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CONJUNCTIVAL ENDOGENOUS MICROBIOTA IN PATIENTS SUBMITTED TO CATARACT SURGERY

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ABSTRACT

Bacterial isolation, identification and antimicrobial susceptibility tests were carried out in ocular material collected with swab and polimethylmethacrylate (PMMA) or silicone intraocular lenses (IOL) from forty six patients submitted to cataract surgery. Seventy six isolates and seven different microorganisms were identified. Coagulase-negative staphylococci (CNS) were the predominant microorganisms isolated from swabs (71.4% of cases), PMMA lenses (81.3%) and silicon lenses (77.8%). Coagulase-negative staphylococci isolates revealed high resistance to penicillin G followed by tetracycline, chloramphenicol and aminoglicosides. However, these isolates displayed great susceptibility to vancomycin, cephalothin and ofloxacin. Except for penicillin G, *Staphylococcus aureus* was very sensitive to the antimicrobial agents including oxacillin. Among Gram-negatives, *Proteus mirabilis* was prevalent and presented high resistance to tetracycline and chloramphenicol. *Enterococcus* isolates were vancomycin sensitive.

Key words: bacteria, conjunctiva, cataract, intraocular lens, antibiotic.

INTRODUCTION

Surgical removal of cataract (facectomy) is the most common eye surgery: in the United States, for example, over 1,000,000 patients are operated a year. Despite great progress has been made in surgical techniques, postoperative endophthalmia is one of the most feared complications of facectomy which may lead to total blindness in up to 50% of diagnosed cases (19,27,35). However, some studies have reported bacterial contamination of the aqueous humour during cataract surgery, without occurring post-operative ocular infection (3,37,43). Conjunctival endogenous microbiota is the main cause of postoperative and post-traumatic eye infections (2,5,8). Molecular techniques have shown a great similarity among strains isolated from vitreous humour, eye anterior chamber and intraocular lens (IOL) in patients with Coagulase-negative staphylococci endophtalmia (17).

Gram-positive microorganisms may be found in up to 70% of endophthalmia cases, with coagulase-negative staphylococci (CNS) being the most common pathogens (7,25,45). It is known that CNS are able to adhere and proliferate on polymeric surfaces, including IOL. Once adhered they secrete a viscous extracellular matrix (biofilm) which protects the microorganisms against the action of antibiotics and the host immune response (12,14,42). When cultures were performed from swabs and lenses after contact with the conjunctiva of patients submitted to facectomy, the number of colonies isolated from IOL was four times higher than that obtained from swabs. This great difference was imputed to electrostatic charges that build up on the IOL metacrylate surface (44).

There is a deep concern with the increase of microbial resistance to drugs around the world. Several authors investigated the antimicrobial susceptibility of microorganisms isolated from ocular infection with different patterns of

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sensitivity occurring (9,20,23,26,32,33), but little information is available in our country (25). The purpose of the present study was to evaluate the conjunctival aerobic microbiota of individuals submitted to cataract surgery and *in vitro* antimicrobial susceptibility of the isolates by using swabs and intraocular lenses for material collection.

MATERIALS AND METHODS

Patients

This procedure was carried out at Hospital de Clínicas da Universidade Federal do Rio Grande do Sul, RS, Brazil, from May to September 2000. Forty-six patients, from different geographical regions, submitted to facectomy followed by implantation of IOL through Brazilian National Health Service (SUS) were included.

Intraocular lenses (IOL)

Forty-six 6.5-mm PMMA IOL (MC50BD model, CILCO®, Alcon Surgical Inc., Forth Worth, Texas, USA), and forty-six 6-mm silicon IOL (SSM26NB model, ALLERGAN MEDICAL OPTICS™, Irvine, California, USA), whose straps were removed, were used in this study.

Ocular material collection

After agreement, collection of ocular material from the patient was carried out in two stages. The first one addressed identification of the endogenous microbiota including samples obtained during preoperative routine procedures. A moist sterile swab was smeared across the conjunctival sac, immediately placed in 2 mL brain-heart infusion (BHI) to send for analysis. The second step, before cataract surgery, was performed in the operating theatre where standard aseptic conditions were strictly observed. The eyelids, eyebrow, nose, cheek and forhead were scrubbed with 10% povidone-iodine in concentric rings outward from the eye. Then, one drop of 5% povidon-iodine was instilled into the eye. One sterile silicon lens and one PMMA intraocular lens were used, for each patient, to collect the samples with help of sterile forceps. The IOL was touched onto the upper conjunctiva for 15 seconds, placed in a tube containing BHI and immediately transported to the laboratory for analysis.

Isolation and microorganism identification

The samples were incubated at 37ºC for 18 to 24h, and then cultured onto 5% sheep blood agar, chocolate agar and MacConkey agar. Subsequently, samples were incubated at 37ºC for 48 hours in microaerophilic environment (candle jar). Each morphologically distinct colony was Gram stained. Standard microbiological methods were used for microorganism identification as described below:

Gram positive cocci isolated on blood agar plates were identified by colonial morphology/hemolysis observation and the following tests: catalase, coagulase, oxidase, bacitracin and novobiocin susceptibility, manitol, 6.5% NaCl, bile esculin and optochin disk (21,24).

The identification of Gram positive rods included esculin hydrolysis, arginine decarboxylation, carbohydrate fermentation (manitol, glucose, maltose, sucrose, trehalose, xylose, arabinose), Voges-Proskauer, urease, gelatin hydrolisis, catalase, SIM, nitrate reduction and Simmons citrate (22).

Gram negative rods were identified by tests of carbohydrate fermentation (glucose, manitol, lactose, maltose, trehalose), gas production, methyl red, Voges-Proskauer, urease, gelatin hydrolisis, oxidase, catalase, SIM, phenylalanine deaminase, nitrate reduction and Simmons citrate (22).

Antimicrobial susceptibility testing

Mueller-Hinton medium (ISOFAR, Rio de Janeiro, Brazil) was used to perform the diffusion test as established in the Kirby-Bauer method (4). The inoculum used in this assay corresponded to 0.5 in the MacFarland scale. The antibiotic discs tested (CEFAR, São Paulo, Brazil) included: chloramphenicol (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), penicillin (10 UI), gentamicin (10 µg), cephalothin (30 µg), tobramycin (10 µg), ofloxacin (5 µg), kanamycin (30 µg), vancomycin (30 µg), and oxacillin (1 µg). Results were interpreted after 18 to 24h incubation, at 37ºC. Microorganisms were typed as sensitive (S), intermediately sensitive (I), or resistant (R) according to NCCLS (31).

Reference strains (ATCC 35984 *S. epidermidis*; ATCC 29213 *S. aureus*; ATCC 27853 *P. aeruginosa*) were used as a quality control for antimicrobials.

RESULTS

Out of 46 swabs used to assess the endogenous microbiota, bacterial growth was observed in 38 swabs (82.6%), with 34 exhibiting pure cultures. In intraocular lenses, 16 (34.8%) were positive for bacterial growth in PPMA lenses and 18 (39.1%) for silicon lenses (Table 1).

Seventy six isolates and seven different microorganisms were identified as shown in Table 2. In swab samples 88% of

Table 1. Occurrence of positive and negative cultures in samples obtained from 46 patients using swab, polymethyl-metacrylate (PMMA) and silicon intraocular lenses (IOL).

	Positive cultures	Negative cultures	Total
Swab	38 (82,6%)[1]	08 (17,4%)	46
PMMA LIO	16 (34,8%)	30 (65,2%)	46
Silicon LIO	18 (39,1%)	28 (60,9%)	46

[1] Mixed cultures occurred in four cases.

Gram-positive microorganisms were detected: coagulase-negative staphylococci (CNS) in 71.4% of cases, followed by *Staphylococcus aureus* (9.5%), *Enterococcus spp.* (4.8%) and *Corynebacterium pseudodiphteriticum* (2.4%). Gram-negatives were present in 12% of swab samples with the following microorganisms being isolated: *Proteus mirabilis*, *Stenotrophomonas maltophilia* and *Acinetobacter calcoaceticus*. In intraocular lenses *Staphylococcus* was isolated in most cases with predominance of CNS (81.3% in PMMA and 77.8% in silicon lenses).

Table 3 shows the antimicrobial susceptibility level of CNS and *S. aureus* isolates. Among antibiotics tested the highest resistance was exhibited against penicillin G for both species, as opposed to vancomycin, cephalothin and ofloxacin. All *S. aureus* isolates were sensitive to oxacillin and slight resistance was observed for CNS. Overall, some degree of resistance to aminoglycosides was detected for staphylococci. *S. aureus* isolates exhibited a better performance than CNS in relation to chloramphenicol and tetracycline.

Table 4 shows the antimicrobial activity of six *P. mirabilis* isolates. High level of resistance was observed to tetracycline and chloramphenicol. Considerable resistance occurred against aminoglycosides but all isolates were sensitive to ofloxacin and ciprofloxacin.

Enterococcus spp. isolates (2) were sensitive to vancomycin, as opposed to tetracycline (data not shown).

Table 2. Bacteria species and respective frequency of isolates (n=76) detected in ocular material from 46 patients using swab, polymethyl-metacrylate (PMMA) and silicon intraocular lenses (IOL).

Bacteria Species	Swab	PMMA IOL	Silicon IOL
Coagulase -negative *staphylococci*	30 (71.4%)	13 (81.3%)	14 (77.8%)
Staphylococcus aureus	4 (9.5%)	2 (12.5%)	2 (11.1%)
Enteroccus spp	2 (4.8%)	-	-
Proteus mirabilis	3 (7.1%)	1 (6.2%)	2 (11.1%)
Stenotrophomonas maltophilia	1 (2.4%)	-	-
Acinetobacter calcoaceticus	1 (2.4%)	-	-
Corynebacterium pseudodiphteriticum	1 (2.4%)	-	-

Table 3. Antimicrobial susceptibility of coagulase-negative staphylococci and *S.aureus* isolates where S= sensitive, I= intermediate and R= resistant.

Antibiotic	Disc concentration	Coagulase-negative staphylococci (n=57)			*Staphylococcus aureus* (n=8)		
		S	**I**	**R**	**S**	**I**	**R**
Ciprofloxacin	5 µg	46	04	07	04	04	0
Ofloxacin	5 µg	52	02	03	08	0	0
Gentamicin	10 µg	43	04	10	04	04	0
Tobramycin	10 µg	44	06	07	05	03	0
Kanamycin	30 µg	45	01	11	08	0	0
Cephalothin	30 µg	53	02	02	08	0	0
Chloramphenicol	30 µg	35	07	15	07	0	01
Tetracycline	30 µg	35	02	20	08	0	0
Penicillin G	10 IU	08	0	49	04	0	04
Oxacillin	1 µg	51	01	05	08	0	0
Vancomycin	30µg	57	00	00	08	0	0

Table 4. Antimicrobial susceptibility of *Proteus mirabilis* isolates where S= sensitive, I= intermediate and R= resistant.

Antibiotic	Disc Concentration	*Proteus mirabilis* (n=6)		
		S	**I**	**R**
Ciprofloxacin	5 µg	6	0	0
Ofloxacin	5 µg	6	0	0
Gentamicin	10 µg	3	2	1
Tobramycin	10 µg	3	2	1
Kanamycin	30 µg	0	6	0
Cephalotin	30 µg	3	2	1
Chloram	30 µg	0	4	2
Tetracyclin	30 µg	1	0	5

DISCUSSION

Results shown in Table 1 reveal that samples collected using swab yielded a greater number of positive cultures when compared to those collected using intraocular lenses but slight differences were observed between silicon and PPMA lenses. This may be explained by the fact that swabs were used to sample the conjunctival sac preoperatively, differently from the procedure adopted to collect samples with intraocular lenses, carried out in the operating theatre, where sample collection followed aseptic care.

Another important aspect to be taken into consideration is that the bacterial population increases as on move away from the limbo into the palpebral margin. Marcon *et al.* (25) observed 45% of conjunctival positive cultures and 100% of positive cultures from samples collected from the palpebral margin. Interestingly, Speaker *et al.* (39) found that bacteria in the anterior chamber of the eye and those causing endophtalmia were identical in molecular terms to bacteria present in the bottom of the conjunctival sac and palpebral margin.

Staphylococcus epidermidis is a common microorganism found on skin and mucosae. As an opportunistic organism may contaminate medical devices such as intraocular lenses, leading to postoperative infections (endophtalmia) in patients who underwent cataract surgery (21). Doyle *et al.* (11) observed that 70% of the microorganisms present in the conjunctival flora in samples collected with swab were CNS, also detected in 82% of PMMA lenses after contacting the conjunctiva immediately following the surgical procedure. Mistlberger *et al.* (28) isolated 75% of CNS present in the conjunctival flora of aspirates collected from the eye anterior chamber. These data are very similar to ours that showed a 75% positivity for CNS. Hughes and Hill (15) reported that Gram-positive microorganisms may be found in up to 70% of the postoperative endophtalmia occurrences, with CNS being the most common one, present in about 40% of cases. *S. aureus* was found in 20% of cases, whereas Gram-negative microorganisms occurred in 16% of the cases. In isolates of ocular infections, Ooshi and Miyao (33) identified Gram-positive cocci in 44.7% of cases, with prevalence of CNS (52.6% of cases) in contrast to 7.4% of Gram-negative bacilli (7,4%).

In spite of the fact that the source of infection can not be determined in the majority of episodes after facectomy, microorganisms present in ocular normal flora and nearby structures are thought to be responsible for the infectious process (7,25,39,40). It was reported that up to 20% of the bacteria present in this flora might be associated with biofilm formation and, once adhered by electrostatic attraction to the intraocular lens, will cause infection (29). When the conjunctival flora of patients submitted to facectomy was assessed prior to surgery, bacteria capable of causing endophtalmia were found in 74% of these individuals (45). On the other hand, it is well known that despite intense preoperative care of patients, either employing antibiotics or polyvinyl-pyrrolidone, the conjunctiva does not become refractory to bacterial growth (2,5,6,8,16). We demonstrated in this study that it may be possible to occur contamination of IOL if they touch the conjunctiva, mainly by staphylococci.

Table 3 shows the antibiotic susceptibility of CNS and *S. aureus*. Vancomycin displayed the best activity, followed by cephalotin, ofloxacin and oxacillin. Higher resistance to aminoglicosides was detected in staphylococci isolates. Not surprisingly great resistance to penicillin G was observed. Kato and Hayasaka (20) detected 978 conjunctival isolates from 628 preoperative patients with no clinical eye infection: in 10 patients methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant staphylococci coagulase-negative (MRCNS) isolates were found to be sensitive to ofloxacin. Different authors examined the antibiotic susceptibility of bacteria isolated from ocular infection with MRSA accounting for 27% and MRCNS for 6.8% of strains. Most MRSA isolates were resistant to penicillin G and ofloxacin but sensitive to vancomycin (33). In our study, five methicillin-resistant CNS isolates (8.8%) were found, but no methicillin-resistant *S. aureus* was isolated.

Fluoroquinolones for ophtalmic use include ciprofloxacin and ofloxacin. In general, ciprofloxacin has been showed to be more effective against the majority of microorganims that cause endophtalmia, though ofloxacin is able to penetrate more efficiently into ocular tissues (9,23,32,38). Marone *et al.* (26) found high anti-staphylococci activity of ofloxacin against coagulase-negative staphylococci and *S. aureus* isolated from eye infections, which was also seen for tobramycin and gentamicin. In our study, better levels of acitivity were obtained with ofloxacin against CNS isolates (Table 3).

In relation to chloramphenicol and tetracycline, CNS exhibited greater resistance when compared to *S. aureus*, which was in turn susceptible to both antibiotics (Table 3). A study including 534 hemolytic staphylococci strains reported resistance to tetracycline in 13% and to chloramphenicol in 2% of occurrences (13). Petersdorf *et al.* (34) stressed a significant ability of staphylococci to develop resistance against chloramphenicol.

In our study, due to the low number of *Enterococcus* spp. and *C. pseudodiphtheriticum* isolates detected, the antibiotic susceptibility will not be discussed here.

Among Gram-negative microorganisms *Proteus mirabilis* predominated, exhibiting great susceptibility to ciprofloxacin and ofloxacin, resistance to tetracycline and intermediate sensitivity to kanamycin and chloramphenicol (Table 4). Ooshi and Miyao (33) observed that the majority of non-fermenter Gram-negative bacilli isolated in patients suffering from ocular infections was likewise susceptible to ofloxacin. Other studies confirmed *in vitro* (10,26,36) and *in vivo* (23) efficiency of ciprofloxacin and ofloxacin against Gram-negatives including *P. aeruginosa*.

Overall, our study demonstrated that coagulase-negative staphylococci were the most commonly isolated aerobic microorganisms in ocular material, with predominance in samples collected with swab than in those collected with intraocular lenses. Among antimicrobials tested, the highest resistance was shown against Penicillin G, while vancomycin displayed the best activity, followed by cephalotin, ofloxacin and oxacillin. Additional studies are needed to evaluate the clinical importance of conjunctival endogenous microbiota on the pathogenesis of endophthalmitis and the influence of antibiotic resistance on treatment. *In vitro* experiments are underway in our laboratory to assess bacteria biofilm production an their interaction with PMMA and silicon intraocular lenses.

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RESUMO

Microbiota endógena conjuntival em pacientes submetidos à cirurgia de catarata

A partir de material ocular coletado de 46 pacientes submetidos à cirurgia de catarata foram realizados isolamento, identificação e teste de susceptibilidade de microrganismos frente a antimicrobianos, utilizando-se suabes e lentes intraoculares (LIO). Foram obtidos 76 isolados e identificados 7 tipos de microrganismos. Estafilococos coagulase-negativos (CNS) foram os microrganismos mais freqüentemente detectados de suabes (71,4% dos casos), lentes de PMMA (81,3%) e lentes de silicone (77,8%). Isolados de CNS apresentaram elevada resistência à penicilina G, seguida por tetraciclina, cloranfenicol e aminoglicosídeos. No entanto, estes isolados mostraram grande sensibilidade à vancomicina, cefalotina e ofloxacina. Com exceção da penicilina G, os isolados de *Staphylococcus aureus* foram bastante sensíveis aos agentes antimicrobianos, incluindo a oxacilina. Entre as gram-negativas, *Proteus mirabilis* foi a bactéria mais freqüente e também se mostrou bastante resistente à tetraciclina e ao cloranfenicol. Os isolados de *Enterococcus* se mostraram sensíveis à vancomicina.

Palavras-chave: bactéria, conjuntiva, catarata, lente intraocular, antibióticos.

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TERMO DE CONSENTIMENTO

A endoftalmite é uma infecção do olho que pode ocorrer em um de cada mil pacientes submetidos à cirurgia para extração da catarata. Esta doença é geralmente causada por bactérias e pode em alguns casos levar à perda total da visão no olho afetado.

Diversos estudos têm sido feitos nos últimos anos na tentativa de encontrar fatores que predisponham ao desenvolvimento desta doença. Isto tem permitido uma diminuição na incidência de endoftalmite, como a que se tem acompanhado década após década.

O Serviço de Oftalmologia do Hospital de Clínicas de Porto Alegre, através do seu corpo clínico, quer conduzir um estudo tentando identificar mais um possível fator de risco para esta doença.

Como você realizará cirurgia de catarata neste serviço, gostaríamos de convidá-lo a participar deste estudo como voluntário. A sua participação consiste em permitir que seja coletada amostra para exame bacteriológico antes da cirurgia, bem como no momento que esta ocorrer. A coleta das amostras será feita primeiramente com um suabe e após com uma lente intra-ocular. É importante ressaltar que a sua participação neste estudo não ocasionará nenhum risco adicional aos da própria cirurgia e será de grande importância para o avanço da ciência.

Eu, ____, fui claramente informado dos objetivos deste estudo e da metodologia empregada. Sei também que poderei retirar esse consentimento a qualquer momento se assim julgar adequado.

Assin. do(a) paciente ____

Assin. do(a) médico responsável ____

Assin. do orientador ____

Porto Alegre, _____ de ___________ de 2000