in ORFs number (unpaired t-test = -1.68; p-value = 0.117), or ORFs length (unpaired t-test = 2.60; p-value = 0.014) (Table S5). Therefore, we can conclude that the SE INPer genome sequences obtained here provide a broad catalog of genes per genome useful for comparative genomics.

**Genomic, pangenomic, and phylogenetic relationships among SE isolates.** To define the genomic similarity between the 17 clinical SE strains, we performed whole-genome pairwise nucleotide identity estimates (Average Nucleotide Identity by Mummer, ANIm) (Richter et al. 2016). The 17 SE showed high genomic ANIm values of about 99%, covering more than 90% of the genome length (Fig. S3). One exception is strain S10, which showed ANIm values of about 97% concerning the rest of the strains.

Besides the genomic identity between SE isolates, we wanted to investigate the extent of their genetic variability by performing a pangenome model. To this end, the SE collection was complemented by the inclusion of 12 complete genomes of SE strains downloaded from GenBank (Table S3, NCBI reference complete genomes). The model obtained using the GET_HOMOLOGUES software package (Contreras-Moreira & Vinuesa 2013), indicated an open pangenome (Fig. S4). The core genome component for the 29 SE strains was predicted to consist of about 1575 gene clusters, whereas the sum of genes unevenly distributed in the 29 SE genomes (accessory component) contains 4360 gene clusters.
To know the phylogenetic relationship of the SE from INPer in the context of reference SE strains, we did an un-rooted ML phylogenetic tree using the predicted 1575 concatenated core proteins (Fig. 2). There were three clades separated by the largest branches in the tree that comprise most of the SE local strains, and one or more SE strains isolated worldwide (Fig. 2, clades B, C, D). The clade marked as D in the tree consisted of two groups, one of which includes only reference SE strains, whereas the other had most of the SE strains of the collections studied. Strain S10, the most different strain by ANIm in the SE collection, was grouped in the clade A with the commensal strains SE ATCC12228 (2) and SE 14.1.R1 isolated from USA and Denmark.

The distribution of accessory genes in individual SE strains coincided with the phylogenetic clades. The SE strains grouped in three phylogenetic clades are closely related by their similitude in the gene presence/absence profile (Fig. 3). Despite the high identity of the SE strains, there is still considerable individual variation that may account for adaptations to local milieu.

**Clonal structure.** To investigate to which clonal ST complex the SE strains belong, we looked for the seven proteins of the S. epidermidis MLST scheme, and compared them with their respective alleles in the Staphylococcus epidermidis MLST database (https://pubmlst.org/sepidermidis/; Table 1; see methods) (Feil et al. 2004; Thomas et al. 2007). The analysis showed a total of 8 different STs; seven of them already recorded in the database. The S10 strain had an unassigned ST in the database and only differed by a single amino acid substitution in the YqiL protein (L370C). The ST2, ST5, and ST23 are worldwide distributed and are the most represented in our sample (Lee et al. 2018; Miragaia et al. 2007). In agreement with global data, ST35, ST59, ST81, ST89 are less frequently represented. The clonal relationships among STs determined by eBURST, indicate that founder clones are ST2 and ST5, whereas the other four STs (ST 59, 81, 89 and 23), are peripheral clones mostly related to each than to the primary founder clones (Fig. S5).

**Virulence genes.** Several known virulence genes of SE are shared by commensal and pathogenic strains (Otto 2009). Among them, we found the cluster icaADBCR (biofilm formation) in nine out of the 17 SE genomes analyzed, except for icaC, absent in strain S05 (Table 1). The phenol-soluble modulins (PSMs), involved in inflammatory response and lysis of leukocytes were present in the genomes of all SE strains (Peschel & Otto 2013). The novel ESAT 6 (esaAB, essABC, and esxAB genes) secretion system implicated in immune system evasion and neutrophil elimination was only present in S10 strain (Burts et al. 2005; Dai et al. 2017; Wang et al. 2016).

Frequently, SE isolates contain the genomic island ACME, composed by arginine deaminase catabolic (arg) and an oligopeptide permease operons (opp) (Miragaia et al. 2009). ACME has been associated with successful adaptation to the skin and mucosal surfaces outcompeting other related bacteria (Planet et al. 2013). The diversity of ACME has been organized in six distinct types according to their gene composition (Table S2 and references therein). We looked for the presence of ACME genes in the 17 SE genomes of INPer collection. By means of BlastP comparisons we identified genes belonging to the ACME Type I (arg+ opp+) in genomes of SE strains S03, S17, and S21, and the ACME Type V that contain the arg+ opp+ and ars+ (arsenate resistance operon), and kpd+, a potassium transporting operon in S24 (Centers for Disease...
Control and Prevention 2019; O'Connor et al. 2018) (Table S2). Even though some other SE strains present genes of the arg and ars operon, they lacked the genes of the opp operon; therefore, they were not assigned to ACME known types.

Antibiotic resistance genotype and phenotype. The SE strains were tested for their susceptibility to methicillin and other β-lactams antibiotics as described in methods. All the 17 SE strains have the β-lactamase gene (blaZ) and their regulators (blaR and I), which are probably responsible for the broad resistance spectrum to penicillin, carbapenems, and cephalosporins determined by the VITEK®2 system (Table 2). Methicillin resistance was evaluated by the disc diffusion method for cefoxitin and corroborated by the presence of mecA in the genomes (Table 1 and Table 2). 12 out of 17 SE strains showed agreement between the cefoxitin resistance phenotype and the mecA genotype. Even thought strains S03, S05, S14, S15, and S21, have the mecA gene in the genome, they were scored as susceptible for cefoxitin (disc diffusion ≥ 25 millimeters). To support that the 15 mecA positive strains are Methicillin-Resistance SE (MRSE) strains, we evaluated their resistance to oxacillin. Commonly, the Methicillin-Resistance S. aureus (MRSA) strains are evaluated by the resistance to oxacillin (Centers for Disease Control and Prevention 2019). In the SE collection, only the S07 strain was susceptible to oxacillin (6 μg/ml) in plate assays, despite the detection of mecA in the genome. Altogether, these results indicate that most of the SE strains (15) studied here are MRSE, identified by the presence of the mecA gene; any phenotypic inconsistencies could be due to lack of expression of the mecA, heteroresistance, or the limitation of the current antibiotic susceptibility testing methods (Band et al. 2019; Harrison et al. 2019; Nicoloff et al. 2019).

The gene mecA encodes for a penicillin-binding protein (PBP) carried in a mobile element known as Staphylococcal Chromosomal Cassette or SCCmec (International Working Group on the Classification of Staphylococcal Cassette Chromosome 2009). By localizing the recombinases ccrA, B, and C, as well the mecA genes in the contigs of the respective genomes, and then constructing phylogenies including known SCCmec recombinase genes, we classified the SCCmec types of the SE INPer strains (Table 1; Fig. S6). The analysis demonstrated the presence of the community-acquired SCCmec type IV cassette in 13 out of 14 methicillin-resistant strains. The SE INPer strains S10 and S16 lack the SCCmec cassette, and no mecA gene was detected. Although the S21 strain has a mecA gene, we were unable to find other gene elements to show the presence of a mec cassette. Moreover, strains S07, S09, S13 strains contain an additional SCCmec type VIII cassette in tandem with the SCCmec IV cassette. The S07 strain carries a contig of 36 535 bps of the SCCmec type IV and VIII, suggesting the probable structure of the recombined cassette (Fig. S7).

The genomic analysis revealed that some SE strains studied here included genes for resistance to fluoroquinolones, macrolides, sulfonamides, aminoglycosides, tetracycline, and other antibiotics not used as the first choice in clinical therapy (Table 2). The results given in Table 2 corroborate that in most of the cases, the probable gene responsible for the resistance is present in the genomes. Besides, non-synonymous mutations in the antibiotic target proteins GyrA and RpoB were identified in some SE strains resistant to quinolones and rifampicin. Despite other SE strains lack these mutations, they still were resistant to these antibiotics. Then, other mutations in the antibiotic target protein or other genetic mechanisms not yet known would be responsible for these resistances.
**Mobile genetic elements.** The genomic variability observed in the SE strains suggests active processes of recombination and gene exchange. To study this concern, we first look for prophages and CRISPR-Cas related systems in the genomes. Prophage footprints were found in 10 out of 17 genomes of the SE strains. The most significant prophage hits detected by the PHAST program, were for genomic regions spanning about 28 to 95 kb that include an attachment site, a signature of lysogenic phages (Arndt et al. 2017) (Table S6). In this analysis, prophages were found integrated into the genomes of some SE strains, such as CNPH82 found in the SE strains S14, S17, and S18 (Daniel et al. 2007). Some other prophages such as SttB20 and SpBeta were present in S03 and S16 strains respectively and, the prophages IPLA5 in strain S07 and IPLA7 in S12 and S15 strains (Gutierrez et al. 2012). In the remaining SE strains, prophages sequences were not detected.

SE strains have also acquired defense mechanisms against phage infection. The search for CRISPR-Cas immune systems identified nine out of the 17 SE INPer strains carrying a CRISPR-Cas Type III system. The system III is composed by the Cas1 and Cas2, responsible for spacer processing and insertion; the ribonuclease Cas6, and the cascade proteins Csm1 to Csm6, involved in the processing of the target transcript (Table S7). The CRISPR-Cas Type III system has been already reported to confer immunity to phages as well as to conjugative plasmids in SE (Marraffini 2015; Marraffini & Sontheimer 2008). Although the enzymatic organization of the CRISPR-Cas systems is remarkably conserved in SE, there are variations in the array of repeats and spacers in the CRISPR loci. Three distinct types of identical repeated units of 30 or 36 nucleotides, associated with specific sequence spacers have been described (Marraffini 2015). Three spacers that correspond to the CRISPR loci found in the strains S02, S05, and S24, match precisely with a sequence in the Staphylococcus phage PH15 genome for the first two strains and the Staphylococcus phage 6ec genome for the last (Aswani et al. 2014; Daniel et al. 2007).

SE strains harbor many ISs, belonging to different families (Table S8). The presence of IS256 has been found within pathogenic SE, associated with biofilm formation and virulence (Kozitskaya et al. 2004; Murugesan et al. 2018). Among the SE strains of our collection, the isolates having the IS256 also contain the ica operon, confirming previous observations. Exceptionally, only the strain S21 has the ica genes but lacks IS256.

**Recombination.** The above results suggest that high frequency of Horizontal Gene Transfer (HGT) and recombination might promote diversification of local SE populations. To evaluate this, we measured the ratio of recombination to mutation (r/m), using the ClonalFrameML program (Didelot & Wilson 2015) in the 17 INPer SE genomes and 16 combined sets of SE genomes downloaded from GenBank (Fig. 2; Table S3). A median average r/m rate of about 6.9 was calculated when the 17 SE were tested, suggesting that nucleotide substitutions by recombination are more frequent than random point mutations (Vos & Didelot 2009). Every COG class shows r/m values equal or higher than the estimate for the complete set of 17 SE genomes. Indeed, the r/m values on virulence or antibiotic resistance gene class result similar to the other COGs involved in housekeeping functions.

To determine whether or not the recombination estimates were affected by the sample composition of SE strains, we design several control tests, with distinct groups of genomes. First, we discarded the most divergent S10 strain of the SE collection and ran the ClonalFrameML test
only with the 16 SE most related SE strains of the collection. As shown in Figure 4, the r/m rate for this set reduced to a median of four, and this value is not significantly different respect to the r/m of SE of the complete genome of SE strains obtained from the GenBank (z-score = 1.4, P-value 0.135) (Fig. 4, boxplot 25). Second, we computed the r/m rate in eight different sets randomly selected among 260 complete and draft genomes of SE strains from the GenBank (Fig. 4, 26-33; Table S3). Some sets (Ctr2 and 8) display the lowest r/m values, whereas the rest control sets have r/m upper than two to four. These results indicate that the strains sample composition influence the recombination estimates.

The RaxML nucleotide phylogenetic tree used as a reference to estimate recombination looks similar to the core protein phylogeny presented in Fig. 2; SE strains within clades maintain cohesive relationships (Fig. 5). However, multiple recombination events were detected in the ancestral nodes leading to the SE strains. The most prominent branch (red dot line in Fig. 5) divided the SE strains into two large clades, one including the clade D and the other constituted by clade B and C. Within different divergent lineages recombination introduces much more nucleotide variants than mutation as presented before (r/m). These results indicate that local hospital settings SE strains may contain enough genomic diversity despite their close relationship with the main clonal ST complexes of worldwide distribution.

Discussion

SE is among the most common bacterial isolates found in the human skin microbiome (Byrd et al. 2018). It is also frequently recovered from bacteremia and sepsis samples in neonatal care clinic units, being its most probable etiological-agent (Byrd et al. 2018; Otto 2009). In clinical practice, it is difficult to assess if SE clones are the causal agents of the disease, are accidental, or opportunistic pathogens (Miragaia et al. 2008; Otto 2017). In this work, we conducted a survey at the Instituto Nacional de Perinatología “INPer” in México City, over a period spanning eight years, to register changes in the antibiotic profile of staphylococci species and strains. We focus on SE because it was the bacteria most frequently found among the staphylococci isolates and showed a remarkable multi-resistance pattern. The number of antibiotic resistances remained very similar year by year with most SE strains being multi-resistant to nine antibiotics; a minor representation of low-resistant strains (< 5 antibiotics) was found. Therefore, the SE population within the INPer hospital was highly stable and led us to question its genetic composition. Specifically, we address the hypothesis that a single or few clones are the basis of the normalization of all the SE multi-resistant strains in the children’s health care unit studied.

We analyzed the genomes of 17 selected SE strains isolated mostly from neonatal patients. Our findings regarding virulence genetic determinants are concordant with those found in SE isolates from hospitals and commensal strains isolated worldwide. The most prominent features of these isolates are their ability to form biofilms, the presence of PMSs, and the multdrug resistance profile displayed (Otto 2014; Xu et al. 2018). Employing a phylogenetic strategy using the ccrA, B, C recombinases, we conclude that most of the INPer SE strains carry the SCCmec type IV. Surprisingly, eight strains of the SE collection analyzed here, harbor neither the ica operon nor the IS256, considered pathogenicity markers in SE (Kozitskaya et al. 2004; Murugesan et al. 2018). Likely, these are commensal SE strains that invaded the patients in the course of their hospital stay even though we cannot discard they use other pathogenicity mechanisms. These strains showed resistance to multiple antibiotics, and three of them contain a composite SCCmec
cassette formed by type IV and VIII gene elements. We propose a probable structure of the combined SCCmec IV and VIII cassette (Figure S7). It is an unusual combination of SCC elements, but there are reports in the literature of mosaic chromosomal staphylococcal mec cassettes (Heusser et al. 2007). However, this SCCmec genetic element should be subject to further corroboration. Classification of SCCmec is still hard to discern due to the variability and presence of repeated elements which difficult the correct assembly of the region (International Working Group on the Classification of Staphylococcal Cassette Chromosome 2009). The chromosomal cassettes might be hot spots of recombination of heavy metal resistance genes, insertion sequences, and antibiotic resistance genes recruited by HGT (Xue et al. 2017).

The genomic antibiotic resistance spectrum of SE strains is very diverse, with some genes present in most strains and others only in few. Examples of diversification of the resistance mechanisms are the presence of membrane efflux pumps (NorAB), which may confer resistance to quinolones as well, and several modifying enzymes (AAC, APH) that inactivate aminoglycosides such as kanamycin. Indeed, the fosB gene, which encodes the resistance to fosfomycin was found in 9 out of 17 SE strains. In several of these instances, the resistance phenotype coincided with the presence of one or more genes, encoding modifying or degrading enzymes and mutant protein targets for some antibiotics (Table 2).

The genome analysis also indicates some mechanisms for antibiotic resistance, including nonsynonymous substitutions in the housekeeping genes gyrA and rpoB. In the gyrase (gyrA) it was found the amino acid S84F change which confers quinolone resistance, whereas, in rpoB (β-subunit of the RNA polymerase), a double amino acid substitution D471E: I527M, and a single I527M were identified. The double mutant rpoB D471E: I527M has been recognized elsewhere as the most common cause of worldwide rifampicin resistance (Lee et al., 2018). Indeed, the presence of this rpoB variant reduces the susceptibility to vancomycin and teicoplanin. In this work, the rpoB double mutant was detected in S02, S05, and S08 all belonging to ST23 clonal type, while the single mutant I527M was observed in the strains S14, S17, S18, and S19, which are within the ST2 clonal type. Both are the clonal lineages worldwide distributed reported by Lee et al., (2019). These INPer strains can form biofilms and contain most of the virulence determinants (Table 1). Therefore, they are very adapted SE strains and continuously present in the INPer.

In the phylogenetic trees reported, pathogenic SE strains are intermingled with commensal SE strains with no pathogenic cluster found (Meric et al. 2018; Miragaia et al. 2005). Recently, Meric et al. suggested, that pathogenic SE subpopulations occur within the commensal SE strains, which contain genes and alleles necessary for colonization at distinct infection sites (Meric et al. 2018). These Genome-wide-association (GWAS) studies showed the enrichment of several genes involved with methicillin resistance, biofilm formation, cell toxicity, and inflammatory response in pathogenic SE isolates (Meric et al. 2018). Therefore, pathogenic and commensal SE strains likely coexist in the same infection site, but current clinical methods of isolation prevent us from distinguishing one from the other. Eight ica out of 17 strains analyzed, could not form biofilms even thought they were recovered from ill patients (Table 1).

Furthermore, here are various footprints of MGEs, including prophages, ISs, and the phage immunity CRISPR-Cas systems in the SE genomes that likely contribute to the adaptability by
the acquisition of virulence and antibiotic resistance factors. As expected, due to its mobile nature, these elements do not follow a uniform distribution in the phylogeny, indicating frequent genetic exchange in the SE population. Together with HGT, homologous recombination may be a factor for genetic diversification of SE in hospital settings. In our work, extensive genome analysis of the rates of recombination versus mutation suggests that recombination affects the whole genome and not only a particular class of genes. It has been estimated that recombination could involve 40% of the genome of SE, whereas in S. aureus recombination comprises the 24% portion (Meric et al., 2015). Although recombination rates depend strongly on the sample of strains used for the analysis, the estimated r/m values reported agrees with other recombination test performed with distinct samples of SE, few or whole-genome markers as well as reported r/m numbers in the literature (Meric et al. 2015; Miragaia et al. 2005). Therefore we can conclude that the SE population despite its whole low level of nucleotide variation (ANIm > 97%) shows cohesive clonal behavior but frequent gene exchange and recombination.

Conclusions

At local hospital settings, pathogenic, and commensal SE strains coexist, but it is hard to discern if they are contaminants, commensal colonizers, or virulent strains (Widerstrom et al. 2016). Indeed, single-colony testing for the identification of SE isolates limits to know the extent of multiclonal or multi-species infections (Harris et al. 2016; Van Eldere et al. 2000). In the present study, some analyzed SE strains came from nosocomial patients, but lack ica genes, a classical virulence marker. However, we cannot exclude that these putative commensal SE strains are pathogens in a non-determined manner, or in other conditions. Likely they are part of the intra-hospital non-pathogenic microbiome. These presumed SE commensal strains, as well as the biofilm formers considered pathogenic SE strains are multi-resistant to antibiotics. The results present here of an 8-year survey, suggest that the multi-resistance to antibiotics might drive adaptation and persistence of specific SE clones in hospital settings. HGT and recombination might play a crucial role in the origin of the pathogenic clones, by moving and recombing antibiotic resistance and virulence genes in distinct genomic clonal backgrounds, including non-pathogenic strains. Therefore, the clinical and genetic factors that influence the adaptability stability and change of SE community overtime should be addressed in detail in future studies.

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References

Antonopoulos DA, Assaf R, Aziz RK, Brettin T, Bun C, Conrad N, Davis JJ, Dietrich EM, Disz T, Gerdes S, Kenyon RW, Machi D, Mao C, Murphy-Olson DE, Nordberg EK, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Santerre J, Shukla M, Stevens RL, VanOeffelen M, Vonstein V, Warren AS, Wattam AR, Xia F, and Yoo H. 2017. PATRIC as a unique resource for studying antimicrobial resistance. Brief Bioinform. 10.1093/bib/bbx083
Arndt D, Marcu A, Liang Y, and Wishart DS. 2017. PHAST, PHASTER and PHASTEST: Tools for finding prophage in bacterial genomes. Brief Bioinform. 10.1093/bib/bbx121

Aswani VH, Tremblay DM, Moineau S, and Shukla SK. 2014. Complete Genome Sequence of a Staphylococcus epidermidis Bacteriophage Isolated from the Anterior Nares of Humans. Genome Announc 2. 10.1128/genomeA.00549-14

Band VI, Hufnagel DA, Jaggavarapu S, Sherman EX, Wozniak JE, Satola SW, Farley MM, Jacob JT, Burd EM, and Weiss DS. 2019. Antibiotic combinations that exploit heteroresistance to multiple drugs effectively control infection. Nat Microbiol 4:1627-1635. 10.1038/s41564-019-0480-z

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, and Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455-477. 10.1089/cmb.2012.0021

Barbier F, Lebeaux D, Hernandez D, Delannoy AS, Caro V, Francois P, Schrenzel J, Ruppe E, Gaillard K, Wolff M, Brisse S, Andremont A, and Ruimy R. 2011. High prevalence of the arginine catabolic mobile element in carriage isolates of methicillin-resistant Staphylococcus epidermidis. J Antimicrob Chemother 66:29-36. 10.1093/jac/dkq410

Ben Zakour NL, Guinane CM, and Fitzgerald JR. 2008. Pathogenomics of the staphylococci: insights into niche adaptation and the emergence of new virulent strains. FEMS Microbiol Lett 289:1-12. 10.1111/j.1574-6968.2008.01384.x

Bosi E, Monk JM, Aziz RK, Fonzi M, Nizet V, and Palsson BO. 2016. Comparative genome-scale modelling of Staphylococcus aureus strains identifies strain-specific metabolic capabilities linked to pathogenicity. Proc Natl Acad Sci U S A 113:E3801-3809. 10.1073/pnas.1523199113

Bouchami O, de Lencastre H, and Miragaia M. 2016. Impact of Insertion Sequences and Recombination on the Population Structure of Staphylococcus haemolyticus. PLoS One 11:e0156653. 10.1371/journal.pone.0156653

Burts ML, Williams WA, DeBord K, and Missiakas DM. 2005. EsxA and EsxB are secreted by an ESAT-6-like system that is required for the pathogenesis of Staphylococcus aureus infections. Proc Natl Acad Sci U S A 102:1169-1174. 10.1073/pnas.0405620102

Byrd AL, Belkaid Y, and Segre JA. 2018. The human skin microbiome. Nat Rev Microbiol 16:143-155. 10.1038/nrmicro.2017.157

Capella-Gutierrez S, and Gabaldon T. 2013. Measuring guide-tree dependency of inferred gaps in progressive aligners. Bioinformatics 29:1011-1017. 10.1093/bioinformatics/btt095

Centers for Disease Control and Prevention C. 2019. Methicillin-resistant Staphylococcus aureus (MRSA). Available at https://www.cdc.gov/mrsa/lab/index.html - anchor _1548429322.

Chessa D, Ganau G, and Mazzarello V. 2015. An overview of Staphylococcus epidermidis and Staphylococcus aureus with a focus on developing countries. J Infect Dev Ctries 9:547-550. 10.3855/jidc.6923

Clinical and Laboratory Standards Institute C. 2019. Performance standards for antibiotic susceptibility testing CLSI approved standard M100. Available at http://em100.edaptivedocs.net/GetDoc.aspx?doc=CLSI M100 ED29:2019&scope=user2019).

Conlan S, Mijares LA, Program NCS, Becker J, Blakesley RW, Bouffard GG, Brooks S, Coleman H, Gupta J, Gurson N, Park M, Schmidt B, Thomas PJ, Otto M, Kong HH,
Murray PR, and Segre JA. 2012. Staphylococcus epidermidis pan-genome sequence analysis reveals diversity of skin commensal and hospital infection-associated isolates. *Genome Biol* 13:R64. 10.1186/gb-2012-13-7-r64

Contreras-Moreira B, and Vinuesa P. 2013. GET_HOMOLOGUES, a versatile software package for scalable and robust microbial pangenome analysis. *Appl Environ Microbiol* 79:7696-7701. 10.1128/AEM.02411-13

Dai Y, Wang Y, Liu Q, Gao Q, Lu H, Meng H, Qin J, Hu M, and Li M. 2017. A Novel ESAT-6 Secretion System-Secreted Protein EsxX of Community-Associated Staphylococcus aureus Lineage ST398 Contributes to Immune Evasion and Virulence. *Front Microbiol* 8:819. 10.3389/fmicb.2017.00819

Daniel A, Bonnen PE, and Fischetti VA. 2007. First complete genome sequence of two Staphylococcus epidermidis bacteriophages. *J Bacteriol* 189:2086-2100. 10.1128/JB.01637-06

Davis JJ, Boisvert S, Brettin T, Kenyon RW, Mao C, Olson R, Overbeek R, Santerre J, Shukla M, Wattam AR, Will R, Xia F, and Stevens R. 2016. Antimicrobial Resistance Prediction in PATRIC and RAST. *Sci Rep* 6:27930. 10.1038/srep27930

Didelot X, and Wilson DJ. 2015. ClonalFrameML: efficient inference of recombination in whole bacterial genomes. *PLoS Comput Biol* 11:e1004041. 10.1371/journal.pcbi.1004041

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792-1797. 10.1093/nar/gkh340

Feil EJ, Li BC, Aanensen DM, Hanage WP, and Spratt BG. 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J Bacteriol* 186:1518-1530.

Forbes BA, and Schaberg DR. 1983. Transfer of resistance plasmids from Staphylococcus epidermidis to Staphylococcus aureus: evidence for conjugative exchange of resistance. *J Bacteriol* 153:627-634.

Gu J, Li H, Li M, Vuong C, Otto M, Wen Y, and Gao Q. 2005. Bacterial insertion sequence IS256 as a potential molecular marker to discriminate invasive strains from commensal strains of Staphylococcus epidermidis. *J Hosp Infect* 61:342-348. 10.1016/j.jhin.2005.04.017

Gutierrez D, Martinez B, Rodriguez A, and Garcia P. 2012. Genomic characterization of two Staphylococcus epidermidis bacteriophages with anti-biofilm potential. *BMC Genomics* 13:228. 10.1186/1471-2164-13-228

Harris LG, Murray S, Pascoe B, Bray J, Mercier G, Mageiros L, Wilkinson TS, Jeeves R, Rohde H, Schwarz S, de Lencastre H, Miragaia M, Rolo J, Bowden R, Jolley KA, Maiden MC, Mack D, and Sheppard SK. 2016. Biofilm Morphotypes and Population Structure among Staphylococcus epidermidis from Commensal and Clinical Samples. *PLoS One* 11:e0151240. 10.1371/journal.pone.0151240

Harrison EM, Ba X, Coll F, Blane B, Restif O, Carvell H, Koser CU, Jamrozy D, Reuter S, Lovering A, Gleadall N, Bellis KL, Uhlemann AC, Lowy FD, Massey RC, Grilo IR, Sobral R, Larsen J, Rhod Larsen A, Vingsbo Lundberg C, Parkhill J, Paterson GK, Holden MTG, Peacock SJ, and Holmes MA. 2019. Genomic identification of cryptic susceptibility to penicillins and beta-lactamase inhibitors in methicillin-resistant Staphylococcus aureus. *Nat Microbiol* 4:1680-1691. 10.1038/s41564-019-0471-0
Heusser R, Ender M, Berger-Bachi B, and McCallum N. 2007. Mosaic staphylococcal cassette chromosome mec containing two recombinase loci and a new mec complex, B2. *Antimicrob Agents Chemother* 51:390-393. 10.1128/AAC.00921-06

Hookey JV, Edwards V, Cookson BD, and Richardson JF. 1999. PCR-RFLP analysis of the coagulase gene of *Staphylococcus aureus*: application to the differentiation of epidemic and sporadic methicillin-resistant strains. *J Hosp Infect* 42:205-212. 10.1053/jhin.1999.0595

International Working Group on the Classification of Staphylococcal Cassette Chromosome E. 2009. Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. *Antimicrob Agents Chemother* 53:4961-4967. 10.1128/AAC.00579-09

Kozitskaya S, Cho SH, Dietrich K, Marre R, Naber K, and Ziebuhr W. 2004. The bacterial insertion sequence element IS256 occurs preferentially in nosocomial *Staphylococcus epidermidis* isolates: association with biofilm formation and resistance to aminoglycosides. *Infect Immun* 72:1210-1215.

Kozitskaya S, Olson ME, Fey PD, Witte W, Ohlsen K, and Ziebuhr W. 2005. Clonal analysis of *Staphylococcus epidermidis* epidermidis isolates carrying or lacking biofilm-mediating genes by multilocus sequence typing. *J Clin Microbiol* 43:4751-4757. 10.1128/JCM.43.9.4751-4757.2005

Lee JYH, Monk IR, Goncalves da Silva A, Seemann T, Chua KYL, Kearns A, Hill R, Woodford N, Bartels MD, Strommenger B, Laurent F, Dodemont M, Deplano A, Patel R, Larsen AR, Korman TM, Stinear TP, and Howden BP. 2018. Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis*. *Nat Microbiol* 3:1175-1185. 10.1038/s41564-018-0230-7

Letunic I, and Bork P. 2019. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res*. 10.1093/nar/gkz239

Marchant EA, Boyce GK, Sadarangani M, and Lavoie PM. 2013. Neonatal sepsis due to coagulase-negative staphylococci. *Clin Dev Immunol* 2013:586076. 10.1155/2013/586076

Marraffini LA. 2015. CRISPR-Cas immunity in prokaryotes. *Nature* 526:55-61. 10.1038/nature15386

Marraffini LA, and Sontheimer EJ. 2008. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. *Science* 322:1843-1845. 10.1126/science.1165771

Martinez-Melendez A, Morfin-Otero R, Villarreal-Trevino L, Camacho-Ortiz A, Gonzalez-Gonzalez G, Llaca-Diaz J, Rodriguez-Noriega E, and Garza-Gonzalez E. 2016. Molecular epidemiology of coagulase-negative bloodstream isolates: detection of *Staphylococcus epidermidis* ST2, ST7 and linezolid-resistant ST23. *Braz J Infect Dis* 20:419-428. 10.1016/j.bjid.2016.05.007

Meric G, Mageiros L, Pensar J, Laabei M, Yahara K, Pascoe B, Kittiwian N, Tadee P, Post V, Lamble S, Bowden R, Bray JE, Morgenstern M, Jolley KA, Maiden MCJ, Feil EJ, Didelot X, Miragaia M, de Lencastre H, Moriarty TF, Rohde H, Massey R, Mack D, Corander J, and Sheppard SK. 2018. Disease-associated genotypes of the commensal skin bacterium *Staphylococcus epidermidis*. *Nat Commun* 9:5034. 10.1038/s41467-018-07368-7

Meric G, Miragaia M, de Been M, Yahara K, Pascoe B, Mageiros L, Mikhail J, Harris LG, Wilkinson TS, Rolo J, Lamble S, Bray JE, Jolley KA, Hanage WP, Bowden R, Maiden...
MC, Mack D, de Lencastre H, Feil EJ, Corander J, and Sheppard SK. 2015. Ecological Overlap and Horizontal Gene Transfer in Staphylococcus aureus and Staphylococcus epidermidis. *Genome Biol Evol* 7:1313-1328. 10.1093/gbe/evv066

Miragaia M. 2018. Factors Contributing to the Evolution of mecA-Mediated beta-lactam Resistance in Staphylococci: Update and New Insights From Whole Genome Sequencing (WGS). *Front Microbiol* 9:2723. 10.3389/fmicb.2018.02723

Miragaia M, Carrico JA, Thomas JC, Couto I, Enright MC, and de Lencastre H. 2008. Comparison of molecular typing methods for characterization of Staphylococcus epidermidis: proposal for clone definition. *J Clin Microbiol* 46:118-129. 10.1128/JCM.01685-07

Miragaia M, Couto I, and de Lencastre H. 2005. Genetic diversity among methicillin-resistant Staphylococcus epidermidis (MRSE). *Microb Drug Resist* 11:83-93. 10.1089/mdr.2005.11.83

Miragaia M, Couto I, Pereira SF, Kristinsson KG, Westh H, Jarlov JO, Carrico J, Almeida J, Santos-Sanches I, and de Lencastre H. 2002. Molecular characterization of methicillin-resistant Staphylococcus epidermidis clones: evidence of geographic dissemination. *J Clin Microbiol* 40:430-438.

Miragaia M, de Lencastre H, Perdreau-Remington F, Chambers HF, Higashi J, Sullam PM, Lin J, Wong KI, King KA, Otto M, Sensabaugh GF, and Diep BA. 2009. Genetic diversity of arginine catabolic mobile element in Staphylococcus epidermidis. *PLoS One* 4:e7722. 10.1371/journal.pone.0007722

Miragaia M, Thomas JC, Couto I, Enright MC, and de Lencastre H. 2007. Inferring a population structure for Staphylococcus epidermidis from multilocus sequence typing data. *J Bacteriol* 189:2540-2552. 10.1128/JB.01484-06

Murugesan S, Mani S, Kuppusamy I, and Krishnan P. 2018. Role of insertion sequence element is256 as a virulence marker and its association with biofilm formation among methicillin-resistant Staphylococcus epidermidis from hospital and community settings in Chennai, South India. *Indian J Med Microbiol* 36:124-126. 10.4103/ijmm.IJMM_17_276

Nicoloff H, Hjort K, Levin BR, and Andersson DI. 2019. The high prevalence of antibiotic heteroresistance in pathogenic bacteria is mainly caused by gene amplification. *Nat Microbiol* 4:504-514. 10.1038/s41564-018-0342-0

O’Connor AM, McManus BA, and Coleman DC. 2018. First description of novel arginine catabolic mobile elements (ACMEs) types IV and V harboring a kdp operon in Staphylococcus epidermidis characterized by whole genome sequencing. *Infect Genet Evol* 61:60-66. 10.1016/j.meegid.2018.03.012

Onishi M, Urushibara N, Kawaguchiya M, Ghosh S, Shinagawa M, Watanabe N, and Kobayashi N. 2013. Prevalence and genetic diversity of arginine catabolic mobile element (ACME) in clinical isolates of coagulase-negative staphylococci: identification of ACME type I variants in Staphylococcus epidermidis. *Infect Genet Evol* 20:381-388. 10.1016/j.meegid.2013.09.018

Otto M. 2009. Staphylococcus epidermidis—the 'accidental' pathogen. *Nat Rev Microbiol* 7:555-567. 10.1038/nrmicro2182

Otto M. 2014. Phenol-soluble modulins. *Int J Med Microbiol* 304:164-169. 10.1016/j.ijmm.2013.11.019

Otto M. 2017. Staphylococcus epidermidis: a major player in bacterial sepsis? *Future Microbiol* 12:1031-1033. 10.2217/fmb-2017-0143
Paez-Espino D, Elof-Fadrosh EA, Pavlopoulos GA, Thomas AD, Huntemann M, Mikhailova N, Rubin E, Ivanova NN, and Kyrpides NC. 2016. Uncovering Earth's virome. *Nature* 536:425-430. 10.1038/nature19094

Peschel A, and Otto M. 2013. Phenol-soluble modulins and staphylococcal infection. *Nat Rev Microbiol* 11:667-673. 10.1038/nrmicro3110

Planet PJ, LaRusca SJ, Dana A, Smith H, Xu A, Ryan C, Uhlmann AC, Boundy S, Goldberg J, Narechania A, Kulkarni R, Ratner AJ, Geoghegan JA, Kolokotronis SO, and Prince A. 2013. Emergence of the epidemic methicillin-resistant Staphylococcus aureus strain USA300 coincides with horizontal transfer of the arginine catabolic mobile element and speG-mediated adaptations for survival on skin. *MBio* 4:e00889-00813. 10.1128/mBio.00889-13

Richter M, Rossello-Mora R, Oliver Glocner F, and Peplies J. 2016. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32:929-931. 10.1093/bioinformatics/btv681

Rolo J, de Lencastre H, and Miragaia M. 2012. Strategies of adaptation of *Staphylococcus epidermidis* to hospital and community: amplification and diversification of SCCmec. *J Antimicrob Chemother* 67:1333-1341. 10.1093/jac/dks068

Rolo J, Worning P, Nielsen JB, Bowden R, Bouchami O, Damborg P, Guardabassi L, Perreten V, Tomasz A, Westh H, de Lencastre H, and Miragaia M. 2017. Evolutionary Origin of the *Staphylococcal Cassette Chromosome Chromosome mec (SCCmec)*. *Antimicrob Agents Chemother* 61. 10.1128/AAC.02302-16

Siguier P, Perochon J, Lestrade L, Mahillon J, and Chandler M. 2006. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* 34:D32-36. 10.1093/nar/gkj014

Stamatakis A. 2015. Using RAxML to Infer Phylogenies. *Curr Protoc Bioinformatics* 51:6 14

11-14. 10.1002/0471250953.bi0614s51

Thomas JC, Vargas MR, Miragaia M, Peacock SJ, Archer GL, and Enright MC. 2007. Improved multilocus sequence typing scheme for *Staphylococcus epidermidis*. *J Clin Microbiol* 45:616-619. 10.1128/JCM.01934-06

Van Eldere J, Peetermans WE, Struelens M, Deplano A, and Bobbaers H. 2000. Polyclonal *Staphylococcal endocarditis* caused by genetic variability. *Clin Infect Dis* 31:24-30. 10.1086/313915

Villari P, Sarnataro C, and Iacuzio L. 2000. Molecular epidemiology of *Staphylococcus epidermidis* in a neonatal intensive care unit over a three-year period. *J Clin Microbiol* 38:1740-1746.

Vos M, and Didelot X. 2009. A comparison of homologous recombination rates in bacteria and archaea. *ISME J* 3:199-208. 10.1038/ismej.2008.93

Wang Y, Hu M, Liu Q, Qin J, Dai Y, He L, Li T, Zheng B, Zhou F, Yu K, Fang J, Liu X, Otto M, and Li M. 2016. Role of the ESAT-6 secretion system in virulence of the emerging community-associated *Staphylococcus aureus* lineage ST398. *Sci Rep* 6:25163. 10.1038/srep25163

Wattam AR, Brettin T, Davis JJ, Gerdes S, Kenyon R, Machi D, Mao C, Olson R, Overbeek R, Pusch GD, Shukla MP, Stevens R, Vonstein V, Warren A, Xia F, and Yoo H. 2018. Assembly, Annotation, and Comparative Genomics in PATRIC, the All Bacterial Bioinformatics Resource Center. *Methods Mol Biol* 1704:79-101. 10.1007/978-1-4939-7463-4_4
Widerstrom M, McCullough CA, Coombs GW, Monsen T, and Christiansen KJ. 2012. A multidrug-resistant Staphylococcus epidermidis clone (ST2) is an ongoing cause of hospital-acquired infection in a Western Australian hospital. *J Clin Microbiol* 50:2147-2151. 10.1128/JCM.06456-11

Widerstrom M, Wistrom J, Edebro H, Marklund E, Backman M, Lindqvist P, and Monsen T. 2016. Colonization of patients, healthcare workers, and the environment with healthcare-associated Staphylococcus epidermidis genotypes in an intensive care unit: a prospective observational cohort study. *BMC Infect Dis* 16:743. 10.1186/s12879-016-2094-x

Xu Z, Misra R, Jamrozy D, Paterson GK, Cutler RR, Holmes MA, Gharbia S, and Mkrtchyan HV. 2018. Whole Genome Sequence and Comparative Genomics Analysis of Multi-drug Resistant Environmental Staphylococcus epidermidis ST59. *G3 (Bethesda)* 8:2225-2230. 10.1534/g3.118.200314

Xue H, Wu Z, Qiao D, Tong C, and Zhao X. 2017. Global acquisition of genetic material from different bacteria into the staphylococcal cassette chromosome elements of a Staphylococcus epidermidis isolate. *Int J Antimicrob Agents* 50:581-587. 10.1016/j.ijantimicag.2017.06.015

Zerbino DR. 2010. Using the Velvet de novo assembler for short-read sequencing technologies. *Curr Protoc Bioinformatics* Chapter 11:Unit 11 15. 10.1002/0471250953.bi1105s31

Ziebuhr W, Krimmer V, Rachid S, Lossner I, Gotz F, and Hacker J. 1999a. A novel mechanism of phase variation of virulence in Staphylococcus epidermidis: evidence for control of the polysaccharide intercellular adhesin synthesis by alternating insertion and excision of the insertion sequence element IS256. *Mol Microbiol* 32:345-356. 10.1046/j.1365-2958.1999.01353.x

Ziebuhr W, Ohlsen K, Karch H, Korhonen T, and Hacker J. 1999b. Evolution of bacterial pathogenesis. *Cell Mol Life Sci* 56:719-728.
Figure 1

Profiles of *S. epidermidis* antibiotic multi-resistance in an eight years period

A-H, percentage of SE strains respect the count of antibiotic resistances by year. I-O, number of strains resistant to each one of twelve antibiotics by year. Percentage of *mecA* strains are showed in the last columns of each plot. The SE strains selected for genome analysis are indicated over the bars.
Figure 2

Phylogenetic relationships of *S. epidermidis* from the Children’s Hospital(INPer) and *S. epidermidis* selected from GenBank.

The ML tree consists of four main clades defined by the longest branches (A to D). They are indicated with color dots: red, clade A; blue, clade B; green, clade C; yellow, clade D. The tree was constructed with 1575 common core proteins using RaxML program as described in methods. The results of bootstrap performed with 1000 replicates are indicated in the branches. Acronyms specify the isolation site of the SE strains: MEX, Mexico; USA, United States of America; FIN, Finland; GER, Germany; DEN, Denmark; IRE, Ireland; CHI, China; AUS, Australia. SE INPer strains are denoted in red. Asterisks indicate ica strains.
Figure 3

Distribution of accessory genes in the *S. epidermidis* genomes.

The heat-map profile was performed with the ggplot2 function in R using the Bray-Curtiss dissimilarity matrix. The Bray-Curtiss dendrogram is indicated at the left. The heat-map at the middle indicates gene presence (blue color); empty cells represent the absence of genes. *S. epidermidis* strains are shown at the left with the same colors of the clades in the core proteins phylogeny (Fig. 1): red, clade A; blue, clade B; green, clade C; yellow, clade D. In the last column, the presence/absence of genomic regions similar to known prophages (Table S5) is indicated.
Figure 4

Rates of recombination/mutation for *S. epidermidis* INPer strains compared with sets of *S. epidermidis* from GenBank.

1. Sixteen strains out 17 *S. epidermidis* of the INPer collection; 2. Seventeen *S. epidermidis* strains of the INPer collection. 3-22, *r/m* rates for the genes encoding proteins classified in COGs in the 17 *SE* INPer: 3. COG C (energy production). 4. COG D (cell division). 5. COG E (amino acid transport and metabolism); 6. COG F (nucleotide transport and metabolism). 7. COG G (carbohydrate transport and metabolism). 8. COG H (coenzyme transport and metabolism). 9. COG I (lipid transport and metabolism). 10. COG J (translation, ribosomal structure and biogenesis). 11. COG K (transcription). 12. COG L (replication, recombination and repair). 13. COG M (cell wall, membrane, envelope biogenesis). 14. COG O (post-translational modification, protein turnover and chaperones). 15. COG P (inorganic ion transport and metabolism). 16. COG Q (secondary metabolites biosynthesis, transport and catabolism). 17. COG R (general function predicted). 18. COG S (function unknown). 19. COG T (signal transduction mechanisms). 20. COG U (intracellular trafficking, secretion and vesicular transport). 21. COG V (defense mechanism). 22. Unassigned COGs. 23. Antibiotic resistance genes predicted in PATRIC server for the *SE* INPer strains. 24. Virulence genes predicted in PATRIC server for the *S. epidermidis* INPer strains. 25. Reference set of 12 complete genomes of *S. epidermidis* strains used through this work. 26-33, subsets of draft *SE* strains from GenBank: 26. Ctr1 (n =36). 27. Ctr2 (n =26). 28. Ctr3 (n =22). 29. Ctr4 (n =35). 30. Ctr-5 (n =36). 31. Ctr6 (n =26). 32. Ctr7 (n =36). 33. Ctr8 (n =36). Descriptions of the *SE* strains included in the control sets and their GenBank accession numbers are in Table S3.
Figure 5

Genome-wide recombination between *S. epidermidis* strains.

ClonalFrameML program detected several events of recombination along 1,354,455 concatenated genomic regions of 29 *S. epidermidis* genomes (17 INPer genomes and 12 GenBank genomes). A. RaxML nucleotide tree is shown at the left of the scheme. Color dots indicate the corresponding clades in the protein phylogeny (Fig. 1). B. Blue bars indicate recombination events along the concatenated genome segments. White bars indicate non-homoplasic nucleotide substitutions; yellow to red bars are probable homoplasic nucleotide substitutions (Didelot and Wilson, 2015). Red dots line indicate an ancestral event of recombination.
**Table 1** (on next page)

General features of the genomes of *S. epidermidis* strains.

a S05 lack of *icaC*; b N prefix indicates strains isolated from neonates; A, isolates from adults; CSF, cerebrospinal fluid; STA, soft tissue aspirate. c U-ST, unassigned ST d ST profile numbers correspond to alleles of ArcC, AroE, Gtr, MutS, TpiA, and YqiL, according to Thomas et al. (2007). e Parentheses indicate the number of complete IS256. NA, indicate the presence of a *ccrC* gene but not assigned to any SCCmec type. SCCmec classification was performed by phylogenetic clustering of the recombinases *ccrA, ccrB*, and *ccrC* with the recombinases present in the SCCmec types.
| Strain a | Origin b | ST c | ST profile d | ica | CRISPR-Cas | Prophages | IS256 e | mecA | ccrA | ccrB | ccrC |
|----------|----------|------|--------------|-----|-----------|-----------|--------|------|------|------|------|
| S02      | N-Catheter | 23   | 7,1,2,1,3,3,1 | +   | 1         | –         | 1 (1)  | +    | IV   | –    | IV   |
| S03      | N-Catheter | 89   | 1,1,2,1,2,1,1 | –   | 0         | +         | 1 (1)  | +    | IV   | –    | IV   |
| S05      | N-Catheter | 23   | 7,1,2,1,3,3,1 | +   | 1         | –         | 1 (1)  | +    | IV   | –    | IV   |
| S07      | A-STA     | 59   | 2,1,1,1,2,1,1 | –   | 0         | +         | –      | +    | IV   | VIII | IV   |
| S08      | N-Blood   | 23   | 7,1,2,1,3,3,1 | +   | 1         | –         | 1 (1)  | +    | IV   | –    | IV   |
| S09      | A-Blood   | 59   | 2,1,1,1,2,1,1 | –   | 1         | –         | –      | +    | IV   | VIII | IV   |
| S10      | A-Blood   | U-ST | 65,48,5,5,8,5,11 | –   | 0         | –         | –      | –    | –    | –    | –    |
| S12      | N-Blood   | 5    | 1,1,2,2,1,1,1 | –   | 3         | +         | 1 (1)  | +    | IV   | –    | IV   |
| S13      | N-Blood   | 81   | 2,17,1,1,2,1,1 | –   | 1         | –         | 1 (0)  | +    | IV   | VIII | IV   |
| S14      | N-Blood   | 2    | 7,1,2,2,4,1,1 | +   | 0         | +         | 2 (1)  | +    | IV   | –    | IV   |
| S15      | N-Catheter | 5    | 1,1,2,2,1,1,1 | –   | 0         | +         | 1 (1)  | +    | IV   | –    | IV   |
| S16      | N-Blood   | 2    | 7,1,2,2,4,1,1 | +   | 0         | +         | 1 (1)  | –    | –    | –    | –    |
| S17      | N-Catheter | 2    | 7,1,2,2,4,1,1 | +   | 0         | –         | 2 (1)  | +    | IV   | –    | IV   |
| S18      | N-Blood   | 2    | 7,1,2,2,4,1,1 | +   | 0         | +         | 2 (1)  | +    | IV   | –    | IV   |
| S19      | N-Catheter | 2    | 7,1,2,2,4,1,1 | +   | 0         | –         | 2 (1)  | +    | IV   | –    | IV   |
| S21      | N-Catheter | 35   | 2,1,2,2,4,1,1 | +   | 1         | –         | –      | +    | NA   | NA   | NA   |
| S24      | N-CSF     | 5    | 1,1,1,2,2,1,1 | –   | 3         | +         | –      | +    | IV   | –    | IV   |

1 Adapted from Table S1, showing the presence of ccrA, mecA, and prophages in various strains.
Table 2 (on next page)

Antibiotic resistance phenotypes and the genotype profile in S. epidermidis strains

Resistant strains are indicated by gray cells (+). Susceptible are in blue cells (-). Strains not tested are indicated as empty cells. Parentheses in the Genotype column indicates the number of strains that present the most probable gene(s) involved in antibiotic resistance. In \textit{gyrA} and \textit{rpoB} the corresponding amino acid substitutions in the protein are indicated. The S10 and S16 strains are denoted as \textit{mecA} by and asterisk. Cefoxitin and Oxacillin antibiotics used for phenotypic characterization of methicillin resistance are marked by a rectangle in the first column. Cefoxitin was evaluated by the disc-diffusion method, considering growth inhibition zones of $\geq 25$ mm as susceptible, and $\leq 24$ mm, resistant (Center for Disease Control and Prevention, CDC 2019).
| Antibiotic       | Class         | S02 | S03 | S05 | S07 | S08 | S09 | S10* | S12 | S13 | S14 | S15 | S16* | S17 | S18 | S19 | S21 | S24 | Genotype                        |
|------------------|---------------|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|--------------------------|
| Ampicillin       | Penicillin    | +   | +   | +   | +   | +   | +   | +    | +   | +   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Penicillin       | Penicillin    | +   | +   | +   | +   | +   | +   | +    | +   | +   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Amoxicillin      | Penicillin    | +   | +   | +   | +   | +   | +   | +    | +   | +   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Oxacillin        | Penicillin    | +   | +   | +   | –   | +   | +   | –    | +   | +   | +   | +   | –    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Cefoxitin        | Penicillin    | –   | +   | –   | +   | +   | +   | –    | –   | –   | +   | +   | –    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Imipenem         | Carbapenem    | +   | +   | +   | +   | +   | +   | +    | +   | +   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Cefazolin        | Cefalosporin  | +   | +   | +   | +   | +   | +   | +    | +   | +   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Ciprofloxacin    | Quinolone     | +   | –   | +   | –   | +   | +   | –    | +   | +   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Levofoxacin      | Quinolone     | +   | –   | +   | –   | –   | +   | –    | +   | +   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Clindamycin      | Lyncosamide   | +   | +   | +   | +   | +   | +   | +    | +   | +   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Tetracycline     | Tetraccline   | –   | +   | –   | +   | –   | +   | –    | –   | –   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Gentamycin       | Aminoglycoside| +   | +   | +   | +   | +   | +   | +    | +   | +   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Trimethoprim/SMX | Sulfonanima    | +   | +   | +   | +   | +   | +   | –    | +   | +   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Chloramphenicol  | Phenicol      | +   | –   | +   | –   | +   | +   | –    | +   | +   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Rifampicin       | Miscellaneous | +   | +   | +   | +   | +   | +   | +    | +   | +   | +   | +   | +    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |
| Vancomycin       | Glycopeptide  | –   | –   | –   | –   | –   | –   | –    | –   | –   | –   | –   | –    | S06 | S07 | S08 | S09 | S10 | blaZ (17), mecA (15)      |

Genes probably involved:
- **blaZ (17)**, mecA (15)
- gyrA S84F (10)
- mphB (3), ermC (11)
- linA (1), ermC (11)
- tetL (4)
- aac/aph (15), aph3 (2), ant9 (3), aadD (11)
- sul3 (17)
- catB (6)
- rpoB D471E, I527M (3); I527M (4)
- vanRI (17)