High-Resolution Genome Profiling Differentiated *Staphylococcus epidermidis* Isolated from Patients with Ocular Infections and Normal Individuals

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Purpose. To investigate the potential phenotypic and genetic differences among the *Staphylococcus epidermidis* isolates obtained from control subjects (lower conjunctival sac; $n = 14$) with those from patients with keratitis (corneal scrapings; $n = 18$) or endophthalmitis (vitreous; $n = 24$).

Methods. Biofilm-forming capacity was detected by PCR for the *icaAB* gene and phenotyping by microtiter plate assay and congo red agar plate. Genotyping was performed by using fluorescence-amplified fragment length polymorphism (FAFLP) and in silico analysis of the FAFLP profiles.

Results. Biofilm phenotyping (congo red agar/microtiter plate) differentiated disease-causing strains from control subjects. PCR assays (*mecA*, *icaAB*) were not useful in differentiating disease-causing strains from that of control subjects. The biofilm-forming capability appeared more critical in the pathogenesis of keratitis than in that of endophthalmitis. Cluster analysis of FAFLP data generated 11 clusters comprising 4 major clusters (I, II, III, and V) and 7 minor ones. FAFLP analysis clearly showed clustering of most of the commensal isolates in cluster I, separate from keratitis and endophthalmitis isolates. In silico analysis mapped signature bands to genes such as *ebh, tagD*, *ptsI*, and *sepA*, which might have a significant role in transforming less virulent populations of *S. epidermidis* to more virulent ones.

Conclusions. The population dynamics of *S. epidermidis* revealed that there are significant genetic variations that can be detected through FAFLP between ocular disease causing isolates and the commensal population. (*Invest Ophthalmol Vis Sci.* 2007;48:3239–3245) DOI:10.1167/iovs.06-1365

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Coagulase negative *Staphylococcus* species (CoNS) are among the most frequently isolated bacterial species in the clinical microbiology laboratory, especially because of their ability to cause nosocomial infections.[1] *S. epidermidis* are normal inhabitants of human skin and mucous membranes and therefore, one of the major challenges of daily diagnostic work is to distinguish clinically significant *S. epidermidis* from contaminant strains. Although several studies have reported a high frequency of *S. epidermidis* in endophthalmitis,[2] only a few studies of keratitis have reported *S. epidermidis* as the most prevalent organism.[3]

Phenotypic and molecular characterization of ocular *S. epidermidis* isolates is essential to understanding the pathogenicity of these isolates that also reside in the extraocular tissues such as lids and conjunctiva. Many techniques have been used to characterize the strains of *S. epidermidis* responsible for bloodstream infections to determine the clonality, and to distinguish conclusively the clinically significant organisms from those of control subjects or contaminants.[4] Such studies are lacking on *S. epidermidis* of ocular origin. Unlike *Staphylococcus aureus*, there are no specific and definite virulence determinants identified in *S. epidermidis*. Molecular characterization of *S. epidermidis* derived from different sources and disease entities may help in identification of new virulence determinants. Although comparison of CoNS obtained from endophthalmitis and CoNS from the conjunctiva of the same eye have been made,[5,6] to date, there have been no genotyping studies in which isolates from patients with keratitis were compared with those from normal conjunctiva.

Whether all strains of *S. epidermidis* have equal disease-invoking potential or invasive disease is associated with particularly virulent genotypes is controversial.[7–9] To assess differences in the virulence potential of various strains of *S. epidermidis*, insights into the natural commensal strain's genetic structure are very essential. Earlier, we observed subtle genetic differences between the normal flora and disease-causing strains and were able to correlate the evolving nature of the pathogenic strains from the commensal strains.[8] Based on these observations we hypothesized that isolates obtained from keratitis and endophthalmitis may be genetically dissimilar and may differ from conjunctival commensal flora of normal subjects. A population study of *S. aureus*, a closely related species obtained from nosocomial infections and the commensal population, has also shown that strains isolated from healthy humans can evolve and transform into pathogens.[10]

Analytical profile index (API; bioMérieux, Marcy-l'Etoile France), antibiotyping, detection of known virulence markers (biofilm formation and methicillin resistance), and fluorescence-amplified fragment length polymorphism (FAFLP) typing techniques can help in reliable identification and characterization of *S. epidermidis* isolates. FAFLP is a PCR-based fingerprinting technology with high resolution and sensitivity that can detect polymorphism at the whole genome level.[11] The present study is designed for the investigation and elucidation

of potential phenotypic and genetic differences among the isolates obtained from control subjects versus those obtained from patients with keratitis or endophthalmitis. An additional objective was to study the genetic variations among invasive endophthalmitis isolates in comparison with those of keratitis isolates. We also attempted to identify genes that are speculated to cause enhanced virulence among *S. epidermidis* isolates such as genes relevant in biofilm formation (*icaA* and *icaB*) and antimicrobial resistance (*mecA*).[7,12,13]

Materials and Methods

Patients, Bacterial Strains, Microbiologic Methods, and Identification

Forty-two patients with ocular infections in the age range of 16 months to 80 years (mean ± SD; 44.003 ± 19.22) with a male-to-female ratio of 29:13 were included in the study. The patients were enrolled and the study was conducted according to the guidelines set forth in the Declaration of Helsinki. Forty-two consecutive isolates from patients attending the outpatient department of the L. V. Prasad Eye Institute (LVPEI) who were clinically diagnosed as having microbial keratitis (n = 18) and endophthalmitis (n = 24) were included in the study. Fourteen commensal isolates (control subjects) were from the conjunctival sac of normal healthy individuals among the students and staff of LVPEI with no history of ocular disease. Three reference strains obtained from ATCC were also included in the study, of which two were biofilm-forming pathogenic strains—namely, ATCC35983 and RP62A[14,15]—and a non–biofilm-forming, nonpathogenic strain ATCC12228.[16] In addition, a single ATCC strain of *P. aeruginosa*, which is phylogenetically distant with different GC content from that of *S. epidermidis*, was selected as an out-group strain for cluster analysis. Identification of *S. epidermidis* was performed by the analytical profiling index system using a kit (API Staph; bioMérieux). Antibiotic susceptibility was determined by the Kirby-Bauer disc diffusion method wherein the following antibiotics were tested: amikacin, cefazolin, ceftazidime, ciprofloxacin, chloramphenicol, gentamicin, methicillin or oxacillin, ofloxacin, and vancomycin. The results of antibiotic susceptibility were interpreted, according to the NCCLS (National Committee for Clinical Laboratory Standards) criteria.[17]

PCR for the *icaAB* and *mecA* Genes

DNA extraction was performed according to the lysis method described earlier.[18] Primers were designed for simultaneous amplification of the fragment encompassing *icaA* and *icaB* genes of *S. epidermidis*, with the help of previously published sequences.[7] The *ica* primers were designed to amplify certain regions of both the *icaA* and *icaB* genes of the *ica* locus. All isolates of *S. epidermidis* were checked for the presence of *mecA* by using PCR corresponding to the unique penicillin-binding protein (PBP2a or PBP2′).[13,19]

Phenotypic Assays for Biofilm Production

Congo Red Agar Plate Method. Congo Red Agar (CRA) plates were prepared as described earlier.[20] All the clinical isolates and standard strains were cultured on CRA plates. The plates were incubated aerobically for 48 hours at 37°C and observed for the color of the colonies.

Microtiter Plate Test for Quantification of Biofilm Production. A microtiter plate assay was performed as described earlier.[21] The cutoff OD (ODc) for the assay was determined according to the procedure described by Stepanovic et al.[21] In this study, isolates classified as weakly adherent were considered negative for biofilm.

Fluorescence-Amplified Fragment Length Polymorphism

Fifty-six isolates along with four standard reference strains (three *S. epidermidis* and one *P. aeruginosa*) were characterized by FAFLP as described previously.[8,22] Using the enzyme combination of *Eco*RI-*Mse*I, we obtained a fingerprint of approximately 44 fragments distributed within the size range of 50 to 500 bp. Primer combinations used were *Eco*RI+0 and *Mse*I+C. The FAFLP experiment and analysis (AFLP Microbial Fingerprinting kit; Applied Biosystems, Inc., [ABI] Foster City, CA) were performed according to the manufacturer's instructions.

FAFLP Data Analysis

Analysis of the data was performed by construction of dendrograms and visual inspection of the common signature bands (GeneScan software; ABI). For construction of the dendrogram data from all isolates including the out-group ATCC *P. aeruginosa* strain were imported into an analysis program (Genotyper; ABI). The percentage similarities/differences between FAFLP amplitypes were calculated using the Dice correlation coefficient. The binary data were converted into a distance matrix, and dendrograms were deduced by using the UPGMA algorithm (unweighted pair group method with arithmetic mean).[22,23]

Signature Fragment and In Silico Analysis

For identification of signature fragments, eight that were closely related representative isolates from each of the three groups included in the study were selected, and their electropherograms were further analyzed (GeneScan; ABI). Three sets of comparisons for the representative isolates were made: the endophthalmitis group versus the keratitis group, the keratitis group versus the commensal group, and the endophthalmitis group versus the commensal group. For each kind of comparison, 16 GeneScan amplitypes were visually analyzed by superimposing color-coded FAFLP amplitypes of isolates. Such analysis was later extended to all the isolates. After identification of signature fragments, corresponding genomic coordinates were identified with the help of in silico AFLP PCR software.[24] These fragments were further analyzed by BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) against the two completed genome sequences (ATCC 12228, NC_004461; RP62A, NC_002976). All the gene names or coding DNA sequence (CDS) types mentioned in this study are from the RP62A genome (NC_002976). To compare the distribution of FAFLP markers among the isolates in three categories, we used the Fisher exact test. A two-tailed $P < 0.05$ was considered significant.

Results

Biotyping, Antimicrobial Susceptibility Testing

API identification helped in accurate species identification of all 56 isolates of *S. epidermidis*. The API identification score and the results of antibiotic susceptibility testing for all the isolates are shown in Table 1.

Presence of *icaAB* and *mecA* in Three Study Groups

Overall, the *mecA* gene was detected in 64.28% (36/56) of isolates and *icaAB* gene was positive in 69.64% (39/56). Both *mecA* and *icaAB* were detected in 66% of endophthalmitis isolates. Keratitis isolates showed 55% positivity for *mecA* and 72% were *icaAB*-positive in this group. Seventy-one percent of isolates from control subjects showed amplification of both *mecA* and *icaAB* (Table 1).

Biofilm Phenotypic Assays

Phenotypic assays for determination of biofilm production by the CRA method and the microtiter plate assay showed commensal isolates having no positivity by the former and 15% positivity by the latter. By the CRA method, biofilm production

TABLE 1. Details of 56 *S. epidermidis* Isolates and Three Reference Strains Included in the Study

Strain	Site of Isolation	API Identification Score	*mecA/icaAB*	*CRA*	Microtitre Plate	Antibiotic Resistance	FAFLP Cluster
L140/05 E	Vitreous	73	+/−	−	−/NA	GEN, CAZ	III
L166/03 E	Vitreous	90.4	+/+	−	−/WA	CAZ, GEN, CIP	VIII
L170/05 E	Vitreous	73	−/+	+	−/NA	Sensitive	III
L172/03 E	Anterior chamber	97	+/+	+	−/NA	GEN. OXA	IV
L173/03 E	Vitreous	84.9	+/+	−	−/NA	GEN, OXA	VIII
L185/05 E	Vitreous	80	+/+	+	−/NA	Sensitive	III
L186/05 E	Vitreous	97	+/−	−	−/NA	Sensitive	V
L187/03E	Vitreous	97	+/+	+	+/NA	Sensitive	IV
L192/04 K	Corneal scrapings	80.5	+/−	+	+/SA	MET	II
L207/05 E	Vitreous	80	−/−	−	−/NA	Sensitive	I
L219/03 E	Vitreous	97.9	+/+	−	+/SA	AMK, CAZ, CIP, GEN, OXA	V
L221/03 E	Anterior chamber	97.9	+/+	−	−/WA	AMK, CAZ, CIP, GEN, OXA	III
L222/03 E	Vitreous	97.9	+/−	−	−/WA	AMK, CAZ, CIP, GEN, OXA	V
L257/05 K	Corneal scrapings	97	+/−	−	−/NA	CIP, GEN, OFX, MET	IX
L282/05 E	Vitreous	97	+/+	NF	NF	GEN, OFX, CAZ, CIP, MET	II
L328/03 K	Corneal scrapings	NA	−/+	NF	NF	Sensitive	VII
L339/03 K	Corneal scrapings	NA	−/−	NF	NF	Sensitive	II
L351/03 K	Corneal scrapings	NA	−/+	−	−/WA	Sensitive	VII
L335/04 E	Vitreous	80.5	+/+	+	+/SA	Sensitive	II
L342/04 E	Vitreous	73.2	−/+	+	−/WA	CFZ, CAZ	III
L807/04 E	Vitreous	80.5	−/−	−	−/NA	AMK, GEN	V
L969/04 E	Vitreous	74.3	−/−	−	−/NA	CAZ	V
L1210/05 K	Corneal scrapings	73.2	−/−	NF	NF	Sensitive	I
L1389/04 K	Corneal scrapings	80.9	−/+	+	+/SA	Sensitive	IV
L1467/04 K	Corneal scrapings	80.9	+/+	+	+/SA	CIP, GEN, OFX	II
L1468/04 K	Corneal scrapings	76	+/+	+	+/SA	CIP, GEN, OFX	II
L1580/05 K	Corneal scraping	80	−/+	+	+	Sensitive	VI
L1687/04 E	Vitreous	50.1	−/−	−	−/NA	Sensitive	V
L1695/04 K	Corneal scrapings	80.5	+/−	NF	NF	CIP, OFX	IX
L1752/04 E	Vitreous	80.5	+/+	−	−/NA	CAZ, CIP, GEN, OFX	II
L1902/04 E	Vitreous	80	+/−	−	−/NA	CHL, CIP, GEN, OFX, CAZ, MET	I
L1916/04 E	Vitreous	80	+/+	+	+/SA	AMK, CIP, GEN, OFX, CAZ, MET	III
L1938/04 E	Vitreous	80.5	−/+	−	−/NA	Sensitive	I
L1957/04 E	Vitreous	80	−/+	−	−/NA	AMK	V
L1994/04 K	Corneal scrapings	80.5	+/+	−	+/SA	OXA	III
L2036/04 K	Corneal scrapings	80.5	−/+	−	−/NA	Sensitive	III
L2087/04 K	Corneal scrapings	80.5	−/+	+	+/SA	CIP, GEN, OFX	X
L2092/04 K	Corneal scrapings	80.5	−/+	+	+/SA	AMK, CIP, OFX, CAZ, MET	III
L2613/04 E	Vitreous	76	+/+	−	+/SA	CFZ, CIP, CAZ, OXA	III
L2795/04 K	Corneal scrapings	80.5	+/+	+	+/SA	CFZ, CIP, OFX, CAZ, MET	I
L2943/04 K	Corneal scrapings	80	+/+	+	+/SA	CIP, GEN. OFX, CAZ	III
L3079/04 K	Corneal scrapings	73	+/+	+	+/SA	CIP, GEN, OFX, OXA	I
C1	Conjunctiva	97.8	+/+	−	−NA	OXA	I
C2	Conjunctiva	80.5	−/−	−	−/NA	Sensitive	I
C3	Conjunctiva	74.3	+/+	−	+/SA	CFZ, GEN, OFX, CAZ, OXA	V
C4	Conjunctiva	69	+/+	−	−/NA	Sensitive	VI
C5	Conjunctiva	67	+/+	NF	NF	Sensitive	II
C6	Conjunctiva	80	+/−	−	−/WA	CIP, OFX, MET	I
C7	Conjunctiva	73.2	+/+	−	+/SA	GEN	III
C8	Conjunctiva	80.5	+/+	−	−/WA	Sensitive	I
C9	Conjunctiva	80	+/−	−	−/NA	GEN, CAZ	I
C10	Conjunctiva	80.5	−/+	−	−/NA	Sensitive	III
C11	Conjunctiva	80.5	−/−	−	−/NA	Sensitive	I
C12	Conjunctiva	73.2	−/+	−	−/WA	Sensitive	I
C13	Conjunctiva	80.5	+/+	−	−/WA	Sensitive	I
C14	Conjunctiva	73.2	+/+	−	−/WA	Sensitive	I
RP62A	Reference strain	97.9	+/+	+	+	CFZ, CIP, GEN, OFX, MET	III
ATCC12228	Reference strain	100	−/−	−	−	Sensitive	I
ATCC35983	Reference strain	100	+/+	−	−	MET	VI

CRA, Congo red agar; AMK, amikacin; CFZ, cefazolin; CAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; MET, methicillin; OFX, ofloxacin; OXA, oxacillin; GEN, gentamicin; NA, nonadherent; WA, weakly adherent; SA, strongly adherent; NF, not found.

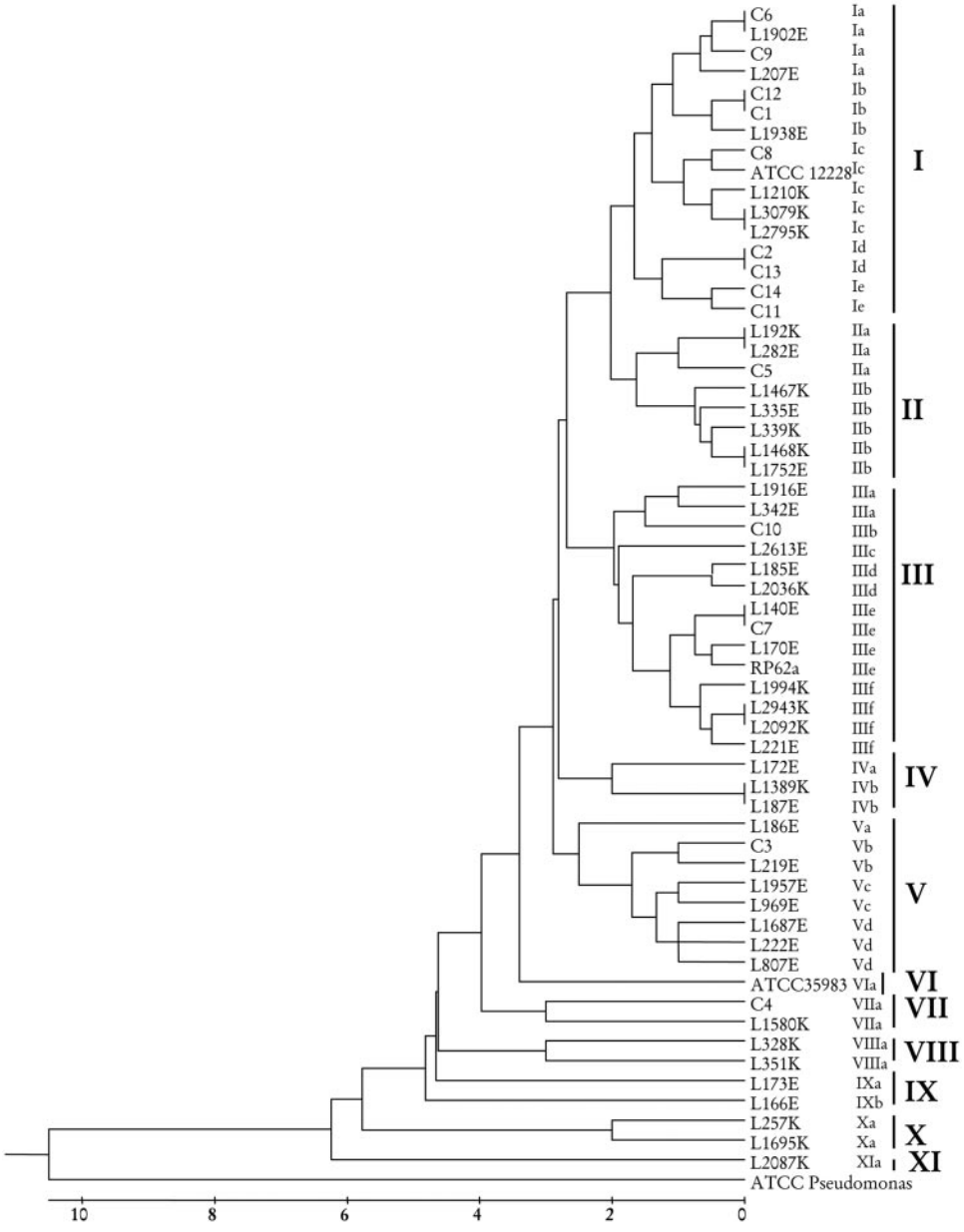

FIGURE 1. Dendrograms constructed by UPGMA showing 11 clusters from all 60 isolates, depicting genetic relatedness among the isolates.

was detected in 71% and 30% of keratitis and endophthalmitis isolates, respectively, whereas it was detected in 78% and 22% by the microtiter plate method.

Fluorescence-Amplified Fragment Length Polymorphism

A total of 60 isolates that were examined by FAFLP with a single primer combination used, generated a total of 31 to 56 differently sized fragments experimentally ranging in size from 50 to 500 bp for all the isolates. FAFLP amplitypes of *S. epidermidis* isolates showed a larger number of small fragments (within a range of 50 to 290 bp). For this study, we defined a cutoff of 99% similarity (S) as the identity level. Therefore, individual isolates of *S. epidermidis* producing FAFLP profiles with $S \geq 99\%$ (having $\leq 1\%$ of difference) are likely to be identical clones.

Dendrograms constructed by UPGMA, by using binary data generated through FAFLP profiles, produced 11 clusters from all the 60 isolates, depicting genetic relatedness among the isolates (Fig. 1). The degree of polymorphism found in *S. epidermidis* isolates in our study is at the level of 6.3%. The maximum number of isolates fell in two main clusters—I ($n = 16$) and III ($n = 14$)—which comprised five and six subclusters, respectively. Cluster I encompassed subcluster Id and Ie, wherein all the isolates were from control subjects. The other three subclusters had commensal isolates grouped with either keratitis (Ib, Ic) or endophthalmitis isolates (Ia). Cluster III consisted of six subclusters, which had a mixture of isolates of keratitis and endophthalmitis. Pathogenic reference strain RP62A was grouped in this cluster. Only two isolates (C7, C10) from the commensal group were present in this cluster. The main clusters II and IV consisted of only two subclusters each. Six isolates belonging to these subclusters were 100% identical. Cluster V had four subclusters, each containing isolates that were 99% identical, and all the isolates in this cluster were from endophthalmitis except one. ATCC 35983 did not group

TABLE 2. Significant Association of Common FAFLP Markers with Any Group of the Isolates and Their Mapping to Corresponding Coding Sequences (CDS) in the Genome

FAFLP Marker: FAFLP Fragment and Corresponding Locus, Gene Name, Putative Identification and Annotation	Controls (n = 14)	Keratitis (n = 18)	Endophthalmitis (n = 24)	ATCC 12228	ATCC 35983	RP 62A	P
98bp, *sepA*,							C vs. K 0.47
Zinc metalloproteinasc aureolysin,	P - 10	P - 10	P - 4	0	0	1	**E vs. C 0.001**
Degradation of proteins.	A - 4	A - 8	A - 20				**K vs. E 0.018**
134bp, *ebh*,							C vs. K 0.06
Fibronectin binding protein,	P - 8	P - 4	P - 19	1	1	1	E vs. C 0.265
Pathogenesis	A - 6	A - 14	A - 5				**K vs. E 0.0004**
139bp, *tagD*,							C vs. K 0.69
Glycero-3-phosphate cytidylyltransferase,	P - 3	P - 6	P - 16	0	0	1	**E vs. C 0.017**
Teichoic acid biosynthesis protein, biosynthesis of degradation of Murein sacculus & Peptidoglycan	A - 11	A - 12	A - 8				K vs. E 0.059
97bp, *SE2041*,							C vs. K 0.124
Glutamate synthetase-related protein	P - 12	P - 10	P - 6	0	0	0	**E vs. C 0.0005**
	A - 2	A - 8	A - 18				K vs. E 0.058
185bp, *pycA*,							C vs. K 1.0
Pyruvate decarboxylase	P - 1	P - 1	P - 8	0	0	0	E vs. C 0.11
Energy metabolism	A - 13	A - 17	A - 16				**K vs. E 0.054**
178bp, *ptsI*,							C vs. K 0.063
Phosphoenol pyruvate phosphotransferase	P - 5	P - 1	P - 12	0	0	0	E vs. C 0.505
Signal transduction	A - 9	A - 17	A - 12				**K vs. E 0.002**
145bp, *spoVG*,							**C vs. K 0.026**
Septation protein	P - 8	P - 3	P - 1	1	0	0	**E vs. C 0.0004**
Cell division	A - 6	A - 15	A - 23				K vs. E 0.297

Data are the number present (P) and absent (A). C, controls; K, keratitis; E, endophthalmitis. Bold faced items indicate the comparisons with significant P value ($P \leq 0.05$).

with any of the isolates included in the study. All other clusters (VI–XI) comprised only one or two isolates. There were two isolates each in clusters VIII, X, and IX, the former clusters (VIII, X) of keratitis and the latter (IX) of endophthalmitis isolates. Overall, the results showed that patients with endophthalmitis and control subjects tended to cluster separately. In contrast, the keratitis isolates were distributed evenly in many clusters, with the exception of clusters VIII and X and subcluster Ic. The neighbor-joining method generated similar clustering of isolates as did the UPGMA method, except one minor cluster of three isolates (IIa: C5, L192K, L282E). These isolates in cluster II of the UPGMA tree grouped in cluster I of neighbor-joining tree.

In Silico AFLP Analysis

Further investigation of all the 56 isolates for the presence of common FAFLP bands/markers specific for any one of the three groups revealed five signature fragments that were highly associated with isolates of one or the other group (Table 2). FAFLP marker bands unique among the maximum number of isolates of a particular group were mapped to corresponding CDS and their annotated functions are tabulated in Table 2. The difference in the presence and absence of signature bands between the diseased and control subjects was statistically significant (Table 2).

DISCUSSION

In the present study, *S. epidermidis* isolates obtained from normal control subjects were found to be sensitive to two or more antibiotics (86%), whereas 50% of the isolates belonging to both the disease groups showed multiple drug resistance (Table 1). Increased sensitivity of control subjects to various antibiotics has been shown earlier.[25,26] Antimicrobial susceptibility testing revealed methicillin resistance (MR) to be high among the isolates belonging to the disease groups (36%).[27]

All the isolates from the commensal group were negative for biofilm production by the CRA method, and only 15% (n = 2) of them were positive by microtiter assay. In contrast, 71% of commensal isolates showed *icaAB* amplification. This indicates that the presence of *icaAB* does not necessarily express biofilm production. Rohde et al.[28] also found that virulent gene detection was not useful in discriminating invasive and commensal isolates. A high percentage of keratitis isolates showed positive results for *icaAB* and biofilm production by CRA and microtiter test in comparison to endophthalmitis isolates (CRA: 71% vs. 30%, P = 0.0116; microtiter plate: 78% vs. 22%, P = 0.0063). This observation implies that adherence or attachment has relatively more important role in surface infections such as keratitis, and hence biofilm-forming capability is probably more critical for the pathogenesis of keratitis than for that of endophthalmitis.

All the subclusters in the major clusters I, II, III, and V are likely to be identical clones as their FAFLP profiles showed ≤1% of difference (S ≥ 99%) except one subcluster IIIb (Fig. 1). Thus, major clusters having isolates from disease groups were homogeneous with all their subclusters comprising identical clones except cluster IV. Minor heterogeneity was seen in the subclusters of the remaining clusters (VI–XI).

FAFLP could not discriminate the isolates of two different disease entities, as the distribution of the keratitis and endophthalmitis isolates in many clusters were overlapping, although in clusters VIII and X, the isolates exclusively belonged to keratitis, and in cluster IX they belonged to endophthalmitis. Isolates from control subjects and endophthalmitis formed the distinct clusters I and V, respectively, which may indicate their unique genetic makeup. Ten isolates among the control subjects, including the reference commensal strain ATCC12228, formed a distinct cluster that appears to be very significant. In

comparison, keratitis isolates were distributed all over the dendrogram. Earlier studies on *S. epidermidis* from nosocomial infections have also shown the existence of genetic differences between the pathogenic and normal isolates.[7,8,29]

Dendrogram analysis of isolates from the three groups revealed that disease-causing isolates may have evolved by clonal expansion of representative isolates of the normal commensal population. At least one isolate from the control group clustered with isolates of all the major clusters. Cluster V comprised only endophthalmitis isolates with the exception of C3 (commensal), a distinct genotypic cluster that may represent isolates with higher invasive capacity (Fig. 1). This C3 was resistant to five antibiotics indicating acquisition of potential genes required for pathogenesis. The apparent differences between the *S. epidermidis* isolates from control subjects and endophthalmitis groups may be because of a varying degree of pathogenicity. All the isolates of endophthalmitis in cluster V had 97% similarity, and isolates from control subjects in cluster I were 99% identical. Such observations imply that the invasive endophthalmitis isolates are clonally expanding with more heterogeneity among themselves, unlike the isolates from the commensal group.

The presence of unique signature bands in the three groups of isolates might give additional information on the factors essential for the development of infection. In silico analysis showed that most of the *S. epidermidis* isolates from endophthalmitis had differential amplification of three genomic regions mapped to the *ebh*, *tagD*, and *ptsI* genes in comparison with keratitis isolates (Table 2). Most of the keratitis isolates did not show amplification of these three genes, perhaps because of mutation. Genes such as *ebh* (or *embp*), *tagD*, and *ptsI* encode for extracellular matrix-binding protein homologue (a fibronectin binding protein), teichoic acid biosynthesis protein, and phospho-enol pyruvate phosphotransferase (involved in energy and signal transduction), respectively. These three molecules seem to have definite roles in human infections.[30–32] The recombinant embp or Ebh protein from both *S. epidermidis* and *S. aureus* has been found to bind human fibronectin specifically.[30] Ebh has been shown to be produced during human infection, as serum samples taken from patients with confirmed *S. aureus* infections were found to contain anti-Ebh antibodies. *S. aureus* Ebh has 57% protein similarity and 39% identity with that of *S. epidermidis* Ebh. TagD is glycerol-3-phosphate cytidylyltransferase, a precursor protein involved in biosynthesis of teichoic acid in *S. aureus*.[31] The penicillin-binding protein (*pbp4*) gene that is responsible for intrinsic β-lactam resistance in *S. aureus* is flanked downstream by the open reading frame *tagD*.[32] It has been shown earlier that precise deletion of *tagD* and controlled depletion of its product, leads to irregular morphology and lysis of *Bacillus subtilis* growing at physiological temperature.[33] *S. epidermidis* TagD has 98% similarity and 95% identity to the *S. aureus* TagD as well 88% similarity and 69% identity to the *B. subtilis* TagD. One of the recent studies showed LD50 of the *S. aureus ptsI* mutant for mice to be more than 10 times higher than the 50% lethal dose for the virulent parent strain, indicating that the mutation affects virulence.[34] *S. epidermidis PtsI* has 96% similarity and 89% identity to the *PtsI* of *S. aureus* and is very likely to have a similar role to enact. Keratitis isolates have modified *ebh*, *tagD*, and *ptsI* genes (Table 2) that may not have vital roles in the disease process, as their disease etiology is only superficial, whereas all of them appear to be essential in the endophthalmitis isolates.

In silico extrapolation of most of the isolates from control subjects had differential amplification of three genomic regions mapped to *sepA*, *spoVG*, and glutamate synthase. These markers showed no amplification among the 72% to 96% of isolates that belonged to endophthalmitis (Table 2). *sepA* encodes zinc metalloproteinase aureolysin, an extracellular elastase. Approximately 84% of endophthalmitis isolates showed no amplification of *sepA*, implying that there is modification of this locus. Seventy-two percent of commensal isolates showed amplification of *sepA*. Normal carriage isolates are usually noninvasive, and it appears that the presence of this exoenzyme alone may not be sufficient to gain invasive capacity. However, with an opportunity such as gaining entry into an intraocular chamber or deeper tissues, disease-causing isolates mutate into more virulent invasive types by recruiting unknown proteins or by modifying certain exoenzymes that help in growth and sustenance in such environments.[35] Endophthalmitis isolates in the present study possibly have faced the same consequences leading to the modification of *sepA*.

In conclusion, this study showed low genetic variability among the *S. epidermidis* isolates distributed in three groups. Biofilm-forming capability was found to be more critical in the pathogenesis of keratitis than in that of endophthalmitis. FAFLP showed that most control isolates formed a distinct cluster revealing the uniqueness in their genomes (cluster I). This finding is likely to be significant, since the in silico extrapolation of the control subject group of isolates also had differential amplification of three genomic regions mapped to *sepA*, *spoVG*, and glutamate synthase (compared to endophthalmitis isolates). Keratitis isolates showed a marked difference from control isolates by the absence of *spoVG*. In addition, there was no amplification of *pstS* and SE0889 CDS in all the control isolates, when compared with endophthalmitis and keratitis isolates, respectively. These observations suggest subtle genetic differences between control and diseased group isolates, since the degree of polymorphism found in *S. epidermidis* isolates in this study is very low (6.3%). It is likely that the *S. epidermidis* genotype that colonizes the periocular region of the human eye can evolve into an ocular pathogen essentially when there is a change in environment with the organism's accidental entry into the sterile inner tissues of the eye; and strains from some clonal lineages may become more virulent than others, because of subtle genetic changes in them. This is the first study to show the comparative genome profiling of ocular isolates. The high association of certain genes/open reading frames (ORFs) in the endophthalmitis isolates detected in this study could indicate their potential to serve as virulence markers; however, such a use should be confirmed by in vitro and in vivo expression studies.

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