Identification of Major Sequence Types among Multidrug-Resistant Staphylococcus epidermidis Strains Isolated from Infected Eyes and Healthy Conjunctiva

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We examined the presence of virulence and antibiotic resistance genes, SCCmec types and determined the genomic diversity among ocular S. epidermidis isolates (patients-23, healthy controls-29). PCR determined the presence of antibiotic resistance genes, virulence genes and SCCmec types among all isolates. MLST and PFGE determined the genomic relatedness among them. All isolates of S. epidermidis showed resistance to at least one class of antibiotics of which 48 isolates were multidrug resistant and carried ARGs. Thirty-five isolates were methicillin resistant and carried mecA gene. Majority of the isolates were resistant to fluoroquinolones and showed mutation in gyrA, parC, and parE genes, however, few isolates showed additional novel mutations in parC gene. Of the MRSE strains, 17 strains carried SCCmec type IV, four type V, two type II, and two UT4. Seven strains carried novel combination of ccr complex and SCCmercury element, not reported earlier. All the S. epidermidis strains harbored icaA and icaD genes, 47 carried ACME operon, and 50 contained IS256. A noteworthy finding was the presence of ST179 among 43% of infected eye isolates an observation rarely reported among S. epidermidis. PFGE and MLST analysis showed genomic diversity among them. Statistical analysis suggests that few healthy conjunctiva isolates had characteristics similar to infected eye isolates.

S. epidermidis strains carrying mecA gene are multidrug resistant, virulent and diverse irrespective of sources of isolation. IS256 cannot be used as marker to differentiate isolates of infected eye from healthy conjunctiva.

Keywords: multidrug-resistant, Staphylococcus epidermidis, ACME, SCCmec typing, PFGE analysis, MLST-genotyping, diversity

INTRODUCTION

Staphylococcus epidermidis an opportunistic pathogen and member of coagulase negative Staphylococci (CoNS) are normal inhabitant of human skin, mucosal and ocular surfaces and cause hospital acquired infections (Graham et al., 2007; Rogers et al., 2009; Le et al., 2014). This organism can cause number of ocular diseases like bacterial endophthalmitis conjunctivitis, blepharitis, and keratitis (Melo et al., 2011; Schimel et al., 2013; Bispo et al., 2014; Park et al., 2015). The predisposing risk factors associated with infections are mostly use of contact lenses,
ocular surgery, and ocular inflammatory diseases (Bourcier et al., 2003; Keay et al., 2006; Park et al., 2015). Although methicillin is not used in the treatment of eye infections, increasing resistance to methicillin is known to contribute significantly to the spread and persistence of multidrug-resistant strains in a given setting (Alekshun and Levy, 2007; Asbell et al., 2008; Cauvoto et al., 2008; Lichtinger et al., 2012). The alteration in penicillin-binding protein (PBPs) confers resistance to methicillin (Hartman and Tomasz, 1984). The mecA gene encodes for PBP2a and is present on the mobile genetic element termed as staphylococcal cassette chromosome mec (SCCMec) that carries a set of recombinase genes (crr) (Katayama et al., 2000). A combination of class of mec gene complex and the ccr gene complex types determines the SCCmec types and diversity among staphylococci (Kondo et al., 2007; Bloemendaal et al., 2010; Martinez-Melendez et al., 2016). Methicillin-resistant S. epidermidis (MRSE) carrying unttypeable SCCmec element are often reported (Ruppé et al., 2009; McManus et al., 2015).

Resistance to fluoroquinolones occurs due to mutation(s) in the quinolone resistance determining region (QRDR) of gyrA, gyrB, parC and parE (Yamada et al., 2008). There are three accomplished mechanisms of macrolide resistance. (i) Modification of the target site, (ii) efflux of antibiotics, and (iii) drug inactivation. Plasmid-borne drug-resistance genes encoding for ABC transporter superfamily efflux pump and msrA can mediate the export of macrolide (Leclercq, 2002). Other plasmid located genes, tetK confers resistance to tetracycline and pC194, pC221, and pC223 inactivates chloramphenicol by converting it to 3-acetyl and 1,3-diacetyl derivatives (Trieu-Cuot et al., 1993; Trzciński et al., 2000).

Pathogenicity of S. epidermidis is attributed to its ability to form biofilm comprised of polysaccharide, protein and eDNA, which confers resistance to antibiotics. During biofilm formation bacteria first, adhere onto abiotic polymeric substances mediated by polysaccharide intercellular adhesin (PIA) encoded by icaADBC locus (Schommer et al., 2011). Arginine catabolic mobile element (ACME) also play a significant role in the pathogenicity by enhancing the fitness of the bacteria by evading the host immune system (Otto, 2009).

Several molecular typing methods have been used to study epidemiology and clonal relationships of S. epidermidis; however, not a single technique alone could discriminate the bacteria because of differences in the degree of typeability, reproducibility, and discriminatory power (Tenover et al., 1994). Pulsed-Field Gel Electrophoresis (PFGE) is known as a “gold standard” and widely used for studying the epidemiological investigations (Hookey et al., 1998). Multi-Locus Sequence Typing (MLST) is currently used for long-term evolutionary research and to study population structure of S. epidermidis isolates (Bloemendaal et al., 2010; Martinez-Meléndez et al., 2016). Although S. epidermidis is rated as one of the emerging ocular pathogens and showing resistance to antibiotics; not much information is available on their molecular diversity. It is not clear how the isolates from healthy conjunctiva are related to and cause ocular infection.

We analyzed antibiotic resistance and virulence profiles of S. epidermidis isolated from infected eye and healthy conjunctiva during 2007–2011 at L.V. Prasad Eye Institute, Bhubaneswar, India. We used PFGE and MLST to determine the genomic diversity and correlated results with antibiotic resistance, SCCmec type, and virulence genes, respectively. Genetic and phenotypic data was used to determine whether isolates from healthy conjunctiva had characteristics similar to infected eye isolates.

MATERIALS AND METHODS

Ethical Statement
The study was approved by Institutional Review Board (IRB) of LV Prasad Eye Institute (LEC/08/110/2009), and the data were analyzed anonymously and reported.

Bacterial Strains
Twenty-three non-duplicate S. epidermidis were isolated from patients with a variety of ocular infections at L.V. Prasad Eye Institute, Bhubaneswar, India, during 2007–2011. These isolates were from patients with keratitis (n = 13), endophthalmitis (n = 2), conjunctivitis (n = 2), marsupialization of cyst (n = 1), chronic dacryocystitis (n = 1), traumatic cataract (n = 1), canaliculitis (n = 1), blepharitis (n = 1) and graft infiltrate (n = 1). Also, 29 strains were isolated from asymptomatic healthy conjunctiva. The study was conducted following the guidelines mentioned in the Declaration of Helsinki. All the 52 isolates were identified by using biochemical tests including Gram staining, catalase production, fermentation of glucose and mannitol and ID32 STAPH strips using ATB™ NEW v.1.0.0 software on an ATB™ reader (bioMerieux, France). The amplification of S. epidermidis nuc gene confirmed the identity of isolates (Hirotaki et al., 2011). S. epidermidis strains ATCC 35984 and ATCC 12228 obtained from American Type Culture Collection (ATCC, Manassas, VA), were used as controls in this study. Staphylococcus aureus ATCC 25293 and Pseudomonas aeruginosa ATCC 27853 was used as quality control strains for antibiotic susceptibility testing.

Antibiotic Resistance and Genetic Characteristics
Antibiotic susceptibility was performed with S. epidermidis for 13 antibiotics; oxacillin, penicillin, chloramphenicol, ciprofloxacin, ofloxacin, gatifloxacin, moxifloxacin, erythromycin, clindamycin, gentamicin, tetracycline, vancomycin, and cefazolin by microbroth dilution methods according to the Clinical Laboratory Standard Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2015). The interpretation criteria for each antibiotic tested were those published previously as CLSI document M100-S25. Isolates showing resistance to three or more than three class of antibiotics were referred here as multidrug resistant (MDR).

Using published methods, PCR determined the presence of blaz, capC221, capC223, ermC, msrA, mphC, aac6′-aph2′, aph3′, and tetK genes encoding for penicillin, chloramphenicol, erythromycin/clindamycin, gentamicin and tetracycline in both phenotypic-resistant and -susceptible strains of S. epidermidis (Schmitz et al., 1999; Schlegelova et al., 2008; Argudin et al., 2011; Duran et al., 2012). We determined the mutations in gyrA, gyrB,
parC, and parE genes by sequencing the PCR product (Yamada et al., 2008). PCR tested all isolates for the presence of icaA, icaD, arca, and opp3AB genes to assess the presence of ica and ACME operons (Arciola et al., 2001; Diep et al., 2008). Each strain was given a particular ACME type depending on the presence of either of the genes (Hellmark et al., 2013). Also PCR determined the presence of insertion sequence element IS256 (Gu et al., 2005).

**PFGE, SCCmec Typing, and MLST**
PFGE of *S. epidermidis* genomic DNA digested with SmaI was carried out by the protocol described for *S. aureus* by Centre for Disease Control and Prevention, Atlanta, USA. Banding patterns were determined by using BioNumerics software, version 7.1 (Applied Maths, Belgium) using the Dice index and un-weighted pair group method with arithmetic average (UPGMA) with 0.5% optimization and 1.5% position tolerance. Isolates showing similarity coefficient of up to 80% were considered belonging to similar pulsotype (Van Belkum et al., 2007).

Two Multiplex PCRs (MPCR-1 and MPCR-2) were performed by the method described previously using specific primers and assigned SCCmec type based on the combination of mec and ccr complexes (Kondo et al., 2007; Zong et al., 2011). Also, PCR determined the presence of SCCmercury element (Kondo et al., 2007).

Internal fragments of seven housekeeping genes, arcC, aroE, gtr, mutS, pyr, tpi, and yqil were amplified using specific primers (Thomas et al., 2007). Amplified fragments were purified using ExoSAP-IT (Affymetrix) and performed sequencing using Life Technology ABI sequencer model 3537 at the sequencing facility of Institute of Life Sciences, Bhubaneswar, India. Sequence alignments were done using MEGA 6. The sequences of the housekeeping genes were analyzed using BioNumerics software version 7.1, and a particular sequence type (ST) was assigned based on the nucleotide polymorphism. Minimum Spanning Tree (MST) was constructed using “MST for categorical data” as analysis template and accordingly assigned ST. We performed partitioning analysis according to the eBURST algorithm. Clonal complex (CC) analysis was performed according to the classification described by Miragaia et al. (2007).

**Indexing Diversity**
Simpson’s index of diversity (SID) was calculated for all the three typing techniques (PFGE, MLST, SCCmec typing) as described by Simpson (1949).

**Statistical Analysis**
We performed Principal Coordinates Analysis (PCoA) and Discriminant Analysis (DA) using PAST program v2.17 (Hammer et al., 2001). We carried out the discriminant analysis using default values to confirm the hypothesis whether two groups of isolates are different. All the genotypic and phenotypic data of the strains are given in the supplementary material (Tables S1, S2).

**RESULTS**

**Antibiotic Resistance Pattern**
Of the 52 strains, 35 strains were resistant to oxacillin and carried mecA gene (Table 1). However, all isolates were resistant to one or more than one class of antibiotics and showed 32 resistance patterns (Table S3). None of the isolates were resistant to vancomycin or cefazolin. Forty-eight strains were multidrug resistant (Table 1) comprising all isolates of MRSE from both sources, all MSSE from the infected eye and six from healthy conjunctiva (Table S3). Twenty-one isolates from the infected eye and 13 from healthy conjunctiva showed resistance

| Antibiotics | Resistance gene marker | No. of strains (%) positive for antibiotic resistance genes | No. of strains showing MIC value of antibiotics (mg/L) |
|-------------|------------------------|----------------------------------------------------------|-----------------------------------------------------|
|             |                        | MRSE | MSSE | Total | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | >64 |
| Oxacillin    | mecA                   | 35 (67%) | 0 (0%) | 35 (67%) | 0 | 4 | 4 | 3 | 0 | 0 | 2 | 0 | 1 | 0 | 21 |
| Penicillin   | blaZ                   | 32 (62%) | 18 (35%) | 50 (96%) | 0 | 0 | 0 | 12 | 0 | 5 | 11 | 3 | 2 | 2 | 6 | 11 |
| Chloramphenicol | catpC223             | 2 (4%) | 2 (4%) | 4 (8%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
|               | catpC221               | 33 (63%) | 14 (27%) | 47 (90%) | 0 | 0 | 0 | 0 | 0 | 5 | 7 | 16 | 4 | 3 | 12 |
|               | catpC223 + catpC221    | 2 (4%) | 2 (4%) | 4 (8%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Erythromycin | ermC                   | 25 (48%) | 15 (28%) | 40 (76.9%) | 2 | 3 | 1 | 4 | 3 | 0 | 1 | 1 | 3 | 0 | 22 |
|               | mssA                   | 14 (27%) | 4 (8%) | 18 (35%) | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 12 |
|               | ermC + mssA            | 13 (25%) | 4 (8%) | 17 (33%) | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 11 |
| Clindamycin  | mphC                   | 16 (31%) | 9 (17%) | 25 (48%) | 15 | 1 | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Gentamicin   | aac6’-aph2’            | 32 (62%) | 14 (27%) | 47 (90%) | 28 | 3 | 1 | 3 | 0 | 3 | 0 | 0 | 4 | 1 | 4 |
|               | aph3’                  | 18 (35%) | 7 (13%) | 25 (48%) | 6 | 5 | 0 | 1 | 1 | 4 | 0 | 0 | 4 | 1 | 3 |
|               | aac6’-aph2’ + aph3’    | 17 (33%) | 5 (10%) | 22 (42%) | 6 | 4 | 0 | 1 | 1 | 3 | 0 | 0 | 3 | 1 | 3 |
| Tetracycline | tetK                   | 32 (62%) | 15 (29%) | 47 (98%) | 0 | 0 | 0 | 4 | 6 | 0 | 4 | 1 | 5 | 21 | 6 |

**TABLE 1** Minimum inhibitory concentration and presence of antibiotic resistance genes of methicillin resistant and susceptible *S. epidermidis* isolated from the infected eye and healthy conjunctiva.

**Resistant: Bold; Intermediate: Bold italic; Sensitive: Normal.**
to fluoroquinolones. However, two from healthy conjunctiva showed intermediate resistance to ofloxacin and ciprofloxacin (Table 2).

### Antibiotic Resistance Genes (ARG) and Gyrase Mutation

Majority of *S. epidermidis* isolates showing phenotypic resistance to multiple antibiotics carried ARGs (Table 1). Of the 36 fluoroquinolone resistant *S. epidermidis*, 32 strains comprising 20 from the infected eye and 12 from healthy conjunctiva showed mutations S84→F/Y in gyrA gene, but none in the gyrB gene (Table 2). Similarly, 31 strains comprising 19 isolates from the infected eye and 12 from healthy conjunctiva showed a known mutation in *parC* gene. All isolates showing high MIC value for fluoroquinolones (especially that ≥ 64 mg/L) had mutation in either *gyrA* gene or *gyrA* and *parC* genes. In addition, five isolates from infected eye also showed novel mutation D84→C and one to V82→L in *parC*, not reported earlier. Of the six strains, five isolates from the infected eye and one from healthy conjunctiva showed mutation N404→S in the *parE* gene (Table 2).

### Virulence Profile of *S. epidermidis*

All the *S. epidermidis* strains harbored icaA and icaD genes. Of the 52 isolates, 20 from infected eye and 27 from healthy conjunctiva contained ACME operon (Table 4). Similarly 22 isolates from the infected eye and 28 from healthy conjunctiva amplified a fragment of IS256 indicating the presence of insertion sequence in *S. epidermidis* (Table S4, Figure 2). These observations thus suggest that virulence determinants were present in *S. epidermidis* strains, irrespective of sources of isolation.

### Table 2 | Results of MIC obtained with fluoroquinolones and mutations identified in *gyrA*, *gyrB*, *parC*, and *parE* among *S. epidermidis* strains isolated from the infected eye and healthy conjunctiva.

| Strain designation (no. of strains) from infected eye | Healthy Conjunctiva | MIC of fluoroquinolones (mg/L) | QRDR showing mutation in genes for |
|------------------------------------------------------|---------------------|--------------------------------|----------------------------------|
|                                                      |                     | MXF | GAT | OFX | CIP | *gyrA* | *gyrB* | *parC* | *parE* |
| 409, 1520, 1429 (3)                                   | N69OD (1)           | 4   | 4   | 32  | >64 | S84→F  | NIL    | S80→F  | NIL    |
| -                                                    | N68OD, N95OS (2)    | 1   | 2   | 8   | 4   |         |        |        |        |
| 1362 (1)                                             | -                   | 0.5 | 2   | 16  | >64 | S84→F  | NIL    | S80→F  | NIL    |
| 1497 (1)                                             | -                   | >64 | >64 | >64 | 32  | S84→F  | NIL    | S80→F  | NIL    |
| 1558 (1)                                             | -                   | 2   | 16  | 16  | 32  |         |        |        |        |
| 1606 (1)                                             | -                   | 2   | 32  | 2   | 2   |         |        |        |        |
| -                                                    | N64OS (1)           | 2   | 2   | 16  | 16  |         |        |        |        |
| 108, 1121, 1184, 1515 (4)                            | -                   | 4   | 4   | 32  | >64 | S84→F  | NIL    | S80→F  | NIL    |
| 866, 1001, 1386 (3)                                  | -                   | 4   | 4   | 32  | >64 | S84→F  | NIL    | S80→F  | NIL    |
| -                                                    | N900S (1)           | >64 | >64 | >64 | >64 | S84→F  | NIL    | S80→F  | NIL    |
| -                                                    | N980S (1)           | 2   | 2   | 8   | 4   |         |        |        |        |
| 1142W (1)                                            | -                   | 1   | 1   | 2   | 32  |         | NIL    | NIL    | N404→S |
| -                                                    | N78OD (1)           | 2   | 4   | 2   | 8   |         |        |        |        |
| 1502 (1)                                             | N79OS (1)           | 4   | 8   | >64 | >64 | S84→F  | NIL    | S80→F  | NIL    |
| -                                                    | N30D (1)            | >64 | >64 | >64 | >64 | S84→F  | NIL    | S80→F  | NIL    |
| -                                                    | N87OS (1)           | 4   | 4   | 16  | 16  |         |        |        |        |
| -                                                    | N74OS, N101OD (2)   | 2   | 2   | 16  | 16  | S84→F  | NIL    | NIL    | NIL    |
| -                                                    | N91OD, N93OSW (2)   | 0.25| 0.25| 1   | 1   |         | NIL    | NIL    | D84→A  |
| 1033 (1)                                             | -                   | 2   | 1   | 32  | >64 | S84→F  | NIL    | S80→F  | NIL    |
|                                                      | 1122 (1)            | -   | >64 | >64 | >64 | 8      | S84→F  | NIL    | N404→S |
| -                                                    | 1123 (1)            | 1   | 2   | 16  | 1   | S84→F  | NIL    | S80→F  | N404→S |
| -                                                    | 1056 (1)            | 2   | 2   | >64 | >64 | S84→F  | NIL    | D84→Y  | N404→S |
| -                                                    | 1150 (1)            | 4   | 4   | 32  | 8   | S84→F  | NIL    | D84→Y  | N404→S |
| -                                                    | N96OD (1)           | 32  | 32  | >64 | 64  | S84→F  | NIL    | S80→F  | NIL    |
| -                                                    | N980D (1)           | 0.125| 0.125| 0.25| 0.25|         | NIL    | NIL    | NIL    |
| 1163, 1528 (2)                                       | N10D, N50D, N6OS, N16OD, N18OD, N41OD, N46OD, N47OD, N52OD, N53OS, N81OD, N86OD, N94OD (13) | 0.125| 0.125| 0.5  | 0.25|         | NIL    | NIL    | NIL    |

MXF, Moxifloxacin; GAT, Gatifloxacin; OFX, Ofloxacin; CIP, Ciprofloxacin. Resistant: Bold; Intermediate: Bold italic; Sensitive: Normal. * Novel mutations found in this study.
**SCCmec Typing**

Of the MRSE strains, 25 belong to known SCCmec types of which 17 strains carried SCCmec type IV, four type V, two type II, and two carried UT4 (Table 3, Figure 1). The remaining 10 MRSE isolates showed four different combinations of mec complex and ccr complex, not reported earlier and thus do not fall within the traditional classification scheme. Of these strains, seven carried multiple ccr complexes and SCC mercury element (SCCmer). One strain from infected eye belonging to UT4 was also positive for SCCmer (Table 3, Figure 1). Although ten of the 17 MSSE isolates failed to amplify mec and ccr complex, five isolates carried ccrA2B2, and one isolate carried ccrC1 but lacked mec complex. One of the MSSE isolates carried more than one ccr type (A2B2, C1) and SCCmer (Table 3, Figure 1). Strains belonging to

### TABLE 3 | Distribution of SCCmec types and SCCmer element among S. epidermidis strains isolated from the infected eye and healthy conjunctiva.

| SCCmec type | No. of strains | No. of strains from | mecA gene | ccr complex | mec complex | SCCmer |
|-------------|----------------|---------------------|-----------|-------------|-------------|--------|
|             | Infected eye   | Healthy conjunctiva |           |             |             |        |
| V           | 4              | 1                   | +         | C1          | C2          | 0      |
| IV          | 17             | 9                   | +         | A2B2        | B           | 0      |
| II          | 2              | 0                   | +         | A2B2        | A           | 0      |
| UT4         | 2              | 1*                  | +         | A2B2, C1    | C2          | 1*     |
| UT11        | 2              | 0                   | +         | A2B2, C1    | B           | 2      |
| UT12        | 5              | 4                   | +         | A2B2, C1    | A           | 5      |
| UT13        | 2              | 0                   | +         | A2B2        | C2          | 0      |
| UT14        | 1              | 1                   | +         | A2B2        | C2+         | 0      |
| UNT-I       | 5              | 1                   | –         | A2B2        | –           | 0      |
| UNT-II      | 1              | 1                   | –         | A2B2, C1    | –           | 1      |
| UNT-III     | 1              | 0                   | –         | C1          | –           | 0      |
| Absent      | 10             | 5                   | –         | –           | –           | 0      |

*UT, Unnamed type; UNT, Untypeable; *One of the UT4 strain carried SCCmer.

**FIGURE 1** | Agarose gel electrophoresis of multiplex PCR employed for (A) ccr complex typing (MPCR1), (B) mec complex typing (MPCR2) of S. epidermidis strains. Lane M1: 100bp ladder; lane 1: S. epidermidis strain 866; lane 2: S. epidermidis strain 1056; lane 3: S. epidermidis strain N60S; lane 4: S. epidermidis strain 1150; lane 5: S. epidermidis strain N91OD; lane 6: S. epidermidis strain 108; lane 7: S. epidermidis strain N60S; lane 8: S. epidermidis strain 1558; lane 9: S. epidermidis strain N91OD; lane 10: S. epidermidis strain 1386; lane 11: S. epidermidis strain 1558; lane 12: S. epidermidis strain 1386; lane 13: S. aureus strain Mu50; lane 14: S. epidermidis strain 1295; lane 15: S. epidermidis strain 12228; lane M2, 1kb ladder.
unreported SCCmec types (UT12 – UT14) showed a high level of oxacillin resistance (data not shown).

Genomic Diversity
Small restriction digestion of DNA from S. epidermidis generated 14–21 bands, classifying the isolates into 35 pulsotypes, 16 subtypes, and three identical pairs (Table 4, Figure 2). Of the 52 S. epidermidis strains, 47 comprising 20 from infected eye and 27 from healthy conjunctiva harbored ACME operon of which 33 belonged to ACME type I, 11 to type II, and three to type III, respectively (Table 4). MLST analysis of the S. epidermidis strains identified 28 distinct STs of which ST179 was found in 43% of infected eye isolates followed by ST59 that was found in 13% of healthy conjunctiva isolates (Table 4). Partitioning analysis of the MST generated two CCs of which CC59 included 12 STs (ST59, ST6, ST210, ST48, ST291, ST142, ST153, ST89, ST5, ST384, ST280 and ST2) and CC197 includes two STs (ST197 and ST564). ST6 seems to be the founder of CC59 according to the eBURST algorithm. The remaining 14 STs were singletons of which ST179 comprised of 11 strains, ST69 and ST173 three strains, ST11 two strains, ST4, ST10, ST19, ST44, ST72, ST183, ST325, ST347, ST488, and ST490 one strain each (Table 4, Figure 3). A good correlation was found between high level resistance to fluoroquinolones and major sequence type, ST179.

SID for MLST, SCCmec, and PFGE
We calculated SID for all the three typing techniques. The values derived for MLST, SCCmec typing and PFGE were 0.95, 0.85, and 0.97, suggesting the existence of genomic diversity among the isolates irrespective of sources of isolation. Overall result suggests that PFGE and MLST were more discriminatory than SCCmec typing to study genomic diversity among S. epidermidis.

Statistical Analysis
PCoA can segregate isolates belonging to the healthy conjunctiva and infected eye, except for few isolates from healthy conjunctiva (axis one was used for the highest percentage of representation), with 33% of explained variance (Figure 4A). From DA graph, it was clear that, whereas isolates from infected eye grouped within more negative values, majority isolates from healthy conjunctiva grouped within positive values (Figure 4B). Predominant biomarkers were determined by calculating the coefficient of discriminant function and considered when the value was equal to 0.5 or >0.5. Whereas infected eye isolates are discriminating in the biomarker of resistance to gatifloxacin (−3.332), ofloxacin (−3.332), and tetracycline (−2.131), the healthy conjunctiva isolates were discriminating in resistance to moxifloxacin (0.64542), ciprofloxacin (0.69144), and erythromycin (1.5531).

DISCUSSION
S. epidermidis has been reported causing indwelling medical device infections, such as prosthetic valve endocarditis, and intra-cardiac abscesses leading to high mortality (Otto, 2009). The incidence of S. epidermidis in ocular disease is more frequent than any other species including S. aureus (Duggirala et al., 2007; Flores-Páez et al., 2015). The frequency of methicillin resistance among disease-associated isolates was higher than commensal strains (Duggirala et al., 2007). Several studies have shown that MDR is more frequent in MRSE than MSSE (Cherifi et al., 2013, 2014; Deplano et al., 2016). Also, strains from the infected eye were more resistant to non-beta-lactam antibiotics than those from the healthy conjunctiva (Duggirala et al., 2007; Cherifi et al., 2013, 2014). This study also showed that S. epidermidis isolates are multidrug resistant, irrespective of methicillin-resistance and sources of isolation. Similar to other workers 70% of isolates comprising isolates from healthy conjunctiva and the infected eye carried catpC221 or catpC223 or both gene(s) encoding for chloramphenicol resistance (Cherifi et al., 2013; Bispo et al., 2014). A good correlation was found between the presence of catp223 and/or catp221 gene(s) and chloramphenicol resistance. The catpC221 gene encoding for the resistance to chloramphenicol was reported previously in

![Table 4](https://example.com/table4.png)

**Table 4** | Distribution of ST, PFGE types, SCCmec types and virulence genes among S. epidermidis isolated from the infected eye and from healthy conjunctiva.

| ST (no. of strains, %) | PFGE types | SCCmec types (no. of strains) | No. of strains showing the presence of virulence factors |
|----------------------|------------|--------------------------------|-------------------------------------------------------|
| **INFECTED EYE**     |            |                                | ACME I ACME II ACME III icaA icaD IS256               |
| ST179 (10, 43.4%)    | 5          | UT14 (1) UT12 (4) IV (5)       | 8  1  1  10  10  9                                    |
| ST197 (2, 8.6%)      | 2          | IV (1) Absent (1)              | 1  0  0  2  2  2                                    |
| ST173 (2, 8.6%)      | 2          | UNT-I (1) UNT-II (1)           | 2  0  0  2  2  2                                    |
| Other STs (9, 39.1%) | 9          | V (1), IV (3), UNT-IV (1), Absent (4) | 5  1  1  9  9  9                               |
| **HEALTHY CONJUNCTIVA** |           |                                |                                                       |
| ST59 (4, 13.7%)      | 4          | IV (3) II (1)                 | 2  2  0  4  4  4                                    |
| ST6 (3, 10.3%)       | 3          | Absent (3)                    | 0  3  0  3  3  3                                    |
| ST48 (2, 6.8%)       | 2          | IV (2)                        | 1  0  0  2  2  2                                    |
| ST69 (2, 6.8%)       | 2          | IV (1) UT12 (1)               | 2  0  0  2  2  2                                    |
| Other STs (18, 62%)  | 16         | IV (2), V (3), II (1), UNT-I (4), UT11 (2), UT13 (2), UNT-IV (1), UNT-III (1), Absent (2) | 12  4  1  18  18  17                                |

ST, sequence type; PFGE, pulsed field gel electrophoresis; MLST, multi-locus sequence typing; UT, Unnamed type; UNT, Untypeable.
FIGURE 2 | Dendrogram representation (Dice co-efficient) for macro-restriction banding patterns of S. epidermidis strains isolated from infected eye and healthy conjunctiva along with ATCC reference strains, generated by Pulsed-field gel electrophoresis of total chromosomal DNA digested with SmaI restriction enzyme. A correlation between Pulsotype, ST, SCCmec type, ACME, ica operon, IS256, and source of isolation is also shown. UT: Unnamed type, UNT: Untypeable, ND: not detected, NK: not known.

CoNS, especially in S. pseudintermedius of veterinary origin (Perreten et al., 2010; Kern and Perreten, 2013). A small percentage of S. epidermidis showed resistance to clindamycin and gentamicin a finding similar to those workers who reported resistance to these antibiotics among isolates from ocular and blood stream infections (Kern and Perreten, 2013; Bispo et al., 2014). In contrast, a high percentage of MLSB resistance gene ermC encoding for macrolide-lincosamide-streptograminB resistance was present among S. epidermidis isolates comprising 12 from the infected eye and 24 from the healthy conjunctiva (Argudin et al., 2011). Interestingly, most frequent ARG found in this study was tetK, though many of the isolates carrying this gene were phenotypically sensitive to tetracycline. Like Argudin et al. (2011) the bifunctional aminoglycoside resistance-conferring gene aac6’-aph2’ was present in majority strains of which only 34% were phenotypically resistant to gentamicin. The commensal isolates were reported showing multidrug resistance (Li et al., 2009). Also, in this study, isolates from the healthy conjunctiva showed resistance to chloramphenicol, fluoroquinolones, erythromycin and tetracycline. These observations thus suggest that even the commensal strains can acquire resistance without being exposed to the antibiotics, therefore, acts as reservoirs for ARGs.

Fluoroquinolones are the preferred antibiotics for the treatment of staphylococcal ocular infections. Use of inappropriate dosage and overexposure of the bacteria to the antibiotic had facilitated the emergence of resistance to this drug. Mutation(s) in the gyrA, gyrB, parC, and parE genes is the primary mechanism of fluoroquinolone resistance. Like other workers, we also did not find any mutation in gyrB among S. epidermidis (Yamada et al., 2008). These workers have shown mutations in QRDR of gyrA, gyrB, parC, and parE genes in ∼50% of S. epidermidis isolates from the human conjunctiva. However, the results of the present study showed an increase in mutation in QRDR up to 70% until 2011. We like other workers also found a mutation in gyrA (Ser84→F/Y) in S. epidermidis strains that showed a significant association with high level of resistance to fluoroquinolones (Yamada et al., 2008; Betanzos-Cabrera et al., 2009; Bispo et al., 2013). In addition to the reported mutation in parC (Ser80→F/Y and D→A),
we found two novel mutations (D84→C and V82→L). These mutations do not alter the level of MIC significantly for any of the tested fluoroquinolones. Although mutation in \textit{gyrA} gene confers resistance to fluoroquinolone, mutations in \textit{parC} or \textit{parE} do not alter the level of fluoroquinolones resistance. We found high MIC value for ciprofloxacin (32 mg/L) in one of the strain 1142W that showed mutation only in \textit{parE} gene but its role in high level of quinolone resistance is not known (Yamada et al., 2008). We also did not find any association of high level of MIC to ciprofloxacin in a strain that had mutation in only \textit{parE} gene. It is likely that other mechanism such as efflux of quinolone may be responsible for quinolone resistance leading to high level of ciprofloxacin resistance in this strain (Betanzos-Cabrera et al., 2009).

Several studies showed that \textit{ica} operon is more prevalent among \textit{S. epidermidis} isolated from a variety of infections than
the commensals (Cherifi et al., 2013; Du et al., 2013). In contrast, all isolates of the present study carried ica operon irrespective of sources of isolation. These findings are similar to those workers who also reported the presence of ica operon among the isolates from both infected eye and healthy conjunctiva (Duggirala et al., 2007; Cherifi et al., 2013; Flores-Páez et al., 2015). Thus, this study support the proposal of eliminating detection of ica gene as a factor for differentiating commensal from invasive isolates (Wisplinghoff et al., 2003). Similarly, ACME operon was present in 47 S. epidermidis strains. This finding is in contrast to those workers who reported less number of S. epidermidis showing the presence of ACME operon (Cherifi et al., 2013; Du et al., 2013; Deplano et al., 2016). These authors also demonstrated that ACME was found more commonly in commensals than those isolated from infections (Du et al., 2013). In contrast, we did not find much difference in the distribution of ACME operon among S. epidermidis with regard to sources of isolation.

The majority of isolates from the infected eye and healthy conjunctiva were methicillin-resistant and carried the mecA gene. We did not find the presence of mecC (a variant of mecA) in any of the S. epidermidis isolates. These findings thus suggest that there is no significant difference of oxacillin resistance and the presence of mecA gene between sources of isolation. MRSE isolates were mostly multidrug resistant and belong to SCCmec type IV. These findings are similar to those workers who also reported the presence of SCCmec type IV in S. epidermidis (Wisplinghoff et al., 2003; Duggirala et al., 2007; Cherifi et al., 2014). The findings of this study thus suggest that there is mobility of SCCmec cassette among S. epidermidis, similar to that reported in S. aureus (Miragaia et al., 2007). Also, nine S. epidermidis isolates comprising six from the infected eye and three from healthy conjunctiva showed amplification of mercury resistance and J-region suggesting the presence of SCCmercury operon. To the best of our knowledge, this is the first report of the presence of SCCmercury among ocular isolates of S. epidermidis, although this element was reported earlier in S. aureus strains (Kondo et al., 2007).

A novel combination of ccr A2B2 and mecC2, designated as UT13, was present among S. epidermidis strains and was not included in the earlier classification scheme (Zong et al., 2011). We also found amplification of more than one ccr complex among few isolates designated as UT11 and UT12 indicating the presence of composite SCCmec types. Although the presence of more than one type of ccr complex was reported earlier in S. epidermidis, 17 MSSE isolates from this study were untypeable because none of the strains amplified either one or both of the complexes (Kondo et al., 2007; Cherifi et al., 2014). Also, we did not find the presence of pseud SCCmec complex designated as a pseudo-SCCmec element, carrying mec complex but lacking ccr complex, in any of the strains. The heterogeneity of isolates including those from healthy conjunctiva indicates that S. epidermidis can act as a reservoir and play a role in the emergence of new types of SCCmec elements. It can happen due to acquisition or loss of SCCmec element which is frequent in CoNS (Zong et al., 2011).

MLST analysis revealed that ST179 is the most common ST found in 43% of infected eye isolates of S. epidermidis. This ST179 was reported earlier in single isolate each from an ocular strain in Brazil in 2009 and Iran in 2016 (Cherifi et al., 2013; Bispo et al., 2014). The second most frequent ST was ST59 and found in one strains from infected eye and three from healthy conjunctiva. This ST59 along with other STs was reported in strains isolated from blood culture, ocular infection and the healthy conjunctiva and suggested their association with the eye disease (Mertens and Ghebremedhin, 2013; Bispo et al., 2014; Soroush et al., 2016). There is good correlation between types of STs, ST179, and ST59 with source of isolation. These findings are in contrast to those workers who reported that lineage ST2 is most frequent in ocular disease and healthy conjunctiva isolates showing a substantial difference between isolates from ocular disease and healthy conjunctiva (Flores-Páez et al., 2015). However, a study conducted on clinical isolates in US hospital on bone and joint infection showed association of ST5 lineage with disease (Mendes et al., 2012; Cremniter et al., 2013). High level
as a marker to differentiate isolates from infected eye and healthy conjunctiva. Statistical analysis suggests that few healthy conjunctiva isolates had characteristics similar to infected eye isolates. A noteworthy finding was the presence of ST179 in 43% of infected eye isolates an observation rarely reported among S. epidermidis. PFGE and MLST analysis indicates genomic diversity among them irrespective of sources of isolation.

**AUTHOR CONTRIBUTIONS**

SJ, SP, and DVS designed the research; SJ and SP participated in most of the experiment; SJ, and SP analyzed the data; KN performed the statistical analysis; SJ drafted the manuscript; SJ, SP, KN, and DVS revised the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2017.01430/full#supplementary-material

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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