Molecular and Phenotypic Characterization of Ocular Methicillin-Resistant *Staphylococcus epidermidis* Isolates in Taiwan

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Purpose. *Staphylococcus epidermidis*, a commensal, has emerged as an important opportunistic pathogen, particularly methicillin-resistant *S. epidermidis* (MRSE). The mechanism behind this transformation remains unclear. This study aimed to investigate the molecular and phenotypic characteristics of MRSE isolated from healthy conjunctiva and ocular infections.

Methods. We collected MRSE isolates from two groups: healthy conjunctiva from patients undergoing cataract surgeries and ocular infections at our hospital. Genotypic analysis included pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*), and biofilm-related genes (*ica*A, *aap*, and *bhp*). Additionally, phenotypic data on biofilm production and antibiotic susceptibility were recorded.

Results. A total of 86 isolates, including 42 from healthy conjunctiva and 44 from ocular infections, were analyzed. MLST identified 21 sequence types (STs), with ST59 being the most frequent ($n = 33$, 39.5%), followed by ST130 ($n = 10$, 11.6%), ST57 ($n = 6$, 7.0%), and ST2 ($n = 6$, 7.0%). All isolates were categorized in 23 PFGE types, and SCC*mec* IV was the most prevalent SCC*mec* type ($n = 52$, 60.5%). The two sources of isolates exhibited overlapping molecular types and phenotypic traits, although the ocular infection isolates exhibited significantly higher multidrug resistance compared to healthy conjunctiva isolates ($P = 0.032$). When contrasting ST59 with non-ST59, ST59 displayed a significantly higher presence of *aap* (100%) and *bhp* (69.7%) while lacking *ica*A (0%). ST59 also showed lower susceptibility to fluoroquinolones compared to non-ST59 (42.4%–54.5% vs. 75.5%–83.0%; $P < 0.01$).

Conclusions. MRSE isolates from healthy conjunctiva and ocular infections demonstrated a degree of resemblance. Specific strains, notably ST59, exhibited distinctive characterizations.

Keywords: antibiotic resistance, multilocus sequence typing (MLST), SCC*mec* typing, *Staphylococcus epidermidis*, virulence genes

Coagulase-negative *Staphylococcus*, particularly *S. epidermidis*, is a prevalent normal inhabitant of human skin and mucosa. However, it has become an important opportunistic pathogen responsible for many health care–associated infections, particularly in situations involving indwelling medical devices.[1,2] *S. epidermidis* also predominantly colonizes the ocular surface.[3,4] Despite its ubiquitous presence and relatively low virulence—leading to limited attention in the past—*S. epidermidis* has increasingly been recognized as an etiological agent in various ocular infections over recent decades.[5] Indeed, coagulase-negative *Staphylococcus*, including *S. epidermidis*, has been identified as the most commonly isolated pathogen for vision-threatening diseases such as endophthalmitis and keratitis.[6–8]

Several factors have contributed to the establishment of clinically significant *S. epidermidis* isolates. First, the majority of *S. epidermidis* has the ability to produce biofilm, which is believed to be a key characteristics of resistance to antibiotics and host immune defenses.[9] Biofilm formation could be mediated by polysaccharide intercellular adhesin (PIA), encoded in the *ica* operon, and also by proteinaceous factors such as accumulation-associated protein (*aap*), biofilm-associated homolog protein (*bhp*), and quorum-sensing system accessory gene regulator (*agr*) in the process of adhesion, accumulation, maturation, and detachment.[10–12] Second, the genotypes of clinically significant *S. epidermidis* are frequently multidrug resistant.[13] There has been an increasing trend toward laboratory resistance to methicillin in *S. epidermidis* (MRSE).[14] This resistance is conferred by

an altered penicillin-binding protein (PBP2a) with reduced affinity for β-lactam antibiotics.[15] PBP2a is encoded by the *mecA* gene carried by a genetic mobile element referred to as the staphylococcal cassette chromosome *mec* (SCC*mec*).[16] Third, the above phenotypic and genotypic characterization of *S. epidermidis* has a high degree of diversity, which could affect the establishment of the isolates as a pathogen. It is plausible that the virulent pathogenic strains carry discriminant markers setting them apart from the commensal strains. Such factors underline the complexity of *S. epidermidis* virulence, highlighting the necessity for comprehensive studies to decipher the mechanisms behind its pathogenicity.

With recent progress in genome and functional studies, we are now able to study the molecular characterizations of ocular *S. epidermidis* and understand the pathogenicity. However, the involvement of *S. epidermidis* derived from the ocular surface in ocular infections remains unclear. Some studies have indicated that the ocular surface is the source of pathogens for ocular infections, with isolates being genetically undistinguishable from those found in ocular infections; conversely, others studies have suggested that the ocular infection isolates bear different features of genotypes or phenotypes compared to those from ocular surface.[17–22] Furthermore, the epidemiologic patterns of infectious diseases can vary across different geographic regions. However, there remains a scarcity of information regarding ocular *S. epidermidis* isolates in Taiwan. Given the global public health concern about methicillin-resistant *staphylococci*, our study sought to fill this gap. We aimed to investigate the genotypic and phenotypic characteristics of ocular MRSE isolates and compare isolates from healthy conjunctiva and ocular infections.

Methods

Clinical Isolates

MRSE isolates were obtained from 2013 to 2018 at Gang Gung Memorial Hospital (CGMH), Taiwan. For healthy conjunctiva isolates, 42 samples were obtained by swabbing from patients undergoing uneventful cataract surgery. There were 16 males (48.5%) and 17 females (51.5%), ranging in age from 46 to 97 years (mean age, 72.1 years). For ocular infection isolates, 44 non-duplicate MRSE samples were isolated from patients with a variety of ocular infections including conjunctivitis ($n = 7$), corneal ulcers/keratitis ($n = 23$), endophthalmitis ($n = 12$), open globe injury wound infection ($n = 8$), and canaliculitis ($n = 2$). The conjunctivitis, open globe injury, and canaliculitis samples were obtained by swabbing, the corneal ulcer samples were obtained by scraping, and the endophthalmitis samples were obtained from aqueous or vitreous tapping. These patients included 24 males (53.3%) and 21 females (46.7%), ranging in age from 19 to 93 years (mean age, 52.9 years).

S. epidermidis was identified in our hospital based on colony morphology, a coagulase test, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics GmbH, Bremen, Germany), which could identify most clinical coagulase-negative *staphylococci* isolates to the species level.[23] The study protocol was approved by the Institutional Review Board of CGMH (no. 201601705B0) and was conducted in accordance with the tenets of the Declaration of Helsinki.

DNA Extraction

Bacterial cells were grown overnight in tryptic soy broth, harvested by centrifugation, and resuspended in 200 µL of lysis solution (20% sucrose; 10-mM Tris-HCl, pH 8; and 10 µg/mL lysozyme). Cells were incubated at 37°C for 40 minutes, and 200 µL of Winston solution (2% Triton X-100; 1% SDS; 10-nM NaCl; 10-mM Tris base, pH 8.0; and 1-mM EDTA) was added. DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1). DNA was subsequently precipitated with a volume of isopropanol and purified by the addition of two volumes of 70% ethanol, then the DNA was resuspended in sterile distilled water.

Molecular Typing

We used pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) to determine the genomic diversity. PFGE is considered the gold standard for molecular typing of *staphylococci*.[24] PFGE of MRSE genomic DNA digested with SmaI was carried out as previously described.[22] The resulting patterns were analyzed using GelCompar II software. MLST has been established for use in long-term evolutionary research and for investigating the population structure of *S. epidermidis* isolates. In our study, genomic DNAs of the isolates were subjected to MLST according to the procedure described by Thomas et al.[25] Briefly, PCRs were performed to amplify fragments (approximately 450 bp) of seven designed housekeeping genes (loci), including *arcC*, *aroE*, *gtr*, *mutS*, *pyr*, *tpi*, and *yqiL*. The amplified products were purified and sequenced by the BigDye Terminator fluorescence kit (Applied Biosystems, Foster City, CA, USA). We implemented the burst detection algorithm to group the MLST-type data based on the allelic profiles and evolutional relatedness (https://pubmlst.org/mlst).

SCC*mec* typing was performed using the PCR schemes as previously published by Zhang et al.[26] A single PCR was performed for each gene. For isolates in which SCC*mec* could not be typed, classes of the *mec* gene complex and the *ccr* gene complex (*ccrAB1*, *ccrAB2*, *ccrAB3*, and *ccrC1*) were examined by additional PCRs using the primers.[26] SCC*mec* types were assigned based on the *mec* complex classes and the *ccr* gene types according to the criteria set for *S. aureus*.[27]

Virulence Profile

PCR was used to test all isolates for the presence of virulence genes by PCR amplification using the primers.[27] The virulence genes included *ica*A, *aap*, and *bhp*. The PCR products were analyzed on agarose gels.

Biofilm Formation Assay

A semiquantitative determination of biofilm formation was performed in 96-well tissue culture plates based on the method reported by Christensen et al.[28] The O-47 *ica*A read by ELISA was used to determine whether isolates were biofilm positive (optical density [OD] > 0.12) or strongly positive (OD > 0.24).[28] Assays were repeated at least three times, and the mean biofilm absorbance values were used.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility was performed with MRSE for six antibiotics, including cefoxitin, erythromycin, clindamycin, trimethoprim/sulfamethoxazole, teicoplanin, and vancomycin by the disk diffusion method in our microbiology laboratory. Cefoxitin was used instead of oxacillin/methicillin to test for β-lactam resistance. The susceptibility to tobramycin and fluoroquinolones, including ciprofloxacin, levofloxacin, moxifloxacin, and gatifloxacin, that were not included in the antibiotic susceptibility profiles for *S. epidermidis* in our microbiology laboratory was evaluated using the ETEST (BioMerieux SA, Marcy-l'Etoile, France). Isolates showing resistance to three or more classes of antibiotics were defined as being multidrug resistant (MDR).

Statistical Analysis

Statistical analysis was performed using SPSS Statistics 25 (IBM Corp., Chicago, IL, USA). Fisher's exact test or the χ^2 test was used to compare positive rates of virulence-associated genes and the antibiotic susceptibility. Comparisons between two groups were done using the two-sided Student's *t*-test. The significance level was set at $P < 0.05$. We performed principal component analysis (PCA) using Prism 8.4 (GraphPad Software, La Jolla, CA, USA).

Results

Molecular Typing of MRSE

In total, 86 MRSE isolates were distributed among 21 sequence types (STs) analyzed by MLST (Supplementary Table S1). Three isolates were not typeable. Lineage ST59 (n = 33, 39.5%) was the most prevalent type among all isolates, followed by ST130 (n = 10, 11.6%), ST57 (n = 6, 7.0%), and ST2 (n = 6, 7.0%). Figure 1 illustrates the burst analysis using all STs available in the MLST database. The algorithm clustered our STs into two major clonal complexes (CCs) and five singletons (ST23, ST173, ST227, ST490, and ST655). ST2 and ST5 represented the putative founders. The first three prevalent STs, including ST59, ST130, and ST57, belonged to CC5. The pulsotypes of MRSE ocular isolates were quite diverse, as there were 23 types from all isolates. SCC*mec* IV was the most prevalent SCC*mec* type (n = 52, 60.5%) in all of the isolates.

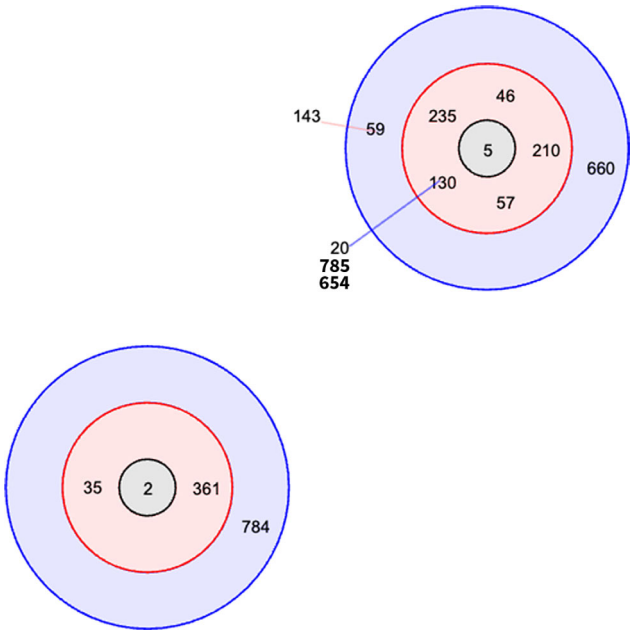

Figure 1. Burst cluster analysis of ocular methicillin-resistant *S. epidermidis* in Taiwan. *Red* indicates single locus variants, and *blue* indicates double locus variants linking the various sequence types (STs).

Comparison of the molecular typing of the MRSE isolates from ocular infection with healthy conjunctiva (Table 1) revealed that there were 10 different STs among the 42 healthy conjunctiva isolates and 17 STs among the 44 ocular infection isolates. The most common molecular types (ST59, ST130, and ST57) were similarly distributed in both groups. Among six isolates of ST2, five (11.9%) were found in conjunctival isolates, but only one isolate (2.3%) was associated with the ocular infections. Regarding pulsotypes, there were 15 different pulsotypes in the healthy conjunctiva and 17 in the ocular infections. SCC*mec* IV was the most prevalent type in both healthy conjunctiva (66.7% of isolates) and ocular infections (54.5% of isolates).

Virulence Profile

All MRSE ocular isolates (except one from ocular infections, ST784) were able to produce biofilm (Table 2), and 90.7% of them were strongly positive for biofilm formation. Among the common identifiable STs, 90.1% of ST59 (30/33), 90% of ST130 (9/10), 83.3% of ST57 (5/6), and 100% of ST2 (6/6) were strongly positive for biofilm formation. The most common biofilm-related gene was *aap*. There were no significant differences in the presence of biofilm-related genes, including *ica*A, *aap*, and *bhp*, between the two sources of isolates. However, when we compared ST59 with non-ST59 isolates (Table 2), ST59 showed a significantly higher presence of *aap* and *bhp* genes than non-ST59 ($P < 0.0001$). In contrast, the presence of the *ica*A gene was 0% in ST59, significantly lower than non-ST59 isolates ($P < 0.0001$). No significant difference in the presence of *ica*A, *aap*, or *bhp* was identified between ST59 from healthy conjunctiva and that from ocular infection isolates (61.1% of conjunctival isolates and 80% of ocular infections for *bhp*; $P = 0.24$). The second common lineage, ST130, had the ability to produce biofilm but did not exhibit any of the biofilm-related genes, including *ica*A, *aap*, and *bhp*. Similarly, neither ST57 nor ST2, the third most common lineages, carried the *bhp* gene (Supplementary Table S2).

Antibiotics Susceptibility

Table 3 shows that all isolates were susceptible to vancomycin and teicoplanin. No significant differences in antibiotic susceptibility were observed between healthy conjunctiva and ocular infection isolates. However, the ocular infection group exhibited a significantly higher percentage of MDR strains compared to the healthy conjunctiva group ($P = 0.032$). When comparing ST59 with non-ST59 isolates, the ST59 lineage demonstrated significantly lower susceptibility than the non-ST59 lineage to levofloxacin, ciprofloxacin, moxifloxacin, and gatifloxacin (all $P < 0.05$). ST130 and ST57, in contrast, were all

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Table 1. Molecular Characteristics of Ocular Methicillin Resistant *Staphylococcus Epidermidis* Isolates From Healthy Conjunctiva and Ocular Infections

Sequence Type (*n*, %)	CC or Singleton	SCC*mec* (Isolates, *n*)	PFGE (Isolates, *n*)
Healthy Conjunctiva (*n* = 42)			
ST59 (18, 42.9)	CC5	IV (14), NT (4)	1 (6), 2 (5), 10 (4), 11 (2), 13 (1)
ST2 (5, 11.9)	CC2	III (1), IV (3), NT (1)	6 (1), 7 (1), 8 (3)
ST130 (5, 11.9)	CC5	IV (5)	6 (4), 14 (1)
ST57 (3, 7.1)	CC5	IV (2), NT (1)	2 (3)
ST35 (3, 7.1)	CC2	NT (3)	9 (3)
Others STs[*] (8, 19)	CC2, CC5, NT	IV (4), V (2), NT (2)	2 (1), 3 (1), 4 (1), 5 (1), 8 (1), 12 (1), 15 (2)
Ocular Infections (*n* = 44)			
ST59 (15, 34.1)	CC5	IV (11), NT (4)	1 (2), 2 (6), 9 (1), 10 (6)
ST130 (5, 10.6)	CC5	IV (3), NT (2)	6 (2), 24 (2), NT (1)
ST57 (3, 6.4)	CC5	IV (3)	2 (2), 10 (1)
ST20 (3, 6.8)	CC20	IV (1), NT (1)	8 (1), 23 (2)
ST173 (3, 6.4)	ST173	NT (3)	5 (3)
ST654 (3, 6.4)	CC20	V (3)	8 (3)
Others STs[†] (12, 27.3)	CC2, CC5, CC20, NT	IV (5), V (2), NT (5)	8 (1), 9 (2), 11 (1), 12 (1), 16 (1), 17 (1), 18 (1), 20 (1), 21 (1), 22 (2)

CC, cluster complex; NT, non-typable; PFGE, pulsed-field gel electrophoresis.
[*] ST5, ST173, ST 227, ST361, ST660 and NT.
[†] ST2, ST5, ST23, ST46, ST143, ST210, ST235, ST490, ST655, ST784, ST785 and NT.

Table 2. Comparison of Biofilm Formation and Biofilm-Associated Genes of MRSE Isolates From Healthy Conjunctiva With Ocular Infection and ST59 With Non-ST59

Isolates	Healthy Conjunctiva (*n* = 42), *n* (%)	Ocular Infection (*n* = 44), *n* (%)	*P*	ST59 (*n* = 33), *n* (%)	Non-ST59 (*n* = 53), *n* (%)	*P*
Biofilm formation						
Positive (OD > 0.12)	42 (100)	43 (97.7)	0.326	33 (100)	52 (98.1)	0.384
Strongly positive (OD > 0.24)	41 (97.6)	37 (84.1)	0.031	30 (90.9)	48 (90.6)	0.958
Virulence genes						
*ica*A	7 (16.7)	12 (27.3)	0.236	0 (0)	19 (35.8)	<0.0001
aap	32 (76.2)	31 (70.5)	0.548	33 (100)	30 (56.6)	<0.0001
bhp	12 (28.6)	13 (29.5)	0.921	23 (69.7)	2 (3.8)	<0.0001

susceptible to the four fluoroquinolones (Supplementary Table S2, Fig. 2).

Relationship Between Virulence Profile and Antibiotics Susceptibility

The presence of the biofilm-related gene *aap* was correlated to the resistance to tobramycin in all isolates ($P = 0.012$). However, there was no significant correlation between the presence of *ica*A, *aap*, and *bhp* genes and the susceptibility to four fluoroquinolones, either in the overall sets of isolate or specifically within ST59 isolates.

Principal Component Analysis

PCA analysis did not exhibit a clear segregation of isolates from healthy conjunctiva and those from ocular infection groups (Fig. 3).

Discussion

In this study, we conducted a genetic and phenotypic analysis of MRSE isolates from both healthy conjunctiva and ocular infections at a tertiary center in Taiwan. This investigation aimed to discern whether the strains colonizing the conjunctiva could potentially act as endogenous reservoirs for clinical infections. Our findings revealed similarities in both molecular and phenotypic characteristics between MRSE isolates derived from healthy conjunctiva and those from ocular infections. Over 98% of the ocular MRSE isolates were capable of producing biofilm, irrespective of their sequence types. Specific strains, including ST59, ST130, and ST57, presented in both healthy conjunctiva and ocular infection isolates, suggesting a potential transition of these strains from being commensals to acting as opportunistic pathogens. ST59, in particular, manifested distinct molecular and phenotypic characterizations.

S. epidermidis is recognized as an important opportunistic pathogen,[29] capable of colonizing human skin or ocular surface and causing invasive infections. Several studies have been conducted to evaluate the factors that contribute to its infectious pathogenicity. Duggirala et al.[30] differentiated *S. epidermidis* isolates from ocular infections, including endophthalmitis and keratitis, and control groups based on the biofilm-forming capability and cluster analysis with fluorescence-amplified fragment length polymorphism. Flores-Páez et al.[19] also reported that the *S. epidermidis* strains causing ocular infections, including blepharitis, conjunctivitis, keratitis, and endophthalmitis, were different from the commensal strains. They found that the ST2 lineage was the most frequent in ocular infection isolates (48.4%), whereas the ST5 lineage (24.4%) was most abundant in healthy conjunctiva isolates. They could be identified

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Table 3. Comparison of Antibiotic Susceptibility Profiles of MRSE Isolates From Healthy Conjunctiva With Ocular Infection and ST59 With Non-ST59

	Susceptibility					
Antibiotic	**Healthy Conjunctiva (n = 42), n (%)**	**Ocular Infection (n = 44), n (%)**	***P***	**ST59 (n = 33), n (%)**	**Non-ST59 (n = 53), n (%)**	***P***
Levofloxacin	29 (69)	25 (56.8)	0.241	14 (42.4)	40 (75.5)	0.002
Ciprofloxacin	29 (69)	25 (56.8)	0.241	14 (42.4)	40 (75.5)	0.002
Moxifloxacin	32 (76.2)	30 (68.2)	0.408	18 (54.5)	44 (83.0)	0.004
Gatifloxacin	29 (69)	25 (56.8)	0.241	14 (42.4)	40 (75.5)	0.002
Tobramycin	11 (26.2)	14 (31.8)	0.566	10 (30.3)	15 (28.3)	0.842
Clindamycin	35 (83.3)	33 (75)	0.342	28 (84.8)	40 (75.5)	0.300
Erythromycin	27 (64.3)	19 (43.2)	0.050	14 (42.4)	32 (60.4)	0.105
Trimethoprim and sulfamethoxazole	32 (76.2)	26 (59.1)	0.091	24 (72.7)	34 (64.2)	0.409
Teicoplanin	42 (100)	44 (100)	N/A	33 (100)	53 (100)	N/A
Vancomycin	42 (100)	44 (100)	N/A	33 (100)	53 (100)	N/A
MDR strain	19 (45.2)	30 (68.2)	0.032	23 (69.7)	26 (49.1)	0.060

N/A, not available.

by several molecular and phenotypic characteristics, including agr type III, agr type II, SCC*mec* type V, SCC*mec* type I, *mecA* gene, resistance to tobramycin, positive biofilm, and being IS256^{+}. Chiquet et al.[20] found that *S. epidermidis* strains from the endophthalmitis patients displayed higher prevalence rates for *aap*, *atlE*, and *mecA* gene carriage and multidrug resistance compared to those from normal conjunctiva. The latter two studies suggest that MRSE

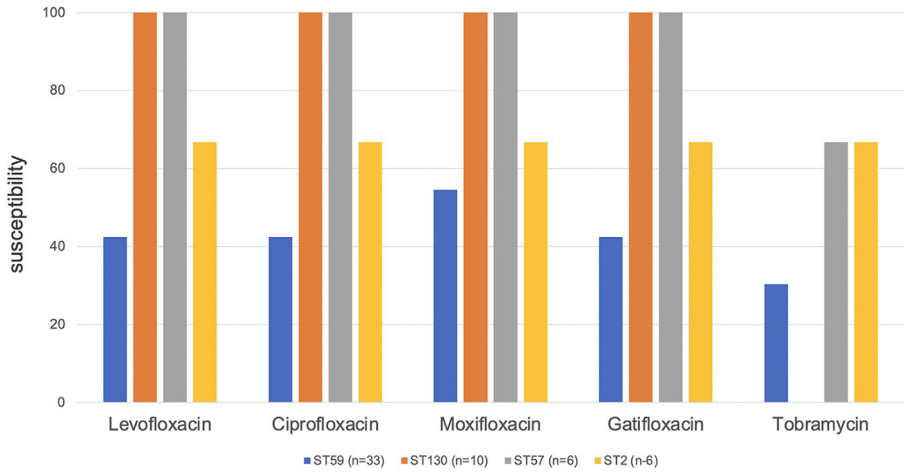

Figure 2. Rates of susceptibility of four fluoroquinolones and tobramycin, which are among the major STs of MRSE.

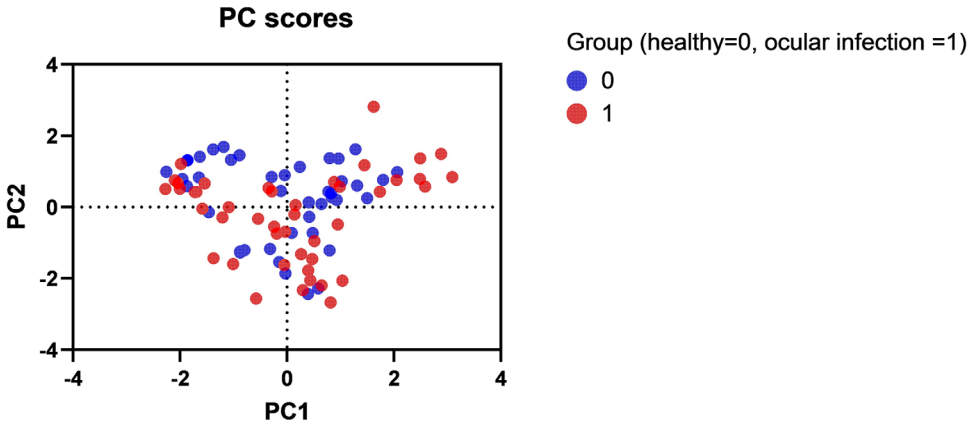

Figure 3. Principal component analysis (PCA) of MRSE isolates from healthy conjunctiva (0, *blue*) and isolates from ocular infection (1, *red*), showing overlap between samples.

isolates colonized on the ocular surface are more likely to transform into invasive pathogens. Our previous study and another study in Germany found that about one-third of the coagulase-negative staphylococci colonized on conjunctiva were methicillin resistant.[31,32] With the increasing resistance to a broad spectrum of antibiotics, it is especially important to evaluate the biological profile of MRSE. Our present study specifically investigated MRSE and found similar molecular and phenotypic profiles between ocular infection and commensal strains (Tables 2, 3). This finding indicates that the source of pathogens for ocular infection is primarily the ocular surface of patients. Similarly, Speaker et al.[17] utilized early genetic techniques to analyze plasmid profiles, DNA fingerprints digested with Hind III, and genotypes hybridized with gene-specific DNA probes. Their results demonstrated that nine *S. epidermidis* isolates from the vitreous were genetically indistinguishable from those derived from the eyelids, conjunctiva, and noses in 11 cases of endophthalmitis. Bannerman et al.[33] also reported that eyelid coagulase-negative staphylococcal isolates were indistinguishable from intraocular isolates in 71 of 105 patients with endophthalmitis (67.7%) using PFGE analysis. Jena et al.[21] reported that some healthy conjunctiva isolates had characteristics similar to those of infected eye isolates. For example, multidrug-resistant *S. epidermidis* strains carried the *mecA* gene and were virulent and diverse whether they were isolates from ocular infections or healthy conjunctiva,[21] which is in agreement with our results.

The discrepancies in the results of the previously described studies may be attributed to differences in study designs. For example, the clinical isolates in these studies were obtained from various sites such as lids, conjunctiva, cornea, aqueous humor, and vitreous, making it challenging to distinguish a contaminant from a pathogen in certain circumstances. In addition, some studies investigated the association between external flora and endophthalmitis using isolates from different sites within the same patients,[17,33] but other studies compared *S. epidermidis* isolates from ocular infections with those from conjunctival flora obtained from different individuals.[20,21,30] Moreover, various genotypic and phenotypic markers have been employed across these studies. Finally, it is essential to consider the impact of geographic epidemiology, as infectious diseases exhibit regional variations. To clarify whether colonized *S. epidermidis* strains can serve as endogenous reservoirs for subsequent infections, further study is needed to explore the correlation between *S. epidermidis* isolates obtained from patients with ocular infections and those from their conjunctiva.

We also observed that the predominant bacterial strains can vary in different regions or countries. Lineage ST59 was the most frequent sequence type in both ocular infection (34.1%) and healthy conjunctiva isolates (42.9%) in our cohort. In a study in India conducted by Jena et al.,[21] the ST179 lineage was found in 43% of the infected eye isolates, whereas ST59 was found in 13% of healthy conjunctiva isolates. The ST179 lineage was reported in a single isolate in a study from Brazil by Bispo et al.,[18] but not in any of our isolates. The study by Bispo et al.[18] examined 30 ocular MRSE isolates from keratitis and endophthalmitis and found that the most frequent isolate was ST59 (30%), grouped within the CC2 subcluster II (equal to CC5 in our study), and SCC*mec* type IV, suggesting that ST59 may possess different characteristics that contribute to ocular infections. ST59 MRSE strains have been characterized by biofilm production and carriage of multiple antibiotic resistance genes.[34,35] It is noteworthy that both our study and the research by Bispo et al.[18] showed that ST59 prevalently carried biofilm-related genes coding for proteinaceous factors, such as *aap* and *bhp*, rather than the *ica*A gene, which is commonly found in *S. epidermis* isolates from blood or catheter-related infections.[36,37] Although *ica*-encoded PIA on the cell surface is an important immune evasion mechanism,[38] this result suggests that biofilm formation may be independent of the *ica* genes in certain MRSE strains or that those without the *ica* locus are more well adapted to the ocular surface environment.[39,40] Interestingly, ST130 did not harbor any of the aforementioned biofilm-related genes such as *ica*A, *aap*, and *bhp*, yet it was still able to produce biofilm and become a pathogenic lineage. Perhaps other factors play a more important role for ST130. For example, the colonization-associated arginine catabolic mobile element was found to be highly prevalent in ST130 lineage, and so did the two other surface-associated autolysin/adhesion genes, *alt*E and *aae*, which help binding to fibrinogen, vitronectin, and fibronectin.[41] Future investigation into these mechanisms is warranted to better understand the pathogenicity of ST130.

Among the tested antibiotics, fluoroquinolones, tobramycin, and erythromycin are commercialized topical medications that are most commonly prescribed by ophthalmologists in clinical practice; however, increasing in vitro resistance to fluoroquinolones has been reported.[42] The fluoroquinolone resistance in *S. epidermidis* is attributed to mutations in the quinolone resistance–determining region of *gyrA* and *parC* genes.[43] Our study showed that the susceptibility to fluoroquinolones was about 65% for all MRSE isolates (Table 3). This percentage is similar to our previous report on MRSA, where approximately 60% of MRSA isolates were susceptible to fluoroquinolones.[44] Therefore, fluoroquinolones are still reasonable options for empirical antibiotics to treat MRSE infections and for perioperative prophylactic use. However, it is important to note that fluoroquinolones may not be effective in certain circumstances, particularly in cases involving ST59, the most common MRSE lineage, which shows particularly low susceptibility to fluoroquinolones (Fig. 2). Fortified vancomycin should therefore be a more appropriate alternative regimen for managing severe ocular infections. Tobramycin is another frequently used topical antibiotic in Taiwan, but it appeared to be least susceptible in our study for both healthy conjunctiva and ocular infection groups. The increased resistance to tobramycin is possibly related to its frequent prophylactic administration.[45] As a result, caution should be exercised when using tobramycin to treat ocular infections. Erythromycin showed slightly higher susceptibility to MRSE compared to tobramycin, making it a viable option when macrolide antibiotics for are needed for mild ocular infections. Furthermore, our study, along with the one conducted by Chiquet et al.,[20] revealed that ocular infection isolates have a significantly higher rate of MDR strains compared to those from healthy conjunctiva. This finding suggests that MDR can be an important factor contributing to colonized MRSE causing ocular infections.

Our study has certain limitations. First, the ocular infections group was comprised of diverse etiologies of infection. The molecular characteristics of the isolates responsible for keratitis and endophthalmitis may differ; therefore, additional studies with sufficient case numbers focusing solely on keratitis or endophthalmitis are warranted to provide more specific insights. Second, clinically it can be

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challenging to discern whether an isolate is a colonizing or infectious pathogen. To address this, we rigorously reviewed the medical records to confirm that all of the clinical samples had a history of active ocular infections requiring treatment. Third, unlike *S. aureus*, which is known for its numerous invasive factors, toxins, and enzymes, *S. epidermidis* appears to have a limited number of virulence factors. We did not test all known virulence factors for MRSE; instead, we initially focused on several biofilm-relevant genes for analysis. Furthermore, beyond the organisms involved, the role of host factors should not be underestimated. For example, systemic chronic disease; the use of cyclosporin, prednisone, or chemotherapy; or conditions such as human immunodeficiency virus infection can all impair the host's immunity.[46] Local factors, such as the balance of ocular microbiome and the tear proteome, can also influence ocular surface health.[47] Conditions such as dry eye, blepharitis, graft-versus-host disease, and Stevens–Johnson syndrome, that disrupt this homeostasis also render the host more susceptible to infection.[48] Another potential bias to be considered arises from the age differences between the participants of both groups in our study. Healthy conjunctiva isolates were obtained from patients undergoing cataract surgeries, consequently representing an older age demographic. Despite these limitations, this study contributes to our current knowledge about *staphylococci* in Taiwan, complementing our previous studies about the clinical and microbiological characteristics of *S. aureus* ocular infections.[44,49] Through our clinical, genotypic, and phenotypic characterization of ocular *S. epidermidis* isolates, we were able to identify certain pathogenic clonal lineages and their relevant virulence factors.

Conclusions

In summary, this study found that ocular MRSE exhibits diverse molecular characteristics, but a certain degree of genetic similarity was shared between healthy conjunctiva and ocular infection MRSE isolates. Virulence-related biofilm formation was universally present in all MRSE strains, regardless of their source of isolation. MDR strains were significantly prevalent in the ocular infection group. Notably, the most common lineage, ST59, displayed unique characteristics, including significantly reduced susceptibility to all fluoroquinolones.

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