Conjunctival bacterial flora and antibiotic resistance pattern in patients undergoing cataract surgery

Flora bacteriana conjuntival e padrão de resistência a antibióticos em pacientes submetidos à cirurgia de catarata

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ABSTRACT

Purpose: To evaluate the conjunctival bacterial flora and its antibiotic resistance pattern in eyes of patients undergoing cataract surgery. **Methods:** From August to October 2004, 50 patients undergoing cataract surgery in the "Fundação Altino Ventura", Recife, Brazil, were prospectively evaluated. Conjunctival material was obtained on the day of surgery, before the application of topical anesthetic, antibiotic or povidone-iodine. The collected material was inoculated and bacterioscopic analysis was carried out. In the cases where there was bacterial growth, antibiotic susceptibility tests and cultures, for isolation and identification of the bacteria, were performed. **Results:** Of the 50 eyes, 43 (86.0%) had positive cultures. The coagulase-negative *Staphylococcus* (CNS), found in 27 (54.0%) eyes, was the most frequent organism. More than 90% of the isolates of this bacterium were susceptible to cephalotin, vancomycin, chloramphenicol, ofloxacin and gatifloxacin; 70 to 90% were susceptible to gentamicin, cefotaxime, oxacillin and ciprofloxacin; and less than 70% were sensible to neomycin. Four (10.5%) of the bacterial isolates were resistant to four or more antibiotics, two of them were CNS. **Conclusion:** The most frequent bacterium in the conjunctival flora is the coagulase-negative *Staphylococcus*. The isolates of this organism showed low susceptibility rate to neomycin, and high susceptibility rates to cephalotin, vancomycin, chloramphenicol, ofloxacin and gatifloxacin.

Keywords: Conjunctiva/microbiology; Antibiotic prophylaxis; Drug resistance, microbial; Cataract extraction; Endophthalmitis; *Staphylococcus*/isolation & purification

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INTRODUCTION

Cataract surgery is one of the most performed operations in the world. In the Unites States of America approximately 1.5 million procedures are performed annually[1]. One of the most feared complications of this surgery is endophthalmitis that, although infrequent, leads to high visual morbidity even with appropriate treatment. For this reason, ophthalmologists adopt several measures for its prophylaxis[2].

Possible sources of postoperative ocular infection are the ocular tear film, ocular adnexa, irrigation solutions, surgical instruments, respiratory and skin flora of the surgeon and assistants and operating room air[2]. Bacteria are the most common group of causative agents of endophthalmitis and gram-positive pathogens are responsible for 60 to 80% of acute infections[2]. Previous studies have shown that most bacteria responsible for postoperative ocular infection are part of the normal microbiota of the

conjunctiva and eyelids of the patient[3-4]. The culture is positive in 50 to 85% of the vitreous aspirates and the coagulase-negative *Staphylococcus* (*Staphylococcus epidermidis*) is the most frequently isolated pathogen, followed by *S. aureus* and *Streptococcus* spp, gram-negative organisms are responsible for 20% of the infections[5]. Studies have shown that the superficial flora penetrates the anterior chamber during cataract surgery but that does not necessarily lead to infection, suggesting that the anterior chamber could eliminate a small inoculum of bacteria without developing endophthalmitis[6-7].

Once superficial flora enters the eye during the cataract surgery, several prophylactic measures are applied to suppress or limit the growth of these microorganisms, which could lead to endophthalmitis. Thus, the use of topical antibiotics before and after cataract surgery is justified as they are efficient in reducing and, sometimes, temporarily eliminating the conjunctival microbiota[2,8]. However, not many studies evaluate the effect of topical antibiotics on endophthalmitis incidence, so the use of these drugs before surgery is considered as probably relevant for infection prophylaxis, but not definitely related to clinical outcome[2].

The choice for the prophylactic topical antibiotic is influenced by factors as spectrum of bacteria covered, the rapidity with which the antibiotic eliminates bacteria from the conjunctival surface, the duration of action, the penetration and toxicity of the antibiotic, the antibiotic susceptibility pattern and the cost[9-11]. Fluoroquinolones are commonly used agents for endophthalmitis prophylaxis, although there is the possibility that the resistance of the ocular bacterial flora to these antibiotics is increasing[9-12].

The purpose of this study was to evaluate the conjunctival bacterial flora and its antibiotic susceptibility pattern in eyes of patients undergoing cataract surgery.

METHODS

From August to October 2004, 50 patients undergoing cataract surgery in the "Fundação Altino Ventura", Recife, Pernambuco, Brazil, were prospectively evaluated. All patients underwent ophthalmologic evaluation and patients with signs of ocular infection were excluded.

Conjunctival material was obtained on the day of surgery before the application of topical anesthetic, antibiotic or povidone-iodine. The inferior conjunctival fornix was swabbed, without touching eyelid or lashes, the swab was then stored in Stuart transport medium. The collected material was inoculated and, when there was bacterial growth, cultures for isolation and identification of the bacteria were made and antibiotic susceptibility tests were carried out. The microbiological investigation followed these steps:

1. Microscopic examination: once collected the swab was rolled across a microscope glass slide, then was rapidly heat-fixed and colored by the Gram stain method.
2. Culture: two plates (blood agar and agar Levine) were used for the bacterial culture and one plate (agar Sabouraud) for the culture of fungus. The material collected by the swab was rolled in a Petri plate with solid culture medium and spread with a platinum wire in lines, covering all surface of the plate. This procedure allowed the isolated growth of the colonies. Later, the plates were incubated at 36±1°C for 24 to 48 hours and if the culture was positive antibiotic susceptibility tests were carried out.
3. Antibiotic susceptibility tests: the sensitivity of the isolated microorganisms was determined by the disc-diffusion method. The reading was made measuring the inhibition halo surrounding the disc.

Statistical analysis was performed using chi-square test, Fisher exact test and Fisher-Freeman-Halton exact test, $p<0.05$ was used for rejecting the null hypothesis.

The study was initiated after the approval by the "Fundação Altino Ventura" Ethics Committee and patients included in this study after their consent was obtained.

RESULTS

Of the 50 patients participating in this study, 56.0% were females and 44.0% males, the mean age was 67.6±11.8 years. The cultures were positive in 86.0% of the eyes.

Forty (88.9%) of the microorganisms isolated were gram-positive and five (11.1%) were gram-negative. The most frequently isolated bacterium was the coagulase-negative *Staphylococcus* (CNS), found in 27 eyes (54.0%). *Bacillus* sp. was isolated from six eyes (12.0%), *S. aureus* from four eyes (8.0%), *Citrobacter freundii* from three eyes (6.0%), and *S. saprophyticus*, *Corynebacterium* sp., *Proteus penneri*, *Enterococcus* sp. and *Morganelia morganii* from one eye (2.0%) each. From two eyes, more than one type of bacterium was isolated.

The antibiotic susceptibility pattern of the isolated colonies is presented in Table 1. The isolates of these organisms, grouped together, showed low susceptibility rate to neomy-

Table 1. Antibiotic susceptibility pattern of all bacterial isolates grouped together

	Neomycin	Gentamicin	Cephalotin	Cefotaxime	Oxacillin	Vancomycin	Chloramphenicol	Ciprofloxacin	Ofloxacin	Gatifloxacin
N	38	38	38	38	38	38	38	38	38	38
S	47.4%	79.0%	94.7%	89.4%	76.3%	89.5%	92.1%	89.5%	97.4%	100.0%
I	34.2%	2.6%	0.0%	5.3%	0.0%	0.0%	0.0%	2.6%	0.0%	0.0%
R	18.4%	18.4%	5.3%	5.3%	23.7%	10.5%	7.9%	7.9%	2.6%	0.0%

N= number tested; S= sensitive; I= intermediate; R= resistant

cin, and high susceptibility rate to cephalotin, chloramphenicol, ofloxacin and gatifloxacin. The isolates of CNS showed low susceptibility rate to neomycin, and high susceptibility rates to cephalotin, vancomycin, chloramphenicol, ofloxacin and gatifloxacin (Table 2). Tables 3 and 4 show the patterns of other isolated pathogens. It was not possible to perform the antibiogram for the anaerobic organisms.

There was no statistical difference in the frequencies of susceptibility of CNS between the fluoroquinolones. Both, ofloxacin and ciprofloxacin had similar susceptibility patterns compared to gatifloxacin (p=1.000 and p=0.236, respectively). The CNS was less sensitive to neomycin than to gentamicin (p=0.003), suggesting a greater resistance of this microorganism to neomycin.

Among the bacterial colonies for which antibiotic susceptibility tests were carried out, 10.5% showed resistance to several antibiotics (four or more), two of these isolates were CNS, both of them were resistant to oxacillin and susceptible to vancomycin and gatifloxacin. Organisms resistant to vancomycin were found in four eyes and all of them were gram-negative (*Citrobacter freundii* in three and *Morganella morganii* in one case).

Microscopic examination was performed in all cases, in 10.0% of them no organisms were found in the glass slide, 74.0% showed gram-positive cocci, 64.0% gram-positive bacilli, 8.0% gram-negative bacilli and in 4.0% of the cases a fungus was found (*Candida* sp.). Some glass slides showed more than one type of organism.

DISCUSSION

The resistance rates to antibiotics are growing with the dissemination and prolonged use of antimicrobial agents. Based on this, the characterization of bacterial ocular flora and its susceptibility pattern is highly justified, as it gives the surgeon a powerful tool to help in the choice of the most appropriate antibiotic to be used in the prophylaxis of his ocular surgeries[9].

It is known that the use of prophylactic antibiotics in cataract surgery reduces the number of organisms in the conjunctiva and eyelids[2-3], other desirable characteristics are good bioavailability, broad-spectrum coverage and favorable susceptibility patterns[9-11].

In this study, the most frequently found organism was the CNS, as shown in other studies[8-9,13-14], this organism is related to approximately 70% of the cases of postsurgical endophthalmitis[15].

There was a low susceptibility rate to neomycin, only 47.4% of the isolates were sensitive to this antibiotic. This may be due to indiscriminate and prolonged use of this agent and the common practice of self-medication found in Brazil. Fluoroquinolones are among the antibiotics commonly used in cataract surgery prophylaxis. We found a reduction in the susceptibility rates to the older quinolones (89.5 and 97.4% of the isolates are sensitive to ciprofloxacin and ofloxacin, respectively), compared to the latest generation quinolone gatifloxacin (100.0% of susceptibility).

Table 2. Antibiotic susceptibility pattern of coagulase-negative *Staphylococcus*

	Neomycin	Gentamicin	Cephalotin	Cefotaxime	Oxacillin	Vancomycin	Chloramphenicol	Ciprofloxacin	Ofloxacin	Gatifloxacin
N	27	27	27	27	27	27	27	27	27	27
S	55.6%	85.2%	100.0%	85.2%	85.2%	100.0%	92.6%	88.9%	96.3%	100.0%
I	33.3%	0.0%	0.0%	7.4%	0.0%	0.0%	0.0%	3.7%	0.0%	0.0%
R	11.1%	14.8%	0.0%	7.4%	14.8%	0.0%	7.4%	7.4%	3.7%	0.0%

N= number tested; S= sensitive; I= intermediate; R= resistant

Table 3. Antibiotic susceptibility pattern of *Staphylococcus aureus*

	Neomycin	Gentamicin	Cephalotin	Cefotaxime	Oxacillin	Vancomycin	Chloramphenicol	Ciprofloxacin	Ofloxacin	Gatifloxacin
N	4	4	4	4	4	4	4	4	4	4
S	50.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
I	25.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
R	25.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

N= number tested; S= sensitive; I= intermediate; R= resistant

Table 4. Antibiotic susceptibility pattern of *Citrobacter freundii*

	Neomycin	Gentamicin	Cephalotin	Cefotaxime	Oxacillin	Vancomycin	Chloramphenicol	Ciprofloxacin	Ofloxacin	Gatifloxacin
N	3	3	3	3	3	3	3	3	3	3
S	0.0%	66.7%	66.7%	100.0%	0.0%	0.0%	100.0%	100.0%	100.0%	100.0%
I	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
R	0.0%	33.3%	33.3%	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%

N= number tested; S= sensitive; I= intermediate; R= resistant

The CNS was more susceptible to oxacillin than it was described in a previous study[9], suggesting low rate of methicillin resistance in the institution where this study was carried out.

The number of CNS isolates resistant to 4 or more antibiotics (7.4%) was lower than the 39% described by Ta et al.[9], and similar to the one found by Pinna et al., (9.0%)[16]. Our results imply low rates of ocular colonization with multiresistant bacteria and demographic differences in the resistance pattern of the ocular bacterial flora.

In the microscopic examinations, as in the cultures, a predominance of gram-positive bacteria was found.

Another point to be considered is that our tests of antibiotic sensitivity were performed in vitro and might not reflect the real efficiency of these antibiotics in vivo. In addition, the disc-diffusion sensitivity test technique is based on the serum concentration of the antibiotics that might be different from the concentration in the conjunctiva with the topical use of eye drops[9]. This suggests that our study can be used by the ophthalmologists as an orientation tool when choosing a prophylactic antibiotic to be used in their surgeries but our results should be confirmed by in vivo tests.

CONCLUSION

This study showed that the bacterium most frequently found in the conjunctival flora of the patients undergoing cataract surgery was the CNS. Isolates of this bacterium had low susceptibility rates to neomycin and high susceptibility to cephalothin, vancomycin, chloramphenicol, ofloxacin and gatifloxacin.

Nowadays, with the considerable increase of bacterial resistance to antibiotics, the understanding of the sensitivity of the conjunctival bacterial flora to antibiotics is of fundamental importance. Additionally, these studies can guide the ophthalmologists when choosing a prophylactic antibiotic to be used in their surgeries.

RESUMO

Objetivo: Avaliar a flora bacteriana conjuntival e seu padrão de resistência a antibióticos em olhos de pacientes a serem submetidos a cirurgias de catarata. **Métodos:** Foram avaliados, prospectivamente, os olhos de 50 pacientes a serem submetidos a facectomias na Fundação Altino Ventura, Recife (PE), durante o período de agosto a outubro de 2004. Foi coletado material para cultura da conjuntiva no dia da cirurgia, antes da aplicação de anestésicos, antibióticos ou iodo povidona tópicos. A partir do material coletado foram realizados bacterioscopias e semeio. Em caso de crescimento bacteriano, foram realizadas culturas para isolamento e identificação das bactérias e preparação de antibiogramas. **Resultados:** Entre os 50 olhos estudados, sete (14,0%) apresentaram culturas negativas e 43 (86,0%) culturas positivas. A bactéria mais freqüentemente isolada foi o *Staphylococcus* coagulase-negativo, encontrada em 27 olhos (54,0%). Entre os isolados desta bactéria, mais de 90% foram sensíveis a cefalotina, vancomicina, cloranfenicol, ofloxacino e gatifloxacino; 70 a 90% destes microrganismos foram sensíveis a gentamicina, cefotaxima, oxacilina e ciprofloxacino; menos que 70% deles foram sensíveis à neomicina. Encontrou-se quatro (10,5%) isolados de bactérias resistentes a quatro ou mais antibióticos, sendo que dois deles foram de *Staphylococcus* coagulase-negativo (7,4% dos isolados desta bactéria). **Conclusão:** A bactéria mais freqüentemente encontrada na conjuntiva foi o *Staphylococcus* coagulase-negativo, sendo que estes isolados mostraram alta resistência aos aminoglicosídeos, principalmente à neomicina, com alta suscetibilidade à cefalotina, vancomicina, cloranfenicol, ofloxacino e gatifloxacino.

Descritores: Conjuntiva/microbiologia; Antibioticoprofilaxia; Resistência microbiana a drogas; Extração de catarata; Endoftalmite; *Staphylococcus*/isolamento & purificação

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