

Prospective randomized study determining whether a 3-day application of ofloxacin results in the selection of fluoroquinolone-resistant coagulase-negative *Staphylococcus*

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PURPOSE. To determine whether a 3-day application of ofloxacin results in the selection of fluoroquinolone-resistant conjunctival coagulase-negative *Staphylococcus*.

Design. Prospective randomized trial.

METHODS. Patients scheduled for ocular surgery were randomized to a control (89 eyes) or study group (70 eyes). The study group received topical ofloxacin (0.3%) four times a day for 3 days. Conjunctival cultures were obtained at baseline (T0) and after 3 days of ofloxacin (T1). Cultures were also obtained at T0 and T1 for the control group, but these eyes did not receive an antibiotic. Bacteria isolated were identified and antibiotic susceptibility was determined.

RESULTS. At T0, 53 out of 89 patients (60%) in the control and 48 out of 70 patients (69%) in the study group harbored coagulase-negative *Staphylococcus*. Among these coagulase-negative *Staphylococcus*, 12 out of 53 in the control and 11 out of 48 in the study group were resistant to ofloxacin ($p > 0.9999$). At T1, significantly fewer coagulase-negative *Staphylococcus* ($p = 0.0003$) were isolated from the study group (18 coagulase-negative *Staphylococcus*), compared the control group (48 coagulase-negative *Staphylococcus*). Of these, 5 out of 17 coagulase-negative *Staphylococcus* in the study group and 9 out of 48 coagulase-negative *Staphylococcus* in the control group were resistant to ofloxacin ($p = 0.5649$). There was no significant difference in the number of coagulase-negative *Staphylococcus* resistant to ciprofloxacin or norfloxacin in the study group compared to the control group at T1.

CONCLUSIONS. Ofloxacin given four times a day for 3 days does not select out for conjunctival fluoroquinolone-resistant coagulase-negative *Staphylococcus*. (*Eur J Ophthalmol* 2006; 16: 359-64)

KEY WORDS. Antibiotic, Bacteria, Conjunctiva, Fluoroquinolone, Ofloxacin, Resistance

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INTRODUCTION

Povidone-iodine and topical antibiotics are commonly used prior to ocular surgery in an attempt to reduce the risk of postoperative endophthalmitis (1-12). The bacteria causing endophthalmitis most likely originate from the eyelids and conjunctiva (13, 14). The most common bacteria causing postoperative endophthalmitis are coagulase-negative

Staphylococcus (15). Although the application of topical antibiotics or povidone-iodine has not been shown in a prospective randomized study to reduce the risk of postoperative endophthalmitis, it is commonly believed that the reduction or elimination of bacteria from the ocular surface may reduce the risk of developing endophthalmitis following intraocular surgery. Both antibiotics and povidone-iodine given preoperatively have been shown to reduce bac-

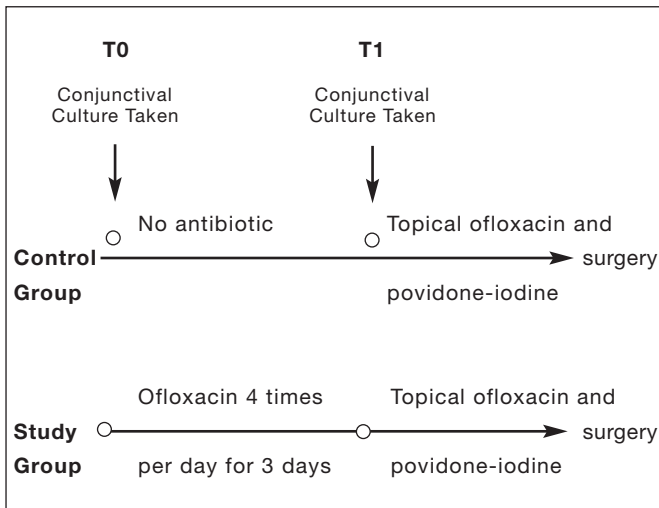


Fig. 1 - Time points of conjunctival cultures for the control and study groups.

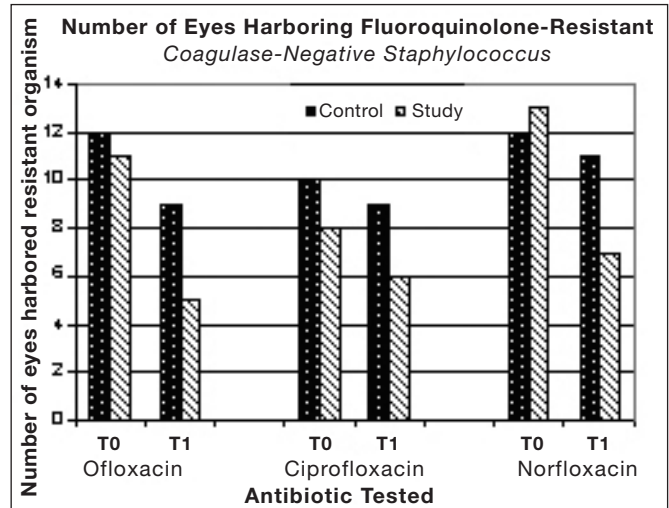


Fig. 2 - Number of eyes harboring fluoroquinolone-resistant coagulase-negative Staphylococcus at baseline (T0) and after treatment with topical ofloxacin for the study group (T1).

terial count from the conjunctiva (4, 9, 16-20). We have previously reported that a 3-day application of topical ofloxacin was more effective in reducing the number of positive conjunctival cultures in the perioperative period compared to an application given 1 hour prior to surgery in patients undergoing cataract surgery (9). One possible adverse effect of using antibiotics for 3 days prior to surgery is the preferential selection of antibiotic-resistant organisms. The possible consequence is the introduction of these organisms into the eye at the time of surgery, resulting in an endophthalmitis caused by antibiotic-resistant bacteria, which may be more difficult to treat. The purpose of this study is to determine whether a 3-day application of topical ofloxacin 0.3% given prior to intraocular surgery increases the likelihood of selecting for conjunctival fluoroquinolone-resistant coagulase-negative *Staphylococcus* compared to eyes that did not receive antibiotic.

DESIGN

Prospective, randomized, controlled trial.

METHODS

All patients scheduled for intraocular anterior segment surgery at the Stanford University School of Medicine Department of Ophthalmology from July 21, 2000, to July

30, 2001, were asked to participate in this prospective study. A total of 169 patients from 181 surgical cases consented to the study. Institutional Review Board approval was obtained for the study prior to enrollment of patients. Exclusion criteria include patients having an allergy to iodine or fluoroquinolone antibiotics, those with active ocular infections, and patients taking topical ophthalmic antibiotics. Random numbers were generated using Microsoft Excel Software (Redmond, WA) and 181 eyes were randomized into a control or study group. Eyes in the study group received one to two drops of 0.3% topical ofloxacin (Allergan, Inc.) four times a day for 3 days prior to surgery. Conjunctival cultures were obtained at baseline prior to antibiotic application (T0), usually 3 to 5 days before surgery, and after 3 days of antibiotics (T1) on the day of surgery for the study group. Conjunctival cultures were also obtained from eyes in the control group at the same time points as eyes in the study group (T0 and T1). However, eyes in the control group did not receive any topical antibiotic. Figure 1 illustrates the time points for the conjunctival cultures. On the day of surgery, eyes from both groups then proceeded with applications of topical 0.3% ofloxacin and 5% povidone-iodine given just prior to surgery.

Conjunctival cultures were obtained using a cotton swab moistened with thioglycolate broth culture media. Culture samples were taken from the inferior conjunctival fornix without touching the eyelids or eyelashes. No topical anesthetic agent was used prior to the culture sam-

pling. The swab was immediately inoculated onto blood and chocolate agar media, as well as thioglycolate broth. All culture media were incubated at 37 °C for 10 days. All bacterial isolates were identified and quantified. The specimens that were culture-positive were then plated onto Mueller-Hinton agar and antibiotic susceptibilities were determined by the Kirby-Bauer disc-diffusion in vitro technique in accordance with the National Committee for Clinical Laboratory Standards (NCCLS). All bacteria isolated were tested for susceptibility to ciprofloxacin, ofloxacin, and norfloxacin. All of the culture media and antibiotic discs were purchased through Fisher Scientific (Los Angeles, CA). The person obtaining the cultures was masked regarding whether the patient was randomized to the control or study group. The microbiologist who interpreted the culture results was not masked regarding the patient's group.

Statistical analysis was performed using the Analyse-it software program (Analyse-It Software, Leeds, England). A power calculation was performed, assuming an alpha error of 0.05 and power of 0.8, which indicated that a total of 66 patients was required in each group to detect a statistically significant increase from 25% resistance rate to 50%. Chi-square test was used for statistical analysis. For the purpose of drawing statistical conclusions, p values of 0.05 or less were considered significant.

RESULTS

Among the 169 patients enrolled in the study, 12 had bilateral surgery, yielding 181 eyes for the study. In 5 of the 12 patients who had bilateral surgery, one eye was randomized to the control and the other to the study group. In the remaining seven patients who had bilateral surgery, only the first eye that underwent surgery was used in the data analysis because both eyes were randomized to the same group. Another 15 eyes were not included in the analysis due to conjunctival cultures not obtained (6 eyes), inadequate recording of data (1 eye), and surgery cancellation (8 eyes). As a result, 159 eyes were available for analysis, with 89 eyes randomized to the control and 70 eyes in the study group.

At baseline (T0), 53 out of 89 eyes (60%) harbored coagulase-negative *Staphylococcus* in the control group. Similarly, 48 out of 70 eyes (69%) in the study group had coagulase-negative *Staphylococcus*-positive cultures. Cultures obtained at T1 were performed on the day of the

surgery. Eyes in the control group have not received antibiotics, whereas eyes in the study group had been treated with ofloxacin for 3 days. Among the 89 eyes in the control group, 48 eyes (54%) were positive for coagulase-negative *Staphylococcus* at T1. In the study group, 18 of the 70 eyes (26%) were found to have coagulase-negative *Staphylococcus*. Table I summarizes the results. There were significantly fewer coagulase-negative *Staphylococcus* isolated from the study group compared to the control group ($p=0.0006$).

Table II summarizes the antibiotic resistant results among the coagulase-negative *Staphylococcus* isolated. The proportion of coagulase-negative *Staphylococcus* resistant to each of the fluoroquinolones tested was similar between the control and study groups for samples obtained at baseline. Furthermore, there were no statistically significant differences among the coagulase-negative *Staphylococcus* samples obtained at T1 from the control versus the study group for any of the antibiotics tested ($p\geq 0.3235$). At baseline, 12 out of 53 coagulase-negative *Staphylococcus* isolated from the control and 11 out of 48 coagulase-negative *Staphylococcus* in the study group were resistant to ofloxacin ($p\geq 0.9999$). For the control group, 9 out of 48 coagulase-negative *Staphylococcus* were resistant to ofloxacin at T1. One sample obtained at T1 was not tested for susceptibility against ofloxacin. Of the remaining 17 coagulase-negative *Staphylococcus*, 5 were resistant to ofloxacin ($p=0.5649$). One sample from the control and study group obtained at baseline was not tested for susceptibility to ciprofloxacin. Table II and Figure 2 summarize the antibiotic resistance results.

DISCUSSION

Our results show a significant reduction in the proportion of eyes with positive conjunctival cultures for coagulase-negative *Staphylococcus* after 3 days of topical ofloxacin compared to no antibiotic treatment. More importantly, the 3-day application of topical ofloxacin did not result in a significant increase in the number of eyes harboring fluoroquinolone-resistant coagulase-negative *Staphylococcus*. Our power calculation indicates that if there were a doubling (from 25% to 50%) in the percentage of eyes harboring fluoroquinolone-resistant coagulase-negative *Staphylococcus* between the control and study group at T1, we would have detected this difference with only 132 eyes, compared to our 159 eyes in our

study. Furthermore, comparing T1 to T0, the absolute number of coagulase-negative *Staphylococcus* samples resistant to fluoroquinolone decreased after 3 days of topical ofloxacin treatment for eyes in the study group. Given the decrease in the number of fluoroquinolone-resistant coagulase-negative *Staphylococcus* isolated after ofloxacin treatment, it is unlikely that a 3-day application of ofloxacin poses additional risk to the patient. The chance of developing endophthalmitis caused by fluoroquinolone-resistant bacteria should not be higher for those in the study group compared to the control group.

Several recent published studies suggest that there may be an increase in the prevalence of bacteria resistant to fluoroquinolones (21-25). Many factors determine the development of antibiotic resistance, including the time period that the drug has been in use, the prescribing dose and duration of use of the medication, and the level of use of the medication, such as whether it is added to animal feed. The relatively high dose of the antibiotic along with short duration of treatment, as in this study, makes it less likely for antibiotic resistant organisms to develop.

Fluoroquinolones kill bacteria by interfering with their DNA topoisomerase, rendering the bacteria incapable of supercoiling and uncoiling the DNA, which is an important

step for protein synthesis and DNA replication (26). Bacteria develop resistance to fluoroquinolones by reducing drug permeability through the bacterial cell wall, developing protection of their DNA topoisomerase from the drug, and efflux of the antibiotic from the cell (27).

It is important to note that the goal of the current study is not to determine whether a 3-day application of topical ofloxacin can induce mutations resulting in the development of fluoroquinolone-resistant coagulase-negative *Staphylococcus*; rather, it is to investigate whether the use of ofloxacin can potentially select out resistant coagulase-negative *Staphylococcus* if the bacteria are already present.

Although our study suggests that there is not a selection bias for fluoroquinolone-resistant coagulase-negative *Staphylococcus* after a 3-day application of topical ofloxacin, it is important to emphasize that antibiotics should be used judiciously. When antibiotics are prescribed to millions of patients, even at the proper therapeutic dose, there is a likelihood of generating drug-resistant bacteria.

Once occurred, the bacteria will spread via patient contacts, bacteriophages, plasmids, or transposons. A potential weakness of our study is that we report only antibiotic

TABLE I - SUMMARY OF CONJUNCTIVAL RESULTS FOR THE CONTROL AND STUDY GROUP

Time points	Description	Control n=89, n (%)	Study n=70, n (%)	p value
Baseline (T0)	Number of eyes with coagulase-negative <i>Staphylococcus</i>	53 (60%)	48 (69%)	0.3139
T1	Number of eyes with coagulase-negative <i>Staphylococcus</i>	48 (54%)	18 (26%)	0.0006

TABLE II - SUSCEPTIBILITY OF COAGULASE-NEGATIVE STAPHYLOCOCCUS TO OFLOXACIN, CIPROFLOXACIN, AND NORFLOXACIN AT BASELINE AND AFTER TREATMENT

	Ofloxacin		Ciprofloxacin		Norfloxacin	
	T0	T1	T0	T1	T0	T1
Control	12/53	9/48	10/52	9/48	12/53	11/48
Study	11/48	5/17	8/47	6/18	13/48	7/18
p value	>0.9999	0.5649	0.9811	0.3527	0.7751	0.3235

The numerator indicates the number of eyes with coagulase-negative *Staphylococcus* that are resistant to the antibiotic tested. The denominator indicates the number of samples available tested. p value indicates statistical comparison between control and study group using chi-square test for each time point

susceptibility for coagulase-negative *Staphylococcus*. However, this organism is by far the most common bacteria isolated from the conjunctiva at baseline and is the cause of endophthalmitis in 70% of the cases (15). The number of eyes in each group was not the same due to the randomization process. In retrospect, the use of block randomization would have yielded similar numbers in the two groups.

In summary, our study showed that a 3-day application of ofloxacin, when used four times a day, does not result in the selection of fluoroquinolone-resistant coagulase-negative *Staphylococcus*. Therefore, it appears that topical ofloxacin could be safely prescribed to individual patients to use four times per day. However, antibiotics should only be prescribed cautiously and with good indications for their use.

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