

Study of the resistance pattern and alteration in the conjunctival flora of patients exposed to repeated antibiotics

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反复使用抗生素患者结膜菌群的变化及耐药模式研究

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摘要

目的: 观察和分析反复使用抗生素患者结膜菌群的变化。此外,确定这种反复暴露对结膜菌群抵抗模式的影响。

方法: 研究纳入 40 例眼科研究所视网膜科患者。所有受试者至少进行了连续 4 次、每月一次的玻璃体腔注射 (IVI), 其中一些患者根据需要延长至 6mo 或 1y。将患者随机分为 3 组, 每组给予氧氟沙星、莫西沙星或头孢他啶任意一种抗生素。使用 Kirby-Bauer 扩散法对所有细菌进行药敏试验。

结果: 研究中, 以表皮葡萄球菌 (51.2%) 为主, 其次是金黄色葡萄球菌 (14%), 微球菌 (12.8%) 和其他凝固酶阴性葡萄球菌 (CONS) 占 13%。结果显示, 在 4 次随访中, 与随机接受头孢他啶治疗的患者的基线培养相比, 接受头孢他啶治疗的患者眼部表皮葡萄球菌的百分比有所增加。相比之下, 与基线相比, 金黄色葡萄球菌的百分比没有明显增加。在氟喹诺酮治疗眼中观察到表皮葡萄球菌的百分比从基线增加。与头孢他啶治疗组相比, 氟喹诺酮治疗组的

金黄色葡萄球菌百分比比较基线升高。然而, 随着新一代和老一代氟喹诺酮类药物的暴露, 眼部菌群组成模式发生了变化。我们注意到, 从基线开始, 莫西沙星治疗组比氧氟沙星治疗组的表皮葡萄球菌增多。暴露于老一代和新一代氟喹诺酮类药物的金黄色葡萄球菌的模式没有差异。

结论: 反复使用眼用抗生素不仅会改变正常眼部菌群的组成, 还会选择耐药菌株。

关键词: 眼部菌群; 眼用抗生素; 表皮葡萄球菌; 耐药性

Abstract

• **AIM:** To investigate and analyze the changes in the conjunctival flora of patients exposed to repeated antibiotic usage. In addition, determining the effects of this repeated exposure on the resistance pattern of the conjunctival flora.

• **METHODS:** This study included 40 patients, admitted to the Retina Unit of the Research Institute of Ophthalmology. All chosen patients had a minimum of 4 consecutive, monthly intravitreal injections (IVI), some were extended to 6mo or 1y as required. The patients were randomly divided into 3 groups and each group received one kind of antibiotic which was either ofloxacin, moxifloxacin or ceftazidime. All bacterial isolates were tested for antibiotic susceptibility using Kirby-Bauer disc diffusion technique.

• **RESULTS:** In this study the conjunctival normal flora at baseline culture varied from a predominance of *Staphylococcus epidermidis* (51.2%), followed by *Staphylococcus aureus* 14% to *Micrococcus* species 12.8% and other coagulase negative staphylococci (CONS) 13%. Our results showed an increase in the percentage of *Staphylococcus epidermidis* among ceftazidime treated eyes during the four visits in comparison to baseline cultures of patients randomized to ceftazidime. In contrast, there was no noticeable increase in *Staphylococcus aureus* percentage from baseline. In fluoroquinolone treated eyes, we also observed an increase in percentage of *Staphylococcus epidermidis* from base line. In contrast to ceftazidime treated eyes, the *Staphylococcus aureus* percentage in fluoroquinolone treated eyes showed an increase from baseline. However, the pattern of the ocular flora composition changed with the exposure to the old and newer generation of fluoroquinolones. We noticed an increase of *Staphylococcus epidermidis* in moxifloxacin treated eyes

than that in ofloxacin treated eyes from baseline. There was no observed difference in the pattern of *Staphylococcus aureus* regarding exposure to older and newer generations of fluoroquinolones.

• **CONCLUSION:** Repeated use of ophthalmic antibiotics not only alters the composition of the normal ocular flora, but also selects for resistant strains.

• **KEYWORDS:** ocular flora; ophthalmic antibiotics; *Staphylococcus epidermidis*; resistance

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INTRODUCTION

Although intravitreal injections (IVI) are generally safe, infectious endophthalmitis is a rare, but devastating complication. Endophthalmitis (EO) rates reported in large multicenter randomized controlled trials of anti-vascular endothelial growth factor. IVI range from 0.019%–0.09% per injection. Even with immediate and appropriate treatment, the risk of vision loss remains high^[1]. The etiology of post-IVI infectious endophthalmitis is based mainly on two mechanisms: direct inoculation at the time of injection or post injection in cases of needle track vitreous incarceration. The correlation between bacteria on the eye surface and the risk of development of endophthalmitis remains to be proven. However, it is believed that most bacterial isolates in endophthalmitis after IVI possibly arise from the bacterial flora of the eye surface^[2]. Consequently, prophylactic measures for minimizing risk of endophthalmitis have been outlined including: use of povidone-iodine on the injection site; topical antibiotics prior to IVI, which remains controversial. As such, more than 80% of retina specialists report using prophylactic topical antibiotics after IVI, and topical antibiotics are routinely prescribed^[3]. The repeated exposure to topical antibiotics may alter the normal bacteria on the eye and increase rates of resistance. This is of increasing concern due to the reports of growing resistance of normal ocular flora to the commonly employed antibiotic classes including macrolides, aminoglycosides and third and fourth generation fluoroquinolones. The issue of increased bacterial resistance has attracted recent attention. It is suggested that repeated exposure to antibiotics after IVI might alter the structure of conjunctival flora and facilitate the development of antibiotic resistant bacterial colonies. The literature contains few studies on the effects of repeated use of antibiotics with regards to the development of resistance. Conjunctival flora and the frequency of resistance to antibiotics changes along with countries and regions. Such findings should be supported with various multicentric studies, and this is why we conducted this research. Also, it is important to repeat such studies

periodically in order to evaluate the flora changes and antibiotic profile patterns^[4].

The purpose of this study was to investigate and analyze the changes in the conjunctival flora of patients exposed to repeated antibiotic usage. In addition, determining the effects of this repeated exposure on the resistance pattern of the conjunctival flora.

SUBJECTS AND METHODS

The study adhered to the tenets of the Declaration of Helsinki and was approved by Ethics Committee of Research Institute of Ophthalmology, Giza, Egypt. All patients were informed regarding the procedure with written consent. This study was carried out after obtaining the approval from the Ethical Committee of Research Institute of Ophthalmology, Giza, Egypt.

The study included 40 patients, with age range 30–75 years old, admitted to the Retina Unit of the Research Institute of Ophthalmology. The subjects enrolled for the study were complaining of age related macular degeneration symptoms including distortion of straight lines progressing to gradual loss of control vision. Also symptoms of choroidal membrane neovascularization in the form of blurring, grey out areas, distorted vision and central blind spot was reported. In addition, complains of diabetic macular edema symptoms such as double vision and floaters, together with central retinal vein occlusion symptoms including visual loss, blurring in part or all of one eye, sudden pain and becoming worse over several hours or days, floaters and painful pressure of the eye was observed. They were diagnosed as age related macular degeneration, choroidal neovascularization and central retinal vein occlusion and prepared to receive either lucentis (ranibizumab injection) or triamcinolone intravitreal injection in one eye only. The patients excluded from enrollment in the study were those using eye drops (of any kind), in either eye within 3d of enrollment or taking systemic antibiotics within 1mo of enrollment. In addition, those wearing contact lenses, or had undergone a previous ocular surgery or had history of ocular infection within the past three months were also excluded. Also, those on long term use of ophthalmic medication were excluded. The outline for the working schedule was, that all chosen patients had a minimum of 4 consecutive, monthly IVI, some were extended to 6mo or one year as required.

The patients were randomly divided into 3 groups and each group received one kind of antibiotic which was either ofloxacin 3 mg, moxifloxacin 5 mg or ceftazidime 5 mg. Procedures for IVI were performed in a standard manner. The protocol for each patient enrolled was as follows: on the day of the first IVI (visit 1), a conjunctival swab and culture of both eyes were taken (baseline culture) prior to the injection. Patients were then instructed to start taking their assigned antibiotic on the day of their injection and continuing for 10d. Ofloxacin (0.3%), moxifloxacin (0.5%) and ceftazidime

(0.5%) were given. All patients returned after 1mo, which is visit one, 2mo (visit 2), 3mo (visit 3) and 4mo (visit 4). At each visit 1, 2, 3 and 4 the patients underwent a bilateral conjunctival culture.

Identification of bacteria strains was performed by Gram staining and testing for the presence or absence of catalase and coagulase/agglutination. *Staphylococcus* identification kit (API Staph kit; bioMérieux, Hazelwood, Missouri) was used to further speciate coagulase-negative staphylococci (CONS). To test resistance, the Kirby - Bauer disc diffusion technique^[5] was conducted in strict accordance with guidelines of the National Committee for Clinical Laboratory Standards. All bacterial isolates were tested to 10 different antibiotics using the Kirby-Bauer disc diffusion technique.

RESULTS

In our study the conjunctival normal flora at baseline culture varied from a predominance of *Staphylococcus epidermidis* (51.2%), followed by *Staphylococcus aureus* 14% to *Micrococcus* species 12.8% and other CONS 13%. Corneal observation of our work showed the normal ocular flora isolated at variable percentages at baseline culture with *Staphylococcus epidermidis* cultured in half of the isolates 51.2%, while the other half was distributed between *Staphylococcus aureus* and CONS and surprisingly two isolates were *Klebsiella* species.

At the start of the intraocular injections and the multiple exposure to the randomly selected antibiotic in the injected eye, we observed a change in the distribution of the ocular flora, most striking was the decline in the percentage of *Staphylococcus epidermidis* among control eyes from visit one to four (51.2% - 25.9%); while in contrast, there was an increase in the percentage of *Staphylococcus epidermidis* among injected eyes from visit one to four (51.2% - 76.5%). Another observation made was the mild increase in *Staphylococcus aureus* percentage among injected eyes from visit one to four (14% - 22.2%) and its disappearance in culture from fellow control eyes from visit one to four, even though it was isolated at baseline cultures at a 14% rate. Interestingly, the two baseline *Klebsiella* species were also recovered from injected eyes from visit one to four. Thus, it is noted a change in the composition of the bacterial flora with repeated antibiotic exposure.

Our results showed an increase in the percentage of *Staphylococcus epidermidis* among ceftazidime treated eyes during the four visits in comparison to baseline cultures of patients randomized to ceftazidime. In contrast, there was no noticeable increase in *Staphylococcus aureus* percentage from baseline. This increase in *Staphylococcus epidermidis* percentage among the ceftazidime treated eyes was at the expense of other CONS which were only recovered from fellow control eyes.

The antibiotic susceptibility of the CONS isolated in controls from visit one to four showed a moderate resistance percentage (46%) to ceftazidime, in comparison to a 100% resistance of

Staphylococcus epidermidis cultured from ceftazidime treated eyes from visit one to four. Likewise, 95% of *Staphylococcus epidermidis* strains cultured from visit one to four after ceftazidime exposure were resistant to the second generation cephalosporins, cefuroxime.

It was noted that all the CONS cultured at baseline were susceptible to cefuroxime, no resistance was recorded. The effects of topical antibiotics on the resistance pattern of the conjunctival flora, after repeated exposure demonstrate that repeated use of ceftazidime selects for resistant conjunctival strains of *Staphylococcus epidermidis*.

In the fluoroquinolone treated eyes, we also observed an increase in the percentage of *Staphylococcus epidermidis* from baseline (78% vs 53.3% respectively). In contrast to ceftazidime treated eyes, the *Staphylococcus aureus* percentage in fluoroquinolone treated eyes showed an increase (yet nonsignificant) from baseline, (20% vs 10% respectively). However, the pattern of the ocular flora composition changed with the exposure to the old and newer generation of fluoroquinolones. We noticed an increase of *Staphylococcus epidermidis* in moxifloxacin treated eyes than that in ofloxacin treated eyes from baseline (82.1% vs 52.6% respectively and 72.8% vs 54.5% respectively). There was no observed difference in the pattern of *Staphylococcus aureus* regarding exposure to older and newer generations of fluoroquinolones. An interesting observation was also made from our work, that there was no significant difference in the recovery rate of both *Staphylococcus epidermidis* and *Staphylococcus aureus* in either ceftazidime or fluoroquinolone treated eyes. The increased fluoroquinolone resistance among CONS isolated during visit one to four was more significant with the fourth generation moxifloxacin, when compared to baseline (77% vs 30.3% respectively).

DISCUSSION

Analysis of the data of this study showed that the repeated local use of ceftazidime (cephalosporins) and fluoroquinolones (ofloxacin and moxifloxacin) antibiotics could alter the conjunctival microbiota. It was observed an increase in the number of *Staphylococcus epidermidis* at the expense of other commensal flora. These changes may have significant clinical importance since *Staphylococcus epidermidis* is a major cause of ocular infection. Most humans harbor at least some bacteria on their ocular surface. The commensal bacteria of the conjunctival sac is similar to those found in the upper respiratory tract and skin, and they are mostly Gram positive bacteria^[6].

Staphylococcus species and *Corynebacterium* species are among the most frequent organisms isolated from the conjunctiva. These ocular commensal bacteria maintain a healthy eye, by protecting it from colonization by pathogenic organisms through a direct inhibition mechanism. *Staphylococcus epidermidis* has a two face role. One, protective by preventing colonization of the host by *Staphylococcus aureus* (a pathogenic organism), a

phenomenon known as “competitive exclusion” seen also in the skin and gastrointestinal tract. The other face is non protective, but rather acting as an opportunistic pathogen. Ironically, this protective/non protective organism is one of the common etiological agents of ocular infections; conjunctivitis, keratitis and endophthalmitis^[7].

Several studies concluded that repeated exposure to antibiotics select for resistant strains in general and specifically for resistant *Staphylococcus epidermidis* as a multiple drug resistant organism. Furthermore, it has been shown that these resistant *Staphylococcus epidermidis* strains are implicated in severe intraocular inflammations than susceptible strains. There has been several theories behind this aggressiveness of the organism, one which is lately accepted is the capability of the organism to biofilm formation which not only acting as a virulence factor in facilitating the avoidance of host defenses, but acting as a privilege for resistance to antibiotics^[8].

Presence and interplay of microbial flora at the ocular surface reveal dynamic and evolving interactions with implications for both ocular surface health and disease. A thorough treatment requires the identification of colonizing organisms^[9]. It has been understood for some time that postoperative surgery, ocular injection, or minor trauma is likely linked to infectious agents resident on the normal ocular surface. The ophthalmic literature has previously demonstrated that surface flora are the major source for postoperative endophthalmitis. Eliminating preoperative flora has been the most effective intervention for reducing postoperative complications^[10].

Normal ocular flora remains consistent among human populations. It could be altered by a variety of factors including: age, immunosuppression, ocular inflammation, contact lens wear, antibiotic use, surgery, climate and geography^[11]. The most commonly repeated bacteria include: CONS, *Staphylococcus aureus*, *Streptococcus* species, *Corynebacterium* species, and *Propionibacterium acnes*. However, the normal flora may also consist of more pathogenic organisms that remain passive colonizers rather than invasive causes of disease. Commonly isolated pathogenic organisms include; gram negative rods such as *Pseudomonas aeruginosa*, *Klebsiella* species and *Haemophilus influenzae*. Consequently, it is difficult to determine the peruse factors that render an organism able to colonize the ocular surface without causing infection^[12].

Miller *et al*^[13] noted that the interaction may depend on an intact surface epithelium as well as immune system regulation based on pathogen – associated molecular patterns. Thus a strong cooperative interaction, likely leads to tolerance of the surface bacteria while uncooperative interactions will stimulate the immune system resulting in pathogenesis.

Eye lids and conjunctiva harbor a significant number of bacteria from the external environment which are called normal flora. They play an important role in normal body functions and health by secreting antibiotics and chemical

mediators to maintain surface homeostasis and immunoregulation. They also out compete pathogenic bacteria for nutrition, thus inhibiting their growth^[14].

A study mentioned that 50% – 80% of vitreous aspirates culture was positive for CONS followed by *Staphylococcus aureus* and *Streptococcus* species, while a study on conjunctival swabs of normal patients found 53% *Staphylococcus epidermidis*, 14.7% *Staphylococcus aureus*, 11.7% diphtheroids and 6.8% *Streptococcus* species. Another study showed 88.8% CONS and 95.3% had *Staphylococcus* species. Sthapit *et al*^[15] reported 51% CONS, followed by *Staphylococcus aureus* and *Micrococcus* species 13%. CONS and *Staphylococcus aureus* are the predominant flora of the conjunctiva.

Similarly in our study the conjunctival normal flora at base line culture varied from a predominance of *Staphylococcus epidermidis* (51.2%), followed by *Staphylococcus aureus* 14% to *Micrococcus* species 12.8% and other CONS 13%. We agree with the statement reported by Sthapit *et al*^[15], justifying the presence of these ocular bacterial species specifically. The fact that these bacterial species are common resident flora of the skin and mucous membrane and are acquired in the conjunctiva from the adjacent eyelid or from the hand explains their predominant presence.

Corneal observation of our work showed the normal ocular flora isolated at variable percentages at baseline culture with *Staphylococcus epidermidis* cultured in half of the isolates 51.2%, while the other half was distributed between *Staphylococcus aureus* and CONS and surprisingly two isolates were *Klebsiella* species.

At the start of the intraocular injections and the multiple exposure to the randomly selected antibiotic in the injected eye, we observed a change in the distribution of the ocular flora, most striking was the decline in the percentage of *Staphylococcus epidermidis* among control eyes from visit one to four (51.2% – 25.9%); while in contrast, there was an increase in the percentage of *Staphylococcus epidermidis* among injected eyes from visit one to four (51.2% – 76.5%). Another observation made was the mild increase in *Staphylococcus aureus* percentage among injected eyes from visit one to four (14% – 22.2%) and its disappearance in culture from fellow control eyes from visit one to four, even though it was isolated at baseline cultures at a 14% rate. Interestingly, the two baseline *Klebsiella* species were also recovered from injected eyes from visit one to four. Thus, it is noted a change in the composition of the bacterial flora with repeated antibiotic exposure.

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It was noted that all the CONS cultured at baseline were susceptible to cefuroxime, no resistance was recorded. The effects of topical antibiotics on the resistance pattern of the conjunctival flora, after repeated exposure demonstrate that repeated use of ceftazidime selects for resistant conjunctival strains of *Staphylococcus epidermidis*. The clinical implications from these findings, concerning the use of ceftazidime in the treatment and management of certain ocular diseases in which CONS are isolated more frequently, and the appearance of selectively resistant strains to ceftazidime as a result of its long term use in these diseases. Consequently, the effectiveness of ceftazidime in the treatment of such conditions is lessened^[16]. It is reported that biofilm formation is one of the virulence factors of *Staphylococcus epidermidis*, for its resistance to antibiotics. Repeated or long term exposure to antibiotic induces *Staphylococcus epidermidis* to increase the transcription of genes specific for biofilm formation and consequently a survival advantage. This was noted in our work, where *Staphylococcus epidermidis* had a survival advantage over *Staphylococcus aureus* and other CONS^[17].

In the fluoroquinolone treated eyes, we also observed an increase in the percentage of *Staphylococcus epidermidis* from base line (78% vs 53.3% respectively). In contrast to ceftazidime treated eyes, the staphylococcus aureus percentage in fluoroquinolone treated eyes showed an increase (yet nonsignificant) from baseline, (20% vs 10% respectively). However, the pattern of the ocular flora composition changed with the exposure to the old and newer generation of fluoroquinolones. We noticed an increase of *Staphylococcus epidermidis* in moxifloxacin treated eyes than that in ofloxacin treated eyes from baseline (82.1% vs 52.6% and 72.8% vs 54.5% respectively). There was no observed difference in the pattern of *Staphylococcus aureus* regarding exposure to older and newer generations of fluoroquinolones. An interesting observation was also made from our work, that there was no significant difference in the recovery rate of both *Staphylococcus epidermidis* and *Staphylococcus aureus* in either ceftazidime or fluoroquinolone treated eyes. This was contrary to the results reported by Gade *et al*^[18]. Our results could be attributed to the later tendency in our location to the frequent usage of the fourth generation fluoroquinolone in association with ceftazidime in the treatment of severe ocular diseases,

promoting resistance to them. The percentage of *Micrococcus* species decline with fluoroquinolone exposure and were likely replaced by more resistant strains of *Staphylococcus epidermidis* and *Staphylococcus aureus*. *Micrococcus* is a non pathogenic organism in immunocompetent hosts, despite some reports. The emerging resistance of CONS ocular flora to the third and fourth generation fluoroquinolones has been observed by several studies^[19].

Our results demonstrate that fluoroquinolone resistant conjunctival CONS emerge rapidly after exposure to their respective antibiotic and are maintained by frequent re-exposure. This has considerable clinical implications. Conjunctival flora are assumed to be the predominant source of post injection endophthalmitis. It has been suggested that antibiotic resistant strains of *Staphylococcus epidermidis* is implicated in intraocular inflammation than antibiotic susceptible strains. The increased fluoroquinolone resistance among CONS isolated during visit one to four was more significant with the fourth generation moxifloxacin, when compared to baseline (77% vs 30.3% respectively). Rapid emergence of fluoroquinolone resistant *Staphylococcus aureus* and CONS was reported after the introduction of ciprofloxacin in the 1980s. This is of concern especially with the greater virulence with resistant *Staphylococcus epidermidis* and *Staphylococcus aureus* infections. The fluoroquinolone resistant strains of *Staphylococcus epidermidis* show expression of biofilm formation, while those of *Staphylococcus aureus* show increased expression of fibronectin thus enhancing their attachment and spread^[20].

Thus we conclude that repeated use of ophthalmic antibiotics not only alters the composition of the normal ocular flora, but also selects for resistant strains. This underlines the need for a more careful and judicious use for ophthalmic antibiotics after intraocular injection to reduce the hazard of antimicrobial resistance.

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