Twenty-seven (57.4%) of the 47 volunteers and 17 (19.1%) of the 89 patients used nonantibiotics eyedrops such as glaucoma medications, artificial tears, and anticataract eyedrops. *Staphylococcus* species were isolated from the conjunctiva of nine (33.3%) of 27 volunteers who used eyedrops and six (30%) of 20 volunteers who did not use eyedrops. *Staphylococcus* species were isolated from the conjunctiva of 12 (57.1%) of 21 patients who used eyedrops and 30 (44.1%) of 68 patients who did not use eyedrops. The differences in the culture-positive rate between the two groups were not statistically significant. Additionally, the number and type of eyedrops did not influence the culture-positive rate.

Staphylococcus species were isolated from the conjunctiva of six (60%) of 10 men and nine (24.3%) of 28 women in the volunteer group, and the conjunctiva of 19 (65.5%) of 29 men in the patient group and 25 (41.7%) of 60 women in the patient group. The culture-positive rate of *Staphylococcus* species in men was significantly higher than in women ($P \leq .05$).

PCR was performed on the DNA of the two reference strains of *S epidermidis*, on the 10 isolates from the conjunctiva of volunteers, on the 40 isolates from the facial skin of volunteers, and on the 36 isolates from the conjunctiva of the precataract patients. As expected, the *icaA* gene was detected in the biofilm-forming strain (GTC1836), but not in the non–biofilm-forming strain (GTC289) (Figure 1).

Six (60%) of 10 isolates from the conjunctiva of volunteers and only six (15%) of 40 isolates from the facial skin of the volunteers carried the *icaA* gene (Table 1). This difference was statistically significant ($P \leq .01$). In the precataract patients, 25 (69.4%) of 36 isolates from the conjunctiva had the *icaA* gene.

The CRA method was applied to the two reference strains, 10 isolates from the conjunctiva of volunteers, 40 isolates from the facial skin of volunteers, and 36 *S epidermidis* strains isolated from the conjunctiva of the precataract patients. Photographs of the reference strains and some of the other isolates are shown in Figure 2. The colonies of the biofilm-positive strain GTC1836 appeared black, and the colonies of the biofilm-negative strain appeared red.

Five (50%) of 10 isolates from the conjunctiva of volunteers formed black colonies, whereas only two (5%) of 40 isolates from the facial skin of the volunteers formed black colonies (Table 1). This difference was statistically significant ($P \leq .01$). Sixteen (44.4%) of 36 isolates from the conjunctiva of the precataract patients formed black colonies. Most importantly, all strains that formed black colonies carried the *icaA* gene.

The biofilm-forming ability was determined quantitatively by the microtiter plate assay. The OD at 490 nm of the biofilm-positive reference strain was 0.8708 and that for the biofilm-negative strain was 0.0220. The mean ODs of the strains from the conjunctiva and skin of volunteers were

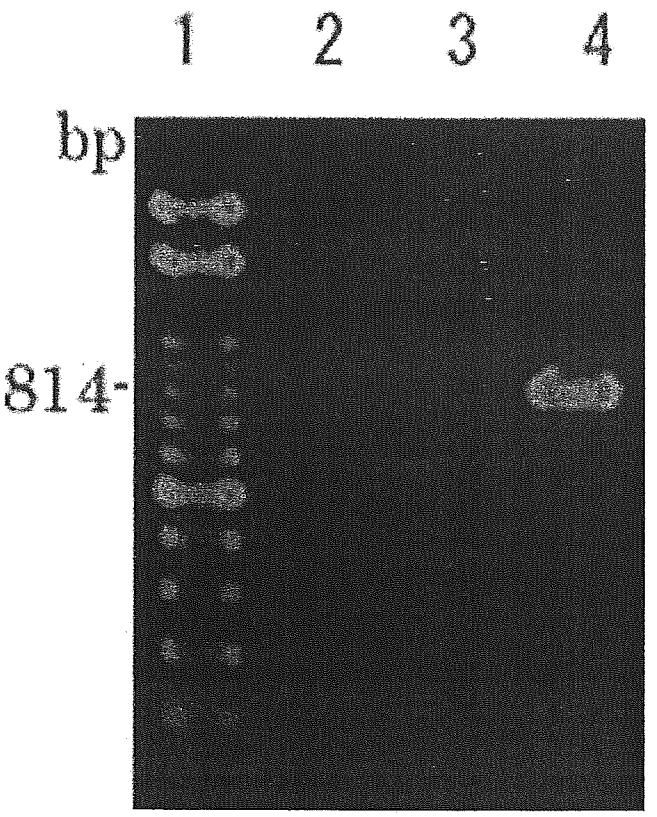

FIGURE 1. Agarose gel electrophoresis of polymerase chain reaction products for *icaA* gene of reference strains. Lane 1, 100-bp molecular marker; lane 2, negative (DNA template absent); lane 3, *S epidermidis* biofilm-negative strain (GTC289); lane 4, *S epidermidis* biofilm-positive strain (GTC1836).

0.2429 ± 0.2781, and 0.0521 ± 0.0375 (Table 1). This difference was statistically significant ($P \leq .05$). The mean ODs of the strains from the conjunctiva and skin of volunteers that were positive with the CRA test were 0.4211 ± 0.3058 and 0.1767 ± 0.0676. Although there were only five CRA-positive strains from conjunctiva and two from the facial skin, the ODs for conjunctival isolates were higher than that of the skin isolates (Figure 3). The mean OD of the strains from the conjunctiva of the precataract patients was 0.2352 ± 0.2832, and the mean ODs of the CRA-positive strains was 0.4831 ± 0.2839 (Figure 3).

Nine strains were isolated from the conjunctiva and facial skin of same volunteers. Six of the nine strains from the conjunctiva carried the *icaA* gene, and five of nine strains grew black colonies on the CRA plates. Only one of the facial skin isolates had the *icaA* gene but not the phenotype of biofilm-forming strains (Table 2, Figure 4)

DISCUSSION

THESE RESULTS CLEARLY DEMONSTRATE THAT THE PREValence of *S epidermidis* strains with biofilm-forming ability

TABLE 1. Relationship Between *icaA* Gene, CRA Test, and Microtiter Plate Assay for Biofilm Production for Conjunctival and Facial Skin Isolates

Sample	*icaA* Positive, n (%)	CRA Positive, n (%)†	OD_{490} (mean ± SD)
Volunteers			
Conjunctival	6/10 (60)*	5/10 (50)**	0.2429 ± 0.2781***
Facial skins	6/40 (15)*	2/40 (5)**	0.0512 ± 0.0375***
Patients			
Conjunctival	25/36 (69.4)	16/36 (44.4)	0.2352 ± 0.2832

OD_{490} = Microtiter plate assay.
†Positive strains for CRA test had *icaA* gene.
*$P = .002$; **$P = 2 \times 10^{-4}$; ***$P = .03$.

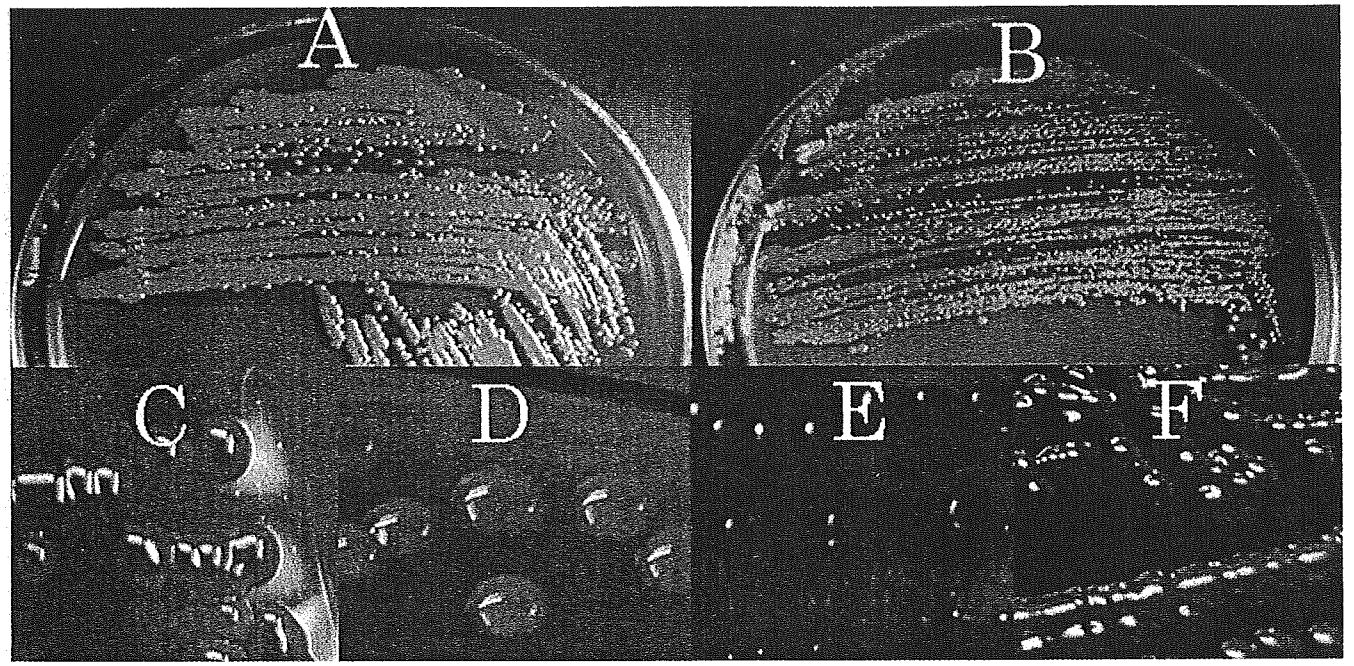

FIGURE 2. Biofilm-forming test using Congo red agar plate of reference strains and some clinical isolates. (A) *S epidermidis* biofilm-negative strain (GTC289). (B) *S epidermidis* biofilm-positive strain (GTC1836). (C) Isolate from facial skin of volunteer. (D) Isolate from facial skin of volunteer. (E) Isolate from conjunctiva of volunteer. (F) Isolate from conjunctiva of precataract patient. Colonies of A, C, and D strain form red colonies, and colonies of B, E, and F strain form black colonies.

was higher in the isolates from the conjunctival sac than from the facial skin in the volunteer group. Our results also demonstrate that a high percentage of strains from the conjunctival sac produced significant amount of biofilm (positive strain of CRA test).

We also investigated the characteristics that could influence the culture-positive rate of *Staphylococcus* species in the conjunctival sac of volunteers and patients. We found a higher rate in men, but not in those who used nonantibiotic eyedrops. These differences influenced the prevalence. The rate in men of the patient group was higher than in the volunteer group, so the culture-positive rate of *Staphylococcus* species in the patients might be higher. Thus, we probably would have obtained a similar prevalence in volunteers if we had cultured *Staphylococcus* species from precataract patient under similar conditions. However, we could not easily confirm that the prevalence of *S epidermidis* strains with biofilm-forming ability might be also higher in the isolates from the conjunctival sac in precataract patients.

These findings are important because the adhesion of bacteria to IOLs during surgery has been described to be an important factor in the pathogenesis of postoperative endophthalmitis and chronic intraocular inflammations in pseudophakic eyes,[14–18] because biofilm formation is well correlated with bacterial attachment to the IOL surface.[9,10]

We cannot fully explain why the prevalence of biofilm-forming *S epidermidis* is higher in the conjunctival sac

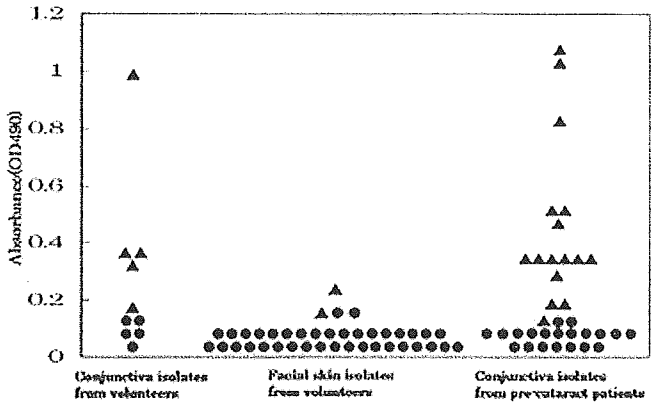

FIGURE 3. Degree of biofilm production by conjunctival and facial skin strains from volunteers and precataract patients. Solid circles, optical density (OD) of strains not positive for CRA test; solid triangles, OD of strains positive for CRA test.

TABLE 2. Comparison of Nine Conjunctival and Facial Skin Isolates From the Same Healthy Volunteer

	Strains From Conjunctiva		Strains From Facial Skin	
Case	*icaA* Gene*	CRA	*icaA* Gene*	CRA Test
1	+	+	+	+
2	+	+	−	−
3	−	−	−	−
4	−	−	−	−
5	+	+	−	−
6	+	+	−	−
7	+	+	−	−
8	−	−	−	−
9	+	−	−	−

*Results of detecting *icaA* gene in Figure 4.

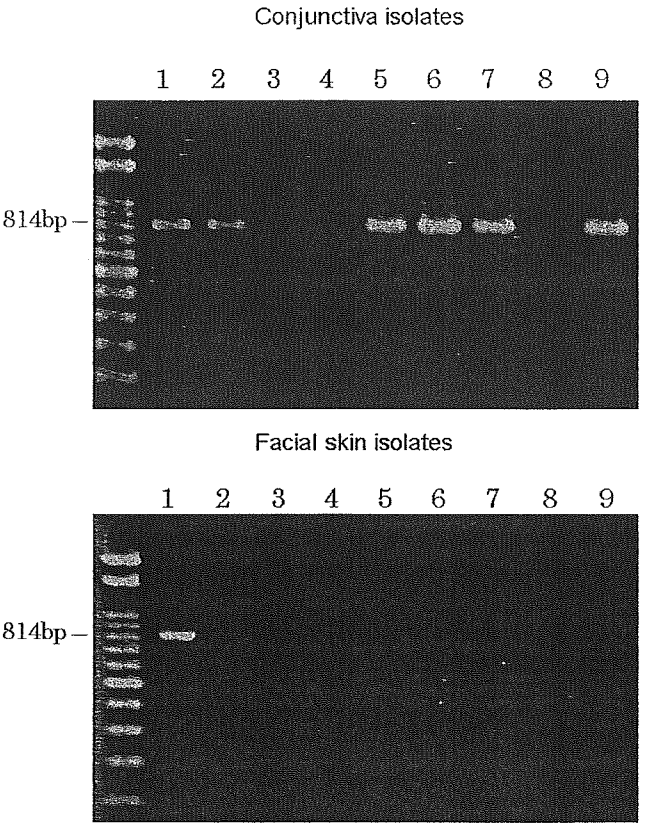

FIGURE 4. Agarose gel electrophoresis of polymerase chain reaction products for *icaA* gene of strains isolated from conjunctiva (top) and facial skin (bottom) of same volunteers. Strain number at top corresponds to strain number at bottom and case number in Table 2.

microflora. Nevertheless, similar observations have been described for the prevalence of biofilm-forming *S epidermidis* strains recovered from the skin.[19,20] In the 52 isolates of *S epidermidis* obtained from blood cultures and the 51 from cerebrospinal fluid,[1] 85% had the *ica* locus, whereas only 2% of the 36 isolates from the skin and mucosa carried the *ica* locus in healthy volunteers.[19] Similarly, 33 (49%) of 68 isolates associated with intravenous catheter-related infections were *icaA* positive, compared with none of the 10 isolates from the skin or mucosa of healthy volunteers.[20] These findings are in good agreement with our observations that the prevalence of biofilm-forming isolates from facial skin was lower than those from the conjunctival sac.

Nine pairs of strains were isolated from the conjunctival sac and skin of the same individual, and six of the conjunctival sac isolates carried the *icaA* gene. It can be postulated that the *S epidermidis* strains in the conjunctival sac may be more prone to acquire the *ica* locus, or strains with the *ica* locus from the other sites of the body can enter and remain in the conjunctival sac as a regular resident. It may be important that the difference in the conjunctival microenvironment from the skin—the presence of tears that contain abundant antimicrobial proteins such as lysozymes and lactoferrin, or mechanical flushing of the conjunctival sac by periodic blinking—can lead to the selective survival of the biofilm-forming bacteria in the conjunctival sac. Further investigations are necessary to test this hypothesis.

The formation of biofilms is thought to occur in two steps: a rapid attachment of the microorganism to the inserted plastic surface, followed by the formation of mature biofilm.[11,21–24] Biochemically, extracellular polysaccharides such as PIA play an important role in biofilm formation. Capsular polysaccharide adhesion (PS/A) molecules,[22] and several other proteins have also been proposed to mediate the initial adherence of *S epidermidis*, whereas the buildup of bacterial cells is due to the production of the PIA proteins.[24] PIA is encoded by the *ica* operon,[24] although recent reports suggest that this operon also encodes PS/A, and PS/A and PIA are chemically related.[6] However, the genetic ability to produce biofilm

does not necessarily mean that the phenotype was expressed in these experiments. We need further investigations to determine what suppresses the expression of the phenotype.

The strains of *S epidermidis* that form biofilm resist many of the antimicrobial agents—for example, ofloxacin—that are commonly used during the perioperative period.[25,26] Thus, if biofilm-forming *S epidermidis* should escape a variety of presurgical prophylaxis treatment, it has an opportunity to enter the anterior chamber attached to the IOLs or surgical instruments during surgery and to grow to develop suppurative endophthalmitis. Thus, an efficient detection of the *icaA* gene as well as the effective suppression of PIA or PS/A synthesis would be feasible strategies for the prevention of the postoperative endophthalmitis.

In recent years, *S epidermidis* has emerged as a common cause of nosocomial infections—for example, the septicemia associated with the use of intravenous catheters and other medical devices.[27] The high prevalence of biofilm-forming *S epidermidis* in the conjunctival microflora is important when the pathogenic mechanisms of extra- or intraocular infections, such as conjunctivitis, keratitis, and endophthalmitis, are considered. However, there is no report describing whether *S epidermidis* strains detected from endophthalmitis cases have biofilm-forming ability. This should be determined in the future.

Our data demonstrate that the prevalence of biofilm-producing strains of *S epidermidis* is higher in the conjunctival than facial skin microflora. This is important because *S epidermidis* is a common pathogen in cases of endophthalmitis. Thus, the best method to eliminate *S epidermidis* before the surgery must be determined.

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