Establishing \textit{in vitro} and \textit{in vivo} Co-culture Models of \textit{Staphylococcus epidermidis} and \textit{Enterococcus faecalis} to Evaluate the Effect of Topical Fluoroquinolone on Ocular Microbes

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\textbf{Purpose:} To establish \textit{in vitro} and \textit{in vivo} ocular co-culture models of \textit{Staphylococcus epidermidis} and \textit{Enterococcus faecalis} and to study how various concentrations of moxifloxacin affect the survival of these two endophthalmitis-causing bacteria.

\textbf{Methods:} Standard strains of \textit{S. epidermidis} and \textit{E. faecalis} were used. Color detection agar plates were employed to distinguish their colonies. To establish the \textit{in vitro} and \textit{in vivo} co-culture models, \textit{S. epidermidis} and \textit{E. faecalis} were co-cultivated at different ratios for various periods. For the \textit{in vivo} model, various volumes and concentrations of either a mono-culture or co-culture were inoculated into the lower conjunctival sac of rabbits. Finally, the newly developed \textit{in vitro} and \textit{in vivo} co-culture models were subjected to the moxifloxacin treatment to access its effect on \textit{S. epidermidis} and \textit{E. faecalis}.

\textbf{Results:} When \textit{S. epidermidis} and \textit{E. faecalis} were cultured separately in tryptic soy broth, their growth peaked and plateaued at approximately 16 and 6 h, respectively. When they were co-cultured, the growth peak of \textit{S. epidermidis} got delayed, whereas the growth peak of \textit{E. faecalis} did not change. The number of \textit{E. faecalis} was significantly higher in the co-culture than that in the mono-culture. Treatment with moxifloxacin in the \textit{in vitro} co-culture model rapidly decreased the number of \textit{S. epidermidis} cells at doses $\geq 0.125 \mu$g/ml. In contrast, the number of \textit{E. faecalis} did not change significantly up to $16 \mu$g/ml moxifloxacin. In \textit{in vivo} co-culture (at 1:1), the \textit{S. epidermidis} count decreased in a pattern similar to that seen in \textit{in vivo} mono-culture and was barely detectable at 24 h after inoculation. In contrast, the \textit{E. faecalis} count increased up to 16 h and then decreased. When moxifloxacin was applied (zero, one, or two times) to this model, the \textit{S. epidermidis} count decreased in proportion to the number of treatments. In contrast, the \textit{E. faecalis} count increased with moxifloxacin treatment.
INTRODUCTION

Cataract surgery is one of the most common surgical procedures worldwide. Owing to advancements in surgical techniques and materials, cataract surgery is now faster and safer (1). Nonetheless, various complications, including posterior capsular tear, loss of endothelial cells, corneal stromal burn, retinal detachment, and postoperative endophthalmitis (PE), still occur (2). Acute PE is a rare prevalence 0.023–0.26% but devastating complication of cataract surgery (3–5).

PE is initiated by the entry of microorganisms into the intraocular space, and the type of invading microorganism is a critical determinant of visual outcomes (6, 7). Because most of the bacteria causing PE come from commensal conjunctival microbes (8), it is important to confirm the presence of certain bacteria on the ocular surface and to monitor changes in this bacterial community.

Recently, it has been reported that the distribution of the causative bacteria of postoperative PE changed in Korea, Sweden, and Taiwan (9–11). These changes may reflect alterations of conjunctival microflora in the PE patients. Nonetheless, it would be difficult to determine the changes in this ocular surface microbial community by human clinical trials because the incidence of endophthalmitis is too low to meet statistical power requirements of a study population. Therefore, we decided to investigate the changes in the causative bacteria of PE by constructing an experimental co-culture model using representative bacteria occurring on the ocular surface. In vitro and in vivo co-culture models based on various microbial species have enabled research into cell-cell interactions among eukaryotic and bacterial species and helped to mimic complex in vivo environment (12).

A healthy and balanced microbial community is essential for maintaining metabolic, physiological, and immunological functions. Commensal microorganisms of the ocular surface provide defense against competitors, by inhibiting the growth of more virulent bacterial strains (13). Nevertheless, a variety of factors, such as age, dry eye, contact lens use, immunocompromising diseases, medications, and environmental factors can shift commensal-microflora composition on the ocular surface (13–16). Such a shift may occur in response to increased use of topical antibiotics intended to prevent ocular infection. Previous studies reporting that the distribution of isolates causing PE is changing from coagulase-negative staphylococci (CNS) to enterococci suggest that external factors have altered the relationship between the two most common bacterial taxa in the conjunctiva (9–11).

Fluoroquinolones are bactericidal antibiotics that directly inhibit bacterial DNA synthesis (17). Topical fluoroquinolones are the prophylactic antibiotics most commonly used in ophthalmic surgery (18). Although no randomized clinical trials of surgical prophylaxis for controlling ocular infection have been conducted, topical fluoroquinolones are routinely employed perioperatively in eye clinics (19). The first three topical fluoroquinolones introduced as topical eye drops were 0.3% ciprofloxacin, 0.3% ofloxacin, and 0.3% norfloxacin. Subsequently, next generation fluoroquinolones, including 0.5% levofloxacin, 0.3% gatifloxin, and 0.5% moxifloxacin, have been introduced. The most important attribute of a later generation fluoroquinolones is their enhanced activity against gram-positive bacteria relative to that of the previous generation of fluoroquinolones (17). Currently, a moxifloxacin solution, a fourth-generation fluoroquinolone, is the most popular topical eye antibiotic for ophthalmic surgery. Of note, the fluoroquinolones possess high antimicrobial activity against staphylococci, as confirmed in some studies; however, these drugs are known to have poor antimicrobial activity against Enterococcus faecalis (20–23). The selective antimicrobial activity of a fluoroquinolone may have an effect on the alteration of microbial community on the ocular surface and induce changes in causative isolates of PE from coagulase-negative staphylococci to enterococci.

At present, however the experimental studies have not been attempted to elucidate the mechanism underlying the changes in causative bacteria of PE. Here, we hypothesized that the major factor altering of causative isolates of PE is perioperative application of ophthalmic antibiotics such as moxifloxacin to the conjunctiva of patients (24). To test this hypothesis, we constructed in vitro and in vivo co-culture models by representative causative pathogens of PE: S. epidermidis and E. faecalis. Then, the models were subjected to a fluoroquinolone ophthalmic antibiotic, and responses of the two bacterial species to the antibiotic were examined.

METHODS

Bacterial Strains and Growth Conditions

Standard strains of E. faecalis (ATCC29212) and S. epidermidis (KCTC1917; equivalent to ATCC12228) were purchased from the American Type Culture Collection and the Korean Collection of Type Cultures, respectively. Both strains were cultured either in tryptic soy broth (TSB) or on tryptic soy agar (TSA) at 37°C. These standard strains were used to set up the in vitro and in vivo co-culture models.

Conclusions: The in vitro and in vivo co-culture models of S. epidermidis and E. faecalis were established to determine the influence of moxifloxacin eye drops on these bacteria. The results clearly show that the moxifloxacin eye drops can make E. faecalis dominant on the ocular surface.

Keywords: Enterococcus, Staphylococcus, fluoroquinolone, endophthalmitis, topical eye drops, co-culture model
In vitro Mono- and Co-cultures of *S. epidermidis* and *E. faecalis*

A single bacterial colony was streaked on a TSA plate and incubated for 24 h at 37°C. Then, colonies were collected and dispersed in TSB, and the suspension was diluted to optical density at 600 nm (OD600) of 0.1. The suspensions of the standard *S. epidermidis* or *E. faecalis* strains were prepared at equal concentrations (2.5 × 10^7 CFU/mL) in 5 ml of TSB.

For the in vitro mono-culture model, each bacterial suspension was incubated for 24 h, and then 100 µl aliquots were collected at 2 h intervals for 12 and 4 h intervals thereafter. The samples were diluted 1:10^5 and 1:10^6 with a 0.85% NaCl solution, and 100 µl of each diluted bacterial suspension was spread on a TSA plate. After incubation for 24 h, the colonies on the plates were counted by means of a colony counter.

For the in vitro co-culture model, the prepared bacterial suspensions of *S. epidermidis* and *E. faecalis* (2.5 × 10^7 CFU/ml each) were mixed in equal volumes and co-cultured for 42 h. The mixture was cultivated in two media in parallel (TSB and PBS). Then, 100 µl of the bacterial suspension was collected at 2 h intervals for 16 h and at 4 h intervals thereafter. The samples were serially diluted, and 100 µl of the diluted suspension was spread on a color detection agar (CDA) plate (MB-C1611; MB-cell, Korea). On such a plate, colonies of *E. faecalis* and *S. epidermidis* can be distinguished by color: *E. faecalis* colonies are blue, whereas *S. epidermidis* colonies are white. After 24 h of incubation, the colonies of each bacterium were counted using the colony counter.

Cultivation of *S. epidermidis* and *E. faecalis* With Various Concentrations of a Culture Supernatant

A single bacterial colony was streaked on a TSA plate and incubated for 24 h at 37°C. After that, colonies were collected and dispersed and diluted in TSB to an OD600 of 0.1. The bacterial suspension was cultivated in a shaking incubator (N-Biotek, South Korea). After cultivation for 24 h, a cell-free culture supernatant was prepared by centrifuging the culture media and passing the supernatant through a 0.2 µm syringe filter (Jet biofil, China). The culture supernatants of *E. faecalis* and *S. epidermidis* were next diluted to various concentrations (50, 80, and 100%) with TSB. To measure the effects of a culture supernatant on bacterial growth, 15 µl of the bacterial suspension (2.5 × 10^7 CFU/mL) and 135 µl of each culture supernatant (separately) were mixed in a 96 well plate and incubated at 37°C with shaking. OD600 was measured on a microplate reader (Infinite M200; Tecan, USA) with 30 min intervals for 24 h to build a growth curve.

Analysis of the Impact of Moxifloxacin on *S. epidermidis* and *E. faecalis* in the in vitro Co-culture Models

*S. epidermidis* and *E. faecalis* suspensions were prepared at equal concentrations (2.5 × 10^7 CFU/ml) in TSB and mixed at a ratio of either 1:1 or 95:5 (S. *epidermidis: E. faecalis*). The co-culture prepared at the ratio of 1:1 was incubated for 18 h, and 100 µl of the resultant suspension was withdrawn and mixed with 900 µl of TSB containing various concentrations of moxifloxacin (0–16 µg/ml; Tokyo Chemical Industry, Japan), and incubated for 14 h. Next, the suspension was serially diluted and plated on CDA. The co-culture prepared at the ratio of 95:5, *S. epidermidis* 95 ul and *E. faecalis* 5 ul, was mixed with TSB (900 ul) containing various concentrations of moxifloxacin and incubated for 18 h. After that, colonies were counted in the same manner as described above.

In vivo Mono- and Co-cultures of *S. epidermidis* and *E. faecalis*

New Zealand white rabbits (4–5 kg; Hyochang Science, Korea) were used to establish in vivo mono- and co-culture models of *S. epidermidis* and *E. faecalis* infection. The rabbits were anesthetized by intramuscular injection of tiletamine-zolazepam (Virbac Korea, France) and xylazine (Bayer, Germany). Each group consisted of five rabbits, and only the right eye was subjected to the experiment.

Either a prepared bacterial suspension or topical antibiotic solution was inoculated into the lower conjunctival sac (Figures 1A,B). To avoid a loss of the solution, tarsorrhaphy was performed using continuous 6–0 nylon (Ethicon, USA) (Figure 1C). To compare the numbers of bacterial cells among the different treatment groups, the bacteria in the inferior cul-de-sac were collected with a sterile, cotton-tipped swab at different time points (4–24 h) after the inoculation (Figure 1D). The swabs were immediately soaked in 1 ml of normal saline in an Eppendorf tube and shaken at 2,800 rpm for 60 s in a microtube homogenizer (BeadBug, Benchmark Scientific, USA) to extract the bacterial cells from the swabs (Figure 1E). Next, the suspension was serially diluted and plated on CDA for differential colony counting (Figures 1F,G). After an overnight incubation at 37°C, the number of *E. faecalis* and *S. epidermidis* colonies were determined as described above (Figure 1H). The study protocol was approved by the Institutional Animal Care and Use Committee of Kosin University College of Medicine (KMAP-18-19). All experiments involving animal subjects were conducted in accordance with the guidelines and regulations of the Association for Research in Vision and Ophthalmology.

For in vivo mono-cultures of *S. epidermidis* or *E. faecalis*, 25 µl of the prepared bacterial suspension (10^9 CFU/ml) was applied to the lower conjunctival sac. For the in vivo co-culture model of *S. epidermidis* and *E. faecalis*, various conditions with different ratios of the two bacteria [1:1, 8:2, or 9:1 (*S. epidermidis: E. faecalis*)], volumes (50, 25, or 12.5 µl), and concentrations (1 × 10^9, 2 × 10^9, or 4 × 10^9 CFU/ml) were applied. Then, the bacteria were collected at 4, 8, and 16 h after the administration as described above.

Evaluation of the Effects of Topical Moxifloxacin Administration on the in vivo Co-culture Model of *S. epidermidis* and *E. faecalis*

To this end, 9 µl of a *S. epidermidis* suspension (4 × 10^9 CFU/ml) and 1 µl of an *E. faecalis* suspension (4 × 10^9 CFU/ml)
were employed. Moxifloxacin (5 µl at 0.5% concentration; Tokyo Chemical Industry Co., Japan) was applied to the lower conjunctival sac once immediately after the inoculation of the bacterial suspension or twice (additional treatment 1 h later). The number of bacterial cells in the lower conjunctiva was determined 4 h after the inoculation of the bacterial solution.

**Statistics**

All *in vitro* experiments were conducted three times independently and animal experiments were conducted at least three to five times with a group of five animals. Paired Student’s *t*-test was performed to assess the significance of differences between two groups. All statistical analyses were performed using GraphPad Prism (version 6.03). Statistical significance was defined as a *p*-value < 0.05.

**RESULTS**

**Growth Curves of *in vitro* Mono-Cultures and Co-cultures of *S. epidermidis* and *E. faecalis***

When *S. epidermidis* was cultivated alone in TSB, its growth peaked and plateaued at approximately 16 h (Figure 2A). When *E. faecalis* was cultured alone in TSB, its growth peaked and plateaued at ~6 h, which was faster than that of *S. epidermidis*. When *S. epidermidis* and *E. faecalis* were cocultivated, peak growth of *S. epidermidis* got delayed to approximately 24 h (Figure 2B). In addition, the concentration of *E. faecalis* cells was 1.86-fold higher in the co-culture with *S. epidermidis* than that in mono-culture.

**Effects of Moxifloxacin on *in vitro* Co-cultures of *S. epidermidis* and *E. faecalis***

When *S. epidermidis* and *E. faecalis* were cocultivated at the ratio of 1:1 or 95:5 for 18 h, the final cellular concentration of the two species was different (Figures 4A,B). The concentrations of *S. epidermidis* and *E. faecalis* cells were 1,925 ± 195 × 10^5 and 338 ± 68 × 10^5 CFU/ml, respectively, when cocultured at 1:1 (*p* < 0.0001, Figure 4A) and 408 ± 158 × 10^5 and 268 ± 55.0 × 10^5, respectively, when cocultured at 95:5 (*p* = 0.028, Figure 4B). This difference was similar to the result shown in Figure 2B, in which the numbers of colonies of the cocultured bacteria, mixed in the ratio of 1:1, are compared over time. In the co-culture, the *E. faecalis* count initially increased sharply at 4–6 h and then decreased, while the growth of *S. epidermidis* was initially delayed and then was good between 12 and 20 h. For this reason, the 1:1 co-culture had a significantly smaller number of *E. faecalis* cells than *S. epidermidis* cells after 18 h. Of note, the numbers of *S. epidermidis* colonies were markedly different between the 1:1 and 95:5 co-cultures, whereas the numbers of *E. faecalis* colonies were similar.

When 1:1 co-cultures were treated with moxifloxacin at various concentrations for 18 h, the numbers of *S. epidermidis*
cells rapidly decreased at 2 µg/ml moxifloxacin, while the numbers of \textit{E. faecalis} cells, did not significantly change even at a moxifloxacin concentration of 16 µg/ml (Figure 4C). On the contrary, when \textit{S. epidermidis} and \textit{E. faecalis} were mixed at 95:5 and immediately treated with moxifloxacin for 18 h (without co-cultivation for 18 h beforehand), the numbers of \textit{S. epidermidis} colonies started to decline at 0.032 µg/ml moxifloxacin, and no \textit{S. epidermidis} colonies were detectable at 0.125 µg/ml. The decrease of \textit{E. faecalis} colonies were observed at 0.125 µg/ml and no colonies at 0.25 µg/ml (Figure 4D). Therefore, when the two bacteria were cocultured for 18 h before the exposure to moxifloxacin, the resistance to this drug strengthened for bacteria. In particular, \textit{E. faecalis} co-cultured with \textit{S. epidermidis} survived with moxifloxacin above 16 µg/ml.

**In vivo Co-culture Modeling With Different Volumes and Ratios of \textit{S. epidermidis} and \textit{E. faecalis} Cell Suspensions**

In the \textit{in vivo} mono-cultures (25 µl) with the inoculum concentration of $10^9$ CFU/ml, the number of \textit{S. epidermidis} colonies gradually diminished at every time point, whereas the number of \textit{E. faecalis} colonies increased until 8 h and then decreased (Figure 5A). When these bacteria were co-cultured, the number of \textit{S. epidermidis} colonies decreased at every time point and was close to zero at 24 h, whereas the number of \textit{E. faecalis} increased until 16 h and then decreased (Figure 5B). In comparison to \textit{in vivo} mono-culture, \textit{E. faecalis} had higher number of cells for longer time in the \textit{in vivo} co-culture. When the inoculum concentration was doubled to $2 \times 10^9$ CFU/ml...
Co-culture Models of S. epidermidis and E. faecalis

**FIGURE 4** | *In vitro* co-culture modeling of S. epidermidis (SE) and E. faecalis (EF) and their responses of bacteria to moxifloxacin treatment. S. epidermidis and E. faecalis were mixed at the ratio of 1:1 and cocultured for 18 h, and their CFUs were quantified (A). Similarly, a mixture in the 95:5 ratio (SE:EF) was cultivated for 18 h (B). For the co-culture with moxifloxacin (MFX) treatment, the bacterial mixture in the 1:1 ratio was cocultured for 18 h and then treated with various concentrations of moxifloxacin. The CFUs were counted after 14 h moxifloxacin treatment (C). When S. epidermidis and E. faecalis were mixed at the ratio of 95:5, the moxifloxacin treatment was started immediately, without the 18 h culturing period, and the CFUs were counted after 18 h moxifloxacin treatment (D). Means and S.D. (n = 3) are shown as columns and error bars, respectively. Asterisks at the top of bars denote statistically significant differences (*, p < 0.05; ***, p < 0.001; student t-test).

by reducing the inoculum volume in half from 25 to 12.5 µl, the number of S. epidermidis colonies were low at 4 h, slightly increased at 8 h and then declined, which differed from the result above, and the E. faecalis count went up until 8 h and then decreased (Figure 5C).

In order to implement the antibiotic treatment, the volume of the suspension of inoculated bacteria was reduced further, and the ratio of the bacteria cells was adjusted. The total volume of the bacterial inoculum was reduced to 10 µl (4 × 10⁹ CFU/ml), and the ratios of S. epidermidis and E. faecalis cells were 8:2 and 9:1 (Figures 5D,E). When the ratio was 8:2, the S. epidermidis count increased until 8 h, whereas the E. faecalis count was stable for 8 h. When the initial ratio was 9:1, the numbers of S. epidermidis and E. faecalis cells were almost identical at 4 h.

**Survival of in vivo Co-cultures After Moxifloxacin Treatment**

The effects of moxifloxacin eye drops were tested on the *in vivo* co-culture model of S. epidermidis and E. faecalis. These microbes (4 × 10⁹ CFU/ml each) were inoculated (9 and 1 µl, respectively), and moxifloxacin was administered zero, one, or two times. The numbers of S. epidermidis and E. faecalis colonies were determined at 4 h post inoculation (Figure 6). The number of S. epidermidis colonies diminished significantly as the number of moxifloxacin treatment increased. In contrast, the number of E. faecalis colonies remained constant, regardless of the moxifloxacin administration scheme. Moreover, when moxifloxacin was administered once, the number of E. faecalis colonies tended to go up.

**DISCUSSION**

In this study, we developed *in vitro* and *in vivo* co-culture models of S. epidermidis and E. faecalis to evaluate the impact of moxifloxacin on the survival of these two bacteria. E. faecalis has significantly higher resistance to moxifloxacin than S. epidermidis does, and administration of moxifloxacin likely allows for selective survival of E. faecalis on the ocular surface. The *in vitro* and *in vivo* co-culture models clearly showed that moxifloxacin treatment reduces the number of S. epidermidis but not the number of E. faecalis. These suggest that if there is a small
number of \textit{E. faecalis} cells in the conjunctiva, then administration of moxifloxacin promotes not only the selective survival of \textit{E. faecalis} but also its domination in conjunctival sac microflora.

Recently, administration of prophylactic antibiotics before and after intraocular surgery became a common practice, even though there is no clear evidence that it prevents infection. If fluoroquinolone is utilized as a prophylactic antibiotic perioperatively, it is expected to be effective against \textit{S. epidermidis} present on the ocular surface but not against \textit{E. faecalis}, hence changing conjunctiva microflora. The changed conjunctiva microflora provides more opportunities for \textit{E. faecalis} to enter the intraocular space through the surgical wounds, resulting PE.

In our study, \textit{in vitro} co-culture of \textit{S. epidermidis} and \textit{E. faecalis} increased the maximum cell concentration of \textit{E. faecalis} in contrast to the mono-cultures. The different growth patterns between mono- and co-cultures is likely related to direct co-culture of the two bacteria. In other words, it is highly likely that the proliferation of \textit{E. faecalis} is induced in the presence of \textit{S. epidermidis}. To explain this difference, the bacteria were cultivated with various concentrations of a culture supernatant from each bacterial species. The experiments using culture supernatants have many limitations to represent the direct co-culture condition. For example, using the culture supernatant of stationary phase may exclude some components of exponential phase, and the nutrient-reduced culture supernatant may hinder normal growth of bacteria. The supernatant of stationary phase rather than exponential phase was chosen because we thought that bacterial growth stage in the \textit{in vivo} condition, where bacteria inhabit on the ocular surface, may be closer to the stationary phase than exponential phase. When the supernatant of stationary phase is used, the effect of nutrient reduction can be an issue. When 0, 50, 80, 100% of supernatant with culture media TSB was used, the effect of nutrient-reduced supernatant to the bacterial growth was shown. However, the purpose of this experiment is to observe the changes in the growth pattern of bacteria affected by bacterial culture supernatant. For this purpose, we were able to observe that the supernatant of \textit{S. epidermidis} culture induced the increase of the cell density of \textit{E. faecalis} above their maximum cell concentration in the standard culture.
The growth patterns shown in the in vitro co-culture model were also observed in the in vivo co-culture model. When E. faecalis and S. epidermidis were administered to the lower conjunctival sac of rabbits, the number of S. epidermidis cells decreased with time, whereas the number of E. faecalis cells increased for up to 16 h. These data suggest that if E. faecalis enters the conjunctival sac before or after intraocular surgery, it is likely to proliferate in the conjunctival sac for at least 16 h. Therefore, great care should be taken not to contaminate the ocular surface with the bacteria before and after intraocular surgery.

We next sought to determine whether there is a change in antibiotic sensitivity when the two bacteria were co-cultured. When the bacteria were mixed (at a ratio of 95:5) and immediately treated with moxifloxacin, without a co-culturing period, no S. epidermidis colonies were detectable in the presence of 0.125 µg/ml moxifloxacin, and there were no E. faecalis colonies in the presence of 0.25 µg/ml moxifloxacin. Of note, when S. epidermidis and E. faecalis were mixed (at a ratio of 1:1), co-cultured for 14 h and only then treated with moxifloxacin, the antibiotic concentration effective against S. epidermidis was much higher, 6 µg/ml and, moreover, E. faecalis became resistant even to 16 µg/ml moxifloxacin. This phenomenon was observed in the in vivo co-culture model too. In the in vivo model, when 0.5% moxifloxacin was administered twice with an 1 h interval, S. epidermidis almost disappeared, but E. faecalis persisted. These findings also imply that not only in the conjunctival sac but also in the cases of endophthalmitis, where the bacteria co-proliferate in the vitreous cavity, E. faecalis may become more resistant to fluoroquinolone antibiotics.

Fluoroquinolone eye drops are widely used before and after intraocular surgery to prevent PE. It is still debatable whether these antibiotics reduce the risk of infectious endophthalmitis. Although these antibiotics are very likely to effectively eliminate CNS, including S. epidermidis, in the conjunctival microflora, our data suggest that moxifloxacin may have little antimicrobial activity toward the Enterococcus spp. that are present in the conjunctiva.

Most of the bacteria that cause PE are those already present in the conjunctiva. Because the most prevalent bacteria in the conjunctiva are CNS, including S. epidermidis, they have been the major causative agent of PE after surgery. On the other hand, several recent reports on PE in Korea, Sweden, and Taiwan indicated that E. faecalis has become the top causative agent of PE (6, 9–11). Enterococcus spp. have been relatively rare but are of special clinical importance because they cause a fulminant and destructive disease course, and poor visual outcomes of infected patients (7, 25–27). In one study, only 17% of patients with E. faecalis endophthalmitis achieved a final visual acuity of 20/400 or better (28). Therefore, the emergence of E. faecalis as a cause of PE may affect visual outcomes after treatment of PE.

This reshuffling of major causative isolates of PE is especially obvious in a series of nationwide, multicenter, prospective studies in Sweden. In the first report, covering the 1998 national prospective survey, the most common bacteria that caused PE were CNS (15/41, 37.37%) (29). In the second report, covering the 1999–2001 survey, CNS were still the most common isolates at 32.97% (30/91), followed by Enterococcus spp. at 25.27% (23/91) (30). In the third report, covering the 2002–2004 survey, CNS were the most common isolates at 39.08% (34/87), again followed by Enterococcus spp. at 28.73% (25/87) (31). In the most recent report, covering the 2005–2010 survey, Enterococcus spp. were the most common isolates at 39.13% (42/115), followed by CNS at 30.43% (35/115) (10). Looking at the results of these four surveys covering 12 years, we can see that the percentage of PE cases caused by Enterococcus spp. gradually increased. Those authors hypothesized that the selection pressure applied by antibiotics has caused the other species to become the leading causative microorganisms of PE (32).

One of key tasks for the researchers in this field is establishing a proper experimental model to investigate the shifts of the major causative isolates of PE. Challenges for the studies on this complex environment of the ocular surface are not limited to the presence of many cell types and bacterial species: there is also substantial variations of the microbiota among individuals. Mimicking such complex ocular surface conditions is difficult in an in vitro model. Therefore, creating in vivo model is essential for researching these changes of ocular pathological processes. Our proposed in vivo animal model may be suitable for studying the shifts of the major causative isolates for understanding PE pathogenesis, and for evaluating novel therapeutics.

In conclusion, we successfully created in vitro and in vivo co-culture models of S. epidermidis and E. faecalis. By means of these models, we confirmed that moxifloxacin, a fourth-generation fluoroquinolone, is effective against S. epidermidis, but not E. faecalis. Our results provide supporting evidences that the administration of fluoroquinolone as perioperative ophthalmic treatment is an important contributing factor of the change in the major causative agent of PE from S. epidermidis to E. faecalis. The co-culture models of this study can be a useful tool to develop an effective antibiotic that can control PE caused by E. faecalis.
DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of Kosin University College of Medicine (KMAP-18-19).

AUTHOR CONTRIBUTIONS

SL conceived of the project and created all instructional materials. SL and HK prepared all figures and wrote the manuscript. JK and IP performed data analysis and critical review the manuscript. All the authors contributed to the article and approved the submitted version.

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