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Characterization of Ocular Methicillin-Resistant *Staphylococcus epidermidis* Isolates Belonging Predominantly to Clonal Complex 2 Subcluster II

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***Staphylococcus epidermidis* is an abundant member of the microbiota of the human skin and wet mucosa, which is commonly associated with sight-threatening infections in eyes with predisposing factors. Ocular *S. epidermidis* has become notorious because of its capability to form biofilms on different ocular devices and due to the evolving rates of antimicrobial resistance. In this study, the molecular epidemiology of 30 ocular methicillin-resistant *S. epidermidis* (MRSE) isolates was assessed using multilocus sequence typing (MLST). Antimicrobial resistance, accessory gene-regulator and staphylococcal cassette chromosome *mec* (SCC*mec*) types, biofilm formation, and the occurrence of biofilm-associated genes were correlated with MLST clonal complexes. Sequence types (STs) frequently found in the hospital setting were rarely found in our collection. Overall, 12 different STs were detected with a predominance of ST59 (30%), ST5 and ST6 (13.3% each). Most of the isolates (93.3%) belonged to the clonal complex 2 (CC2) and grouped mainly within subcluster CC2-II (92.9%). Isolates grouped within this subcluster were frequently biofilm producers (92.3%) with a higher occurrence of the *aap* (84.5%) and *bhp* (46.1%) genes compared to *ica*A (19.2%). SCC*mec* type IV (53.8%) was predominant within CC2-II strains, while 38.4% were nontypeable. In addition, CC2-II strains were frequently multidrug resistant (80.7%) and demonstrated to be particularly resistant to ciprofloxacin (80.8%), ofloxacin (77%), azithromycin (61.5%), and gentamicin (57.7%). Our findings demonstrate the predominance of a particular MRSE cluster causing ocular infections, which was associated with high rates of antimicrobial resistance and particularly the carriage of biofilm-related genes coding for proteinaceous factors implicated in biofilm accumulation.**

Staphylococcus epidermidis is the main colonizer of the human skin and wet mucosa, where it maintains a benign relationship with the host. Although found as a common constituent of the ocular surface microbiota (1), *S. epidermidis* is the leading cause of bacterial endophthalmitis following trauma and intraocular procedures (2, 3) and has been frequently associated with infectious keratitis worldwide in patients with predisposing risk factors, including contact lens wear, trauma, surgery, and ocular surface inflammatory diseases (4, 5). The success of *S. epidermidis* as a pathogen is particularly attributed to its capability to form agglomerations of cells embedded in and protected by an extracellular matrix composed of polysaccharides and/or proteins, known as biofilm, which confers resistance to antibiotic action and host immune defenses (6). Biofilm formation is a process that begins first with adhesion of bacterial cells to abiotic or protein-coated biotic surfaces mediated by numerous surface proteins, followed by an accumulation process mediated by a polysaccharide intercellular adhesin (PIA) encoded by the *icaADBC* locus and also by proteinaceous factors such as Aap (accumulation-associated protein) (7). The biofilm-associated homologue protein, Bhp, is also possibly involved in biofilm accumulation (8). In addition, the association between biofilm formation capability and the emerging resistance to antibiotics used routinely for prophylaxis and treatment of ocular infections (9, 10) increases the success of *S. epidermidis* as an ocular pathogen. Although beta-lactams are not frequently used in ophthalmology, methicillin resistance is a key mechanism of resistance in staphylococci and is significantly associated with higher resistance rates to other non-beta-lactam agents, contributing to the spread and persistence of multidrug-resistant strains in several settings. The rates of methicillin resistance among ocular staphylococci isolates are currently on the rise (11–13). Resistance to methicillin in both *S. aureus* and coagulase-negative staphylococci (CoNS) is conferred by an altered penicillin-binding protein (PBP2a) with reduced affinity for beta-lactam antibiotics (14). PBP2a is encoded by the *mecA* gene, which is carried in the mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*) (15). In addition, SCC*mec* also carries a set of recombinase genes (*ccr*), which are implicated in the recombination events of SCC*mec* with the staphylococcal chromosome (15). Currently, SCC*mec* types are usually determined by a combination of the *ccr* gene complex type and the class of *mec* gene complex (16). To date, 11 SCC*mec* types have been identified among methicillin-resistant *S. aureus* isolates (http://www.sccmec.org/Pages/SCC_TypesEN.html). In CoNS, including *S. epidermidis*, the variability of SCC*mec* regions is greater than in *S. aureus*, with a number of isolates carrying multiple *ccr* types or *ccr/mec* complex combinations not yet described in *S. aureus*. As a result, methicillin-resistant *S. epidermidis* (MRSE) carrying nontypeable SCC*mec* elements by current protocols are frequently found (17).

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Several molecular typing methods have been used to study the molecular epidemiology of *S. epidermidis* (including methicillin-resistant strains), but most of them do not allow the inference of epidemiological relationships in long-term investigations and the population structure (18). Pulsed-field gel electrophoresis (PFGE) is the most discriminatory method for characterizing *S. epidermidis* and has been widely used for studying the dissemination of MRSE clones in hospitals and community (19–22). However, its application has been more restricted to short-term epidemiology for characterization of hospital outbreaks (18, 21). Multilocus sequence typing (MLST) scheme is currently applied for long-term evolutionary analysis and has provided additional insights into the population structure of *S. epidermidis* isolates from hospital and community settings (22, 23). However, despite the importance of *S. epidermidis* as an ocular pathogen and its emerging rates of resistance to antimicrobial agents, no information on the molecular diversity of ocular isolates is available. Therefore, this study was performed to assess the molecular epidemiology of MRSE isolates from keratitis and endophthalmitis using the currently used MLST scheme. Molecular typing results were correlated with antimicrobial resistance, accessory gene-regulator (*agr*) types and SCC*mec* types, and the occurrence of biofilm-associated genes and *in vitro* biofilm formation capability.

MATERIALS AND METHODS

Bacterial isolates. A total of 30 consecutive, nonduplicate MRSE isolates recovered from keratitis ($n = 27$) and endophthalmitis ($n = 03$) cases seen at the Department of Ophthalmology, Federal University of Sao Paulo, in 2009 were included. Isolates stored at −80°C in tryptic soy broth (TSB) with 15% glycerol were inoculated on 5% sheep blood agar before testing. Species-level identification was accomplished by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry with a Microflex mass spectrometer (Bruker Daltonik, Bremen, Germany) using MALDI Biotyper 2.0 software as previously published (24).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed by broth microdilution methods according to the Clinical and Laboratory Standards Institute (CLSI) (25). Quality control was performed by testing *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *P. aeruginosa* ATCC 27853. The interpretative criteria for each antimicrobial agent tested were those published previously as CLSI document M100-S13 (26).

***In vitro* polystyrene adherence.** *In vitro* biofilm formation was determined by the modified microtiter-plate quantitative protocol as previously described (27). *Staphylococcus epidermidis* ATCC 12228 and ATCC 35984 were used as negative and positive controls, respectively. Absorbance was read at 570 nm using a Synergy HT Multi-Mode microplate reader (BioTek Vermont, USA). Each sample and positive and negative (only broth) controls were cultured in triplicate and in three independent experiments. The results of all experiments were averaged.

DNA extraction. DNA extraction was performed using Chelex 100 molecular biology resin (Bio-Rad) as previously published with slight modifications (28). Briefly, isolates from fresh cultures on 5% blood agar were harvested and grown in 5 ml of TSB overnight. An aliquot of 1 ml was centrifuged for 5 min at 13,000 rpm and then washed twice using 1× phosphate-buffered saline. Pellets were resuspended in 300 μl of 10% Chelex 100 resin and incubated for 30 min at 95°C. After centrifugation for 5 min at 13,000 rpm, a 1-μl aliquot of the supernatant was collected, diluted 1:10 and then used in the PCR amplification protocols.

SCC*mec* typing. The SCC*mec* types were determined by a combination of multiplex PCR designed to classify the *mec* complex and *ccr* complex using a previously published protocol (16). For each multiplex PCR assay, reference *S. aureus* strains for SCC*mec* types I (NCTC10442), II (N315), III (85/2082), IV (CA05), and V (WIS) were included. SCC*mec* was considered nontypeable if *mec* and/or *ccr* complex gave no amplification results or if the isolate carried more than one *ccr* or *mec* complex and also if there was an *mec*/*ccr* complex combination not previously described.

Detection of biofilm-related genes and *agr* typing. Amplification of the *icaAD* (29), *aap* (30), and *bhp* genes and the insertion sequence IS*256* (31) were carried out according to previously published protocols. The *agr* locus was typed by a multiplex PCR protocol using specific primers designed to amplify *agr* loci I, II, and III from *S. epidermidis* (32).

MLST. MLST was performed for all MRSE isolates as previously described (33). This MLST scheme is based on the sequencing of internal fragments of seven housekeeping genes, including *arcC*, *aroE*, *gtr*, *mutS*, *pyr*, *tpi*, and *yiqL*. The PCR products were purified (QIAquick PCR purification kit; Qiagen), and both strands were sequenced using the BigDye fluorescent terminator with an ABI 3000 genetic analyzer (Applied Biosystems, CA). The sequences obtained were edited using SeqMan (DNASTAR, Madison, WI) and sequence types (STs) were assigned using the *S. epidermidis* MLST database (http://www.mlst.net). Clonal complexes (CC) were determined using the eBURST algorithm.

RESULTS

Organisms and MLST. Species-level identification for all isolates included in the present study ($n = 30$) was confirmed by MALDI-TOF. Moreover, all isolates were confirmed as MRSE by amplification of the *mecA* gene in a singleplex PCR protocol. The majority of the isolates included were recovered from corneal ulcers of patients with clinically diagnosed bacterial keratitis ($n = 27$), and the others were recovered from vitreous fluids ($n = 3$) of patients with acute endophthalmitis following intravitreal injection, trauma, and one case of keratitis progressing to endophthalmitis (Table 1). Overall, 12 different STs were detected, including the new ST493. The most common STs found in the present study were ST59 ($n = 9$), ST6 ($n = 4$), ST5 ($n = 4$), ST20 ($n = 3$), and ST81 ($n = 3$). Six other STs were found (ST2, ST35, ST57, ST174, ST179, and ST198), each corresponding to one isolate. STs were grouped in CC by using eBURST and separation of subclusters within the same CC was used in the present study as previously proposed (25, 27). The vast majority of STs belonged to CC2 (28/30; 93.3%) in the eBURST analysis, which was mainly comprised of subcluster CC2-II (26/28; 92.9%).

SCC*mec* and *agr* typing. SCC*mec* type IV was the most prevalent (15/30; 50%) and was found mainly for isolates belonging to CC2-II (14/26; 53.8%). The isolate grouped in the CC365 (ST174) carried an SCC*mec* type IV. SCC*mec* type II was detected in two isolates (ST35-CC2-I and ST20-CC2-II) and SCC*mec* type V in one isolate (ST198-CC2-II), while the remaining isolates (12/30; 40%) were nontypeable. Isolates belonging to *agr* type I were the most common (22/30; 73.3%), followed by *agr* type II (6/30; 20%), and 2 isolates were nontypeable. *agr* type III was not detected. All ST5 isolates were *agr* type II, while the remaining isolates clustered within CC2-II carried the *agr* type I locus, with the exception of one ST59 isolate that was *agr* type II. A correlation between SCC*mec* types and *agr* types was not seen, since isolates carrying SCC*mec* type IV were *agr* type I (11/15; 73.3%%) or type II (4/15; 26.6%).

Biofilm formation and biofilm-related genes. The ability for biofilm formation measured by the degree of adherence to polystyrene microtiter plates was found for 93.3% of isolates. Most of the isolates (50%) produced weakly adherent biofilms followed by isolates forming moderately (26.6%) and strongly (16.6%) adherent biofilms (Table 2). Only 20 and 26.6% of isolates carried the

TABLE 1 Molecular typing, virulence, multidrug resistance, and source data for all MRSE isolates included in this study[a]

Sequence type (no. of isolates)	CC or singleton	SCC*mec* and *agr* typing (no. of isolates)		No. of isolates positive for:					No. of MDR isolates[b]	Source(s) (no. of isolates)
		SCC*mec*	*agr*	Biofilm formation	*icaA*	*aap*	*bhp*	IS*256*		
ST59 (9)	2-II	IV (7), NT (2)	I (8), II (1)	8	0	9	8	1	6	Cornea (8), vitreous humor (1)
ST5 (4)	2-II	IV (3), NT (1)	II (4)	4	0	4	4	0	2	Cornea (4)
ST6 (4)	2-II	IV (3), NT (1)	I (4)	3	0	0	0	4	4	Cornea (4)
ST20 (3)	2-II	II (1), NT (2)	I (3)	3	3	3	0	0	2	Cornea (2), vitreous humor (1)
ST81 (3)	2-II	NT (3)	I (3)	3	0	3	0	0	3	Cornea (3)
ST2 (1)	2-I	IV (1)	I (1)	1	1	1	0	1	1	Cornea (1)
ST35 (1)	2-I	II (1)	I (1)	1	1	1	0	0	1	Cornea (1)
ST57 (1)	2-II	IV (1)	I (1)	1	0	1	0	0	1	Cornea (1)
ST174 (1)	365	IV (1)	II (1)	1	0	0	0	0	1	Vitreous humor (1)
ST179 (1)	2-II	NT (1)	I (1)	1	1	1	0	0	1	Cornea (1)
ST198 (1)	2-II	V (1)	NT (1)	1	1	1	0	0	1	Cornea (1)
ST493 (1)	S493	NT	NT	1	1	1	0	0	1	Cornea (1)

[a] Abbreviations: ST, sequence type; CC, clonal complex; S, singleton; NT, nontypeable.
[b] Multidrug-resistant (MDR) isolates are defined as isolates resistant to at least three antimicrobial classes.

IS*256* element and *icaA* gene, respectively, whereas 83.3% were positive for *aap* and 40% for *bhp*. The *aap* gene was widely distributed among the STs found in the present study, being negative only for the ST6 isolates and the single ST174 isolate. The carriage of *bhp* was demonstrated to be dependent on the genetic background, since it was detected only for ST59 (8/9; 88.8%) and ST5 (4/4 100%), which were clustered within CC2-II (Table 1). The presence of the *icaA* gene was also correlated with specific STs. Isolates grouped in CC2-I were positive for *icaA* (2/2), while for the isolates grouped within CC2-II, carriage of *icaA* was positive only for the ST20 (3/3), ST179 (1/1) and ST198 (1/1). The isolate belonging to the new ST493 was also *icaA* positive (Table 1).

Antimicrobial resistance. MIC values and susceptibility profile of all MRSE and CC2-II isolates are shown in Table 3. There was a high rate of multidrug resistance phenotype for all isolates (76.6%) and also among isolates clustered within CC2-II (80.7%). Overall, resistance rates were particularly high for ciprofloxacin (76.7%; MIC_{90} >16 μg/ml), ofloxacin (73.3%; MIC_{90} 16 μg/ml), moxifloxacin (33.3%, plus 30% intermediate; MIC_{90} 2 μg/ml), azithromycin (63.3%; MIC_{90} >32 μg/ml) and gentamicin (53.3%; MIC_{90} >32 μg/ml). Lower resistance rates were found for trimethoprim-sulfamethoxazole (13.8%; MIC_{90} 4 μg/ml), tobramycin (20.1%; MIC_{90} 16 μg/ml), chloramphenicol (26.7%; MIC_{90} 64 μg/ml), and clindamycin (26.6%; MIC_{90} >16 μg/ml). All isolates were susceptible to linezolid (MICs ranging from 0.5 to 2 μg/ml; MIC_{90} 2 μg/ml) and vancomycin (MICs ranging from ≤0.5 to 2 μg/ml; MIC_{90} 2 μg/ml). As the majority of isolates were grouped within CC2-II, resistance rates in this group were similar to the overall profile, as follows: ciprofloxacin (80.7%), ofloxacin (77%), moxifloxacin (33.3%), azithromycin (61.5%), and gentamicin (57.7%) (data not shown).

DISCUSSION

Using an MSLT scheme to characterize the molecular epidemiology of MRSE isolates from ocular infections, we demonstrated that a major subcluster of CC2, subcluster CC2-II, grouped the vast majority of isolates in our collection. Strains belonging to this subcluster possibly represent lineages well adapted and widespread in the community setting (34). The majority of isolates examined in the present study differ from the most common clones isolated from the hospital environment, which are also part of CC2, but are frequently *ica*-positive isolates belonging to ST2 (subcluster CC2-I) (20, 35, 36). Among our isolates, only two strains belonging to subcluster CC2-I were found. Interestingly, only five isolates (19.2%) clustered within subcluster CC2-II (STs 20, 179, and 198) were *ica* positive. Conversely, a higher occurrence of protein-associated biofilm factors such as *aap* (84.6%) and *bhp* (46.1%) was found among CC2-II strains, demonstrating that their genetic background is more suitable for acquisition of *aap* and *bhp* genes than the *ica* locus, except for STs 20, 179, and 198. Almost all CC2-II isolates (92.3%) were able to form biofilm *in vitro*, but only 4 (15.4%) demonstrated a strong phenotype, compatible with biofilms formed by *ica*-positive isolates. The other isolates produced weak or moderate biofilms, compatible with biofilms formed by proteinaceous factors (7). Although the sample size in the present study was small, it seems that distinct MRSE strains belonging to CC2-II use different mechanisms of adaptation in the community setting, and it is tempting to infer

TABLE 2 Frequency of biofilm formation and -associated genes among 30 MRSE isolates according to the clonal complex

Clonal complex (no. of isolates)	No. of isolates (%) with the following degree of biofilm formation:				No. of isolates (%) positive for:			
	Weak	Moderate	Strong	All phenotypes	*icaA*	*aap*	*bhp*	IS*256*
CC2-II (26)	13 (50)	7 (27)	4 (15.4)	24 (92.3)	5 (19.2)	22 (84.6%)	12 (46.1)	5 (19.2)
All isolates (30)	15 (50)	8 (26.6)	5 (16.6)	28 (93.3)	8 (26.6)	25 (83.3)	12 (40)	6 (20)

TABLE 3 Antimicrobial susceptibility profile of all 30 MRSE isolates (76.6% MDR) included in this study[a]

Antimicrobial agent	MIC (μg/ml)			Susceptibility profile (%)[b]		
	Range	MIC_{50}	MIC_{90}	S	I	R
Ciprofloxacin	0.5 to >16	>16	>16	23.3	-	76.7
Ofloxacin	0.25 to >16	8	16	26.7	-	73.3
Moxifloxacin	≤0.12 to >16	1	2	36.7	30.0	33.3
Azithromycin	0.5 to >32	>32	>32	36.7	-	63.3
Tobramycin	≤0.25 to >32	4	16	50.0	29.9	20.1
Gentamicin	≤0.25 to >32	16	>32	43.3	3.4	53.3
Clindamycin	≤0.12 to >16	≤0.12	>32	70.0	3.4	26.6
Chloramphenicol	2 to 64	4	64	73.3	-	26.7
Trimethoprim-sulfamethoxazole	≤0.25 to >32	≤0.25	4	86.2	-	13.8
Linezolid	0.5 to 2	1	2	100	-	-
Vancomycin	≤0.5 to 2	2	2	100	-	-

[a] Multidrug-resistant (MDR) isolates are defined as isolates resistant to at least three antimicrobial classes.
[b] Interpretive criteria as published by Clinical and Laboratory Standards Institute in 2013 (M100-S23): S, susceptible; I, intermediate; and R, resistant.

that MRSE strains lacking the *ica* locus are more well adapted to the ocular surface environment than isolates carrying this locus (since most of our isolates were recovered from keratitis cases). Recently, it has been demonstrated that *ica*-negative isolates were also predominant among *S. epidermidis* recovered from ocular tissues of patients suffering with distinct ocular infections in Mexico (37). Carriage of the *ica* locus by *S. epidermidis* implicates a fitness cost, and isolates lacking *ica* genes are able to outcompete strains carrying this locus (38). Moreover, no association has been demonstrated between PIA expression and colonization, whereas Aap, in addition to its role in biofilm accumulation, has been also demonstrated to be an important surface adhesin for skin colonization by promoting adhesion to corneocytes (39). Thus, the absence of the *ica* locus in the majority of CC2-II isolates compensated by the carriage of the *aap* gene is likely to confer a selective advantage for MRSE in community-associated ocular infections.

The spread of different *S. epidermidis* clones in community and hospital environments is probably an effect of a different selective pressure for each setting. Patients admitted to a hospital may become colonized with more virulent subpopulations of commensal *S. epidermidis* due to different hospital selective pressure (40, 41). Selective pressure for both strong biofilm formation ability and antimicrobial resistance is likely to have a major impact on selecting *S. epidermidis* causing hospital-acquired device-related infections, which can explain the widespread occurrence of multidrug-resistant strains carrying the *ica* locus and IS*256* element isolated from hospitalized patients (20, 35, 36). In addition, the site of infection may play a role in selecting isolates with specific mechanisms of biofilm formation. This hypothesis is supported by a study demonstrating that ST2 isolates from hospitalized patients encompassed exclusively methicillin-resistant, *ica*- and IS*256*-positive strains, which were strongly associated with bacteremia and catheter-related infections and rarely isolated from cerebrospinal fluids (36). On the other hand, although biofilm formation represents a key factor for protection against host immune response and antimicrobial activity, the adaptive power of hospital-associated strains seems to be restricted to this environment, since selective pressure in the community setting may vary according to the group of patients and type of disease. *S. epidermidis* causing ocular infections usually belongs to the patient's periocular microbiota, which is the main source of isolates causing endophthalmitis (42) and possibly keratitis (1). Organisms of the periocular microbiota can reach internal ocular tissues after trauma caused by different conditions. For corneal infections, the most important predisposing risk factors include contact lens wear, surgical and nonsurgical trauma, and the presence of inflammatory ocular surface diseases (4, 5). Organisms that are part of the ocular surface microbiota can access the anterior chamber and vitreous cavity following a breach due to intraocular procedures such as cataract, glaucoma, and other surgeries, intravitreal injections, and trauma caused by plant matter and other objects.

Patients presenting with corneal ulcer are immediately started on eye drop antibiotics, usually a new 8-methoxyfluoroquinolone, including moxifloxacin and gatifloxacin, whereas patients submitted to intraocular procedures are commonly subjected to a 7-day regimen (or more) of topical fluoroquinolone for prophylaxis of endophthalmitis (43, 44). The impact of biofilm formation on ocular infections is well known and undoubtedly plays a major role in the pathogenesis of such infections (45). In fact, most of the isolates included in the present study were positive for at least one biofilm-related gene, especially *aap* and *bhp*, both associated with protein-related mechanism of biofilm maturation. The fact that both the *ica* locus and IS*256* insertion sequence are infrequent among isolates included in the present study, as well as among ocular isolates independently studied (37), reinforces the idea that *S. epidermidis* isolates recovered from ocular tissues are not subjected to a selective pressure for strong biofilm formation, since maturation via intercellular protein adhesins results in weaker biofilms compared to PIA-dependent mechanisms (7). However, since the number of isolates recovered from endophthalmitis in the present study was small, this conclusion could be true for keratitis-associated strains only. Corroborating our results, previous reports have also found a low occurrence of the *ica* locus and IS*256* element among *S. epidermidis* comprising CC2-II (19, 36).

Taking into consideration the widespread use of fluoroquinolone agents for the prevention and treatment of ocular infections as mentioned above, it is more reasonable to associate the patterns of antibiotic use with selection of specific MRSE strains causing ocular infections acquired in the community setting. We found strikingly elevated rates of nonsusceptibility among our MRSE isolates to the fluoroquinolones ciprofloxacin, ofloxacin and moxifloxacin and other topically used antibiotics such as azithromycin and gentamicin. Previous reports have also demonstrated markedly higher rates of resistance among MRSA and methicillin-resistant CoNS (including MRSE) isolates from ocular infections (12, 46–48). In addition, extensive use of fluoroquinolones is a known risk factor associated with increased incidence of MRSA carriage and infections (49, 50). Thus, fluoroquinolone selective pressure is likely to play an important role in the selection of successful MLST strains of MRSE causing ocular infections in a setting with increased use of fluoroquinolones.

The genetic diversity of typeable SCC*mec* elements in our collection was low, and there was a similar distribution of types as found in the community setting (17, 22). Most of the MRSE isolates included in the present study (15/30; 50%) carried a type IV SCC*mec*, while SCC*mec* types II and V were identified in only 2 and 1 isolate, respectively. However, a large number of nontypeable isolates carrying more than one *ccr* allotype were found, corroborating previous findings that MRSE isolates from the community setting exhibit a remarkable diversity of SCC*mec* elements and may represent natural reservoirs for methicillin resistance de-

terminants (17). In addition, as previously demonstrated, antibiotic exposure is associated with increased expression of *ccr* genes, which could result in increased recombination between SCC*mec* elements (51).

Taken together, our findings demonstrate the predominance of a particular MRSE cluster causing ocular infections that is associated with high rates of antimicrobial resistance, particularly to fluoroquinolone agents, and carriage of biofilm-related genes coding for proteinaceous factors implicated in biofilm accumulation. Selection by antibiotic pressure of *ica*-negative multidrug-resistant MRSE strains with survival advantages to outcompete other strains in the ocular environment may contribute to the establishment of strains belonging to CC2-II as successful ocular pathogens.

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