Conjunctival flora growth in intravitreal injection settings.

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Abstract

Purpose: To study the conjunctival flora in patients undergoing repeated intravitreal injections.

Methods: Prospective observational study. Patients undergoing intravitreal injections were randomized into two groups: 1) preparation with povidone-iodine 5% alone, or 2) preparation with povidone-iodine 5% with Maxitrol eye drops. Our standard injection technique included topical anesthesia, a solid bladed lid speculum, and commercially prepared topical povidone-iodine 5% applied for 2 minutes. Cultures were obtained from the conjunctival cul-de-sac on routine office visits prior to any preparation and immediately after injection.

Results: A total of 110 Eyes of 86 patients were enrolled in the study. Fifty-five were irrigated with povidone-iodine 5% only, and 55 were irrigated with a combination of povidone-iodine 5% and Maxitrol drops. Compared with the povidone-iodine only group, the Maxitrol and povidone-iodine group did not lead to a statistically significant reduction in patients with positive cultures after injection, (p=0.19). The most common organism cultured was coagulase-negative staphylococcus; however, Enterococcus, Pseudomonas, Staphylococcus aureus, and Bacillus were also cultured. Thirty-seven percent of organisms were resistant to 4th generation quinolones. No cases of endophthalmitis were recognized. Of the positive cultures, 33% were resistant to over 4 antibiotics tested.

Conclusion: In this routine clinical setting, conjunctival antisepsis was difficult to achieve despite the use of topical povidone-iodine with or without Maxitrol. No infectious complications were recognized.

Keywords: Intravitreal injection, Conjunctival flora, Conjunctival culture, Endophthalmitis.

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Introduction

The use of Intravitreal Injection (IVI) has become the main treatment modality for a variety of ocular conditions, including choroidal neovascularization and macular edema secondary to diabetes, vein obstruction, and many other causes. The most feared complication of intravitreal injections is endophthalmitis. The reported incidence of endophthalmitis following IVI is low, ranging from 0.01 to 0.26% [1-8]. Up to 80% of perioperative endophthalmitis is secondary to indigenous ocular floral contamination [9].

Increasing resistance of normal ocular flora to commonly used antibiotics, such as aminoglycosides, macrolides and third-generation fluoroquinolones, has led to the use of high potency fourth generation fluoroquinolones namely, moxifloxacin 0.5% (Vigamox, Alcon, Fort Worth, TX) and gatifloxacin 0.3% (Zymar, Allergan Pharmaceuticals, Irvine, CA) [10-12]. Over time resistance to these fourth-generation fluoroquinolones has also emerged [13-19]. In this study our aim was to evaluate the ocular flora and patterns of antibiotic resistance of our patients undergoing routine intraocular injections in an office setting.

Methods

Prospective observational study performed from January 9th, 2014 to May 30th, 2017.

Patients undergoing intravitreal injection during routine office visits were enrolled in the study. Indications for injection included choroidal neovascularization (CNV) secondary to macular degeneration, histoplasmosis syndrome and myopia, as well as macular edema (CME) secondary to diabetes, vein obstruction and other vascular diseases. None of the patients had any signs of blepharitis or external infection. Cultures were obtained from the conjunctival cul-de-sac by swabbing with a dry cotton tip applicator and then placing this applicator immediately in trypticase soy broth. This was performed prior to the instillation of any topical agents. Patients were then randomized to regular preparation with povidone-iodine 5% alone before injection or with povidone-iodine and Maxitrol®, (Alcon, Texas) (neomycin, polymyxin B sulfates and dexamethasone) eye drops prior to injection. Our standard injection technique included topical anesthesia, a solid bladed lid speculum, and commercially prepared topical povidone-iodine 5% applied for 2 minutes. The eye was not irrigated after the preparation with povidone iodine, and the injector was not wearing a mask. Cultures were then obtained a second time in a similar fashion with a dry cotton tip applicator immediately after injection. All isolates were identified; sensitivities were obtained using Kirby-Bauer disc diffusion technique. The study has been approved by the University of Louisville Institutional Review Board, and all patients signed an informed consent prior to enrolling in the study.

Statistics

Descriptive statistics were utilized for group characteristics. Group comparisons, when appropriate, were performed using the Fisher exact test. A p-value of <0.05 was considered to be statistically significant. Statistical analysis was performed using GraphPad Prism 6® (GraphPad Inc., La Jolla, CA, USA).

Results

A total of 110 Eyes of 86 patients were enrolled in the study. There were 48 females and 38 males with a mean age of 76 ± 10 years. Bevacizumab was used in 91 eyes, Ranibizumab in 4 eyes, Aflibercept in 8 eyes, Kenalog in 4 eyes, and Ozurdex in 3 eyes.

Of 110 eyes, only 37 samples (34%) had positive cultures before injection. Of those 37 samples, 12 still had positive cultures after injection. A total of 25 eyes of the 110 (23%) had positive cultures after injection, meaning that 13 eyes converted from no growth to growth of bacteria post injection.

Of the 55 eyes irrigated with povidone-iodine only, 19 eyes (35%) had growth pre injection. Of those 19 eyes 8 eyes still had positive cultures after injection. A total of 17 eyes (31%) had growth post injection, meaning that 9 eyes converted from no growth to growth of bacteria post injection. Of the 55 eyes irrigated with povidone-iodine and Maxitrol 18 eyes (33%) had growth before injection and only 4 of those 18 eyes had growth post injection. Of the 55 eyes prep total of 8 eyes of these 55 eyes (15%) had growth post injection, meaning that 4 more eyes in the group converted from no growth to growth of bacteria post injection. Positive cultures consisted of rare growth of organisms in 38% of pre-injection cultures and 60% rare growth of organisms in the post-injection cultures.

Compared with the povidone-iodine only group, the Maxitrol and povidone-iodine group did not lead to a statistically significant reduction in patients with positive cultures after injection, (p=0.19). Looking at the positive cultures of coagulase-negative staphylococcus, in the povidone-iodine alone group the number of cultures decreased only slightly, by 12.5% (from 16 to 14 positive samples), while in the povidone-iodine with Maxitrol group the number of patients with coagulase-negative staphylococcus decreased by 72.4% (from 14 to 4 positive samples).

Of the 62 total positive cultures, 49 (63%), were of coagulase-negative *staphylococus* 7 (9%) Staphylococcus aureus and 6 (8%) Cornybacterium. Other pathogens included Enterrococcus, Pseudomonas, Acinetobacter, Rhizobium and Bacillus (Table 1).

Bacteria isolated	n	%
Coagulase-negative staphylococcus	49	63%
Staphylococcus aureus	7	9%
Corynebacterium	6	8%
Enterococcus	5	6%
Acinetobacter	3	4%
Bacillus	2	3%
Pseudomonas	2	3%
Rhizobium	2	3%
Serratia	1	1%
Micrococcus	1	1%
Total	78	100%

Table 1. *Conjunctival flora pathogens.*

Sensitivities were checked for multiple antibiotics. Forty-four percent of organisms were resistant to over 3 antibiotics tested, and 33% were resistant to over 4 antibiotics tested, with the highest resistance to erythromycin (69%) and the lowest resistance to gentamycin (6%). Mean sensitivity to all antibiotics tested was 58%, with higher sensitivities to clindamycin, tobramycin and gentamycin. The sensitivities of all organisms to moxifloxacin and gatifloxacin were 58% and 52% respectively, with 37% resistant to both 4th generation quinolones, and 36% resistant to 3 generation (group 3B) cephalosporin (ceftazidime) (Figure 1).

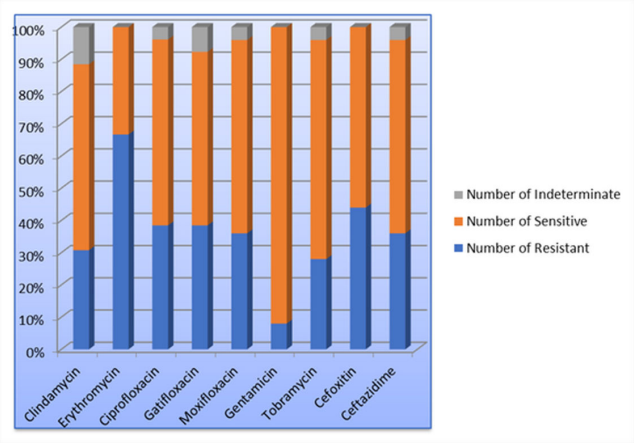

Figure 1. *Post injection resistance patterns.*

Discussion

One of the most common bacteria found on the surface of the eye is coagulase-negative staphylococcus (CoNS), with Staphylococcus epidermis being the predominant species. Other commensal organisms commonly constituting the ocular flora are Staphylococcus aureus, Propionibacterium, Corynebacterium, Pseudomonas aeruginosa and Haemophilus influenza [20,21]. Other organisms identified by PCR are Rhodococcus, Klebsiella, Propionibacterium and Erwinia species. [16,20] We found a similar spectrum of bacteria in this study, with the highest rates of growth being coagulase-negative staphylococcus and Staphylococcus aureus, which are known to be the most common cause of endophthalmitis post-surgery/intraocular injection.[21] We also found more virulent organisms such as Serratia, Pseudomonas, Acenitobacter and Escherichia coli. None of the patients in this study had any

signs of blepharitis or external infection. The growth rate prior to preparation was 34% (37 of 110 patients).

The species of bacteria seen in our study are in agreement with the pathogens found in the study by Walker et al. [22]. However, both the data by Walker et al. and Graham et al. did not show positive cultures to Serratia, Acinetobacter and Rhizobium species [20,22], which may cause ominous prognosis if result in endophthalmitis. Moreover, in the study by Walker, the percentage of patients with positive culture growth of conjunctival swabs going into elective ocular surgery was much higher (74%) than in our study [22]. Our rate of positive cultures may have been higher if we had used a moistened cotton tip applicator instead of a dry one. However, cultures show positive or negative bacterial growth, and not the quantity of bacteria present. It is believed that whether or not endophthalmitis develop depends on the virulence and number of bacterial present. In the current study, a positive culture of 1 colony-forming unit is the same as 1000 colony-forming unit. This may explain why there are persistent positive culture results after instilliation of povidone-iodine. Moreover, a large quantity of the positive cultures actually had only rare growth of organisms in the pre-injection samples (38%), and much larger (60%) in the postinjection samples.

All cultures		
Pre-injection	Post-injection	Total (N)
No-growth	No-growth	60
Growth	No-growth	25
Growth	Growth	12
No-Growth	Growth	13
Cultures without Maxitrol		
Pre-injection	Post-injection	Total (N)
No-growth	No-growth	27
Growth	No-growth	11
Growth	Growth	8
No-Growth	Growth	9
Cultures with Maxitrol		
Pre-injection	Post-injection	Total (N)
No-growth	No-growth	33
Growth	No-growth	14
Growth	Growth	4
No-Growth	Growth	4

Table 2. *Culture growth pre and post injection.*

In this study the growth rate decreased after the preparation by only 67.5% in the group which had growth pre-injection, however some cultures came back positive after the initial pre-injection culture was negative (Table 2). We were somewhat surprised and disappointed that we were unable to more completely sterilize the ocular surface with Povidone-iodine prior to injection. The decrement in positive cultures in eyes whose samples had positive culture prior to injection was larger in the combined povidone-iodine with Maxitrol group and reached 78% vs. 57% in the povidone-iodine alone group, however this was not statistically significant. Our study is in agreement with the findings of Caro et al., who cultured sites and needles from patients undergoing intravitreal injections and found that 2% of needles were contaminated with bacteria. Injection site prophylaxis with antibiotic significantly reduced positive cultures in their study from 43% to 13% [23]. Moreover, the decrement in growth expected with the use of prophylactic antibiotics may only occur with longer prophylaxis than a few minutes prior to injection. The correct time for antibiotic prophylaxis is not known, however, A study testing the differences between ocular flora with maxitrol 1 day or 1 hour before cataract surgery found no differences in the reduction of positive cultures compared with control eyes [24]. It is not surprising that there was no difference in positive culture rate between the povidone-iodine alone group and the povidone-iodine and Maxitrol groups.

Povidone-iodine has been known to lower endophthalmitis rates significantly in intraocular surgery and intra-ocular procedures. In the Age-Related Macular Degeneration Treatment Trials, the rate of endophthalmitis was ten times higher among patients who were not irrigated with Povidone-iodine compared with those who were [25]. Another retrospective review also demonstrated a higher rate of endophthalmitis in injections without preparation with povidone iodine [26].

There is recent evidence that prophylactic antibiotic use may not benefit, and even harm patients in the long run. In the studies of the DRCR net [27] and by Cheung et al. [28], a higher rate of endophthalmitis was reported in patients treated with antibiotics. On the other hand, Bhatt et al. found no difference in endophthalmitis occurrence regardless of antibiotic use [29]. In a meta-analysis by Sigford et al., culture-positive endophthalmitis was significantly increased by the reported use of antibiotics [30]. In a second, more recent metanalysis, evaluating the use of topical antibiotics has found an increased odds ratio of 1.33 to develop endophthalmitis with antibiotic prophylaxis compared to with no prophylaxis [31]. A third meta-analysis of 174,159 intravitreal injections did not report a significant difference in the prevalence of clinical endophthalmitis between groups with and without topical antibiotics, they concluded that antibiotic prophylaxis is not required in intravitreal injections [32].

Repeated exposure to ophthalmic antibiotics has been shown to select for resistant strains of ocular and nasopharyngeal flora, [13,14,16,33] and may also cause drug-resistant strains to be more virulent [34]. This is in agreement with our findings of culture resistant bacteria to 4th generation quinolones in 37% of isolates in our study. Our findings are similar to the rates found in the Vanderbilt study, where a third of the organisms were resistant to 4th generation quinolones. [13] Although antibiotic use may decrease the growth of coagulase-negative staphylococcus, it may cause resistant pathogens and is not routinely recommended.

The limitations of the study include a small study size as the rates of positive cultures were low. A much larger sample size would be needed to evaluate the true rates of endophthalmitis and association with different sterilizing techniques. The strengths of this study are its prospective nature, and randomization into two treatment groups.

Conclusion

In conclusion, this study performed in real life settings, shows a variety of pathogens not commonly identified in the conjunctival fornix both prior and post injections, with a high rate of resistance to a variety of antibiotics, including 4th generation quinolones, and 3rd generation cephalosporin. Additional use of Maxitrol to regular preparation with povidone-iodine did not alter the incidence of positive cultures and is not routinely recommended.

Compliance with Ethical Standards

- The authors have no conflict of interest or financial interest to disclose.
- Funding: Supported by an unrestricted grant from Research to Prevent Blindness.
- Ethical approval: All procedures performed in this study were in accordance with the ethical standards of the University of Louisville IRB.
- Informed consent was obtained from all individual participants included in the study.

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