• Clinical Research •

Conjunctival flora in anophthalmic patients: microbiological spectrum and antibiotic sensitivity

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Abstract

• AIM: To identify the spectrum and susceptibility pattern of isolated microorganisms from conjunctival flora of anophthalmic patients.

• METHODS: A cross-sectional clinical study including 60 patients with unilateral anophthalmia. Patients with use of antibiotic drops in their socket during the last month were also included. From each patient, three microbiological samples were taken from the lower conjunctival sac (healthy eye, pre-prosthesis, and retro-prosthesis space of socket). The 180 samples obtained were cultured. Isolates were identified and their antibiotic sensitivities were determined.

• RESULTS: A total of 251 isolates were recovered (62 isolates from healthy eye, 93 from pre-prosthesis, and 96 from retro-prosthesis space). The most common organism was *Staphylococcus epidermidis,* in both healthy eyes (64.5%) and sockets (45.5%). Altogether, coagulase-positive *Staphylococci, Streptococci*, and Gram-negative bacteria accounted for less than 15% of isolates in healthy eyes and more than 35% in sockets. Regarding the antibiotic sensitivities, there were no significant differences between isolates from sockets and healthy eyes. Nine patients recognized the use of self-prescribed antibiotic drops in their socket. In the healthy eyes of these subjects, Gram-positive microorganisms showed significantly greater resistance to aminoglycosides and tetracycline.

• CONCLUSION: Sockets of anophthalmic patients show a greater number of pathogens compared to healthy eyes. The use of antibiotic drops in the socket promotes a resistant flora not only in the socket but also in the healthy eye. Quinolones and macrolides may be better therapeutic options than aminoglycosides for treating conjunctivitis of anophthalmic sockets, since these antibiotics are less active against *Staphylococcus epidermidis*.

• **KEYWORDS:** socket; ocular prosthesis; antibiotic resistance; microflora; conjunctival dysbiosis

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INTRODUCTION

Ocular prostheses improve the esthetic alteration that causes the loss of an eye. However, anophthalmic patients often refer chronic discharge and irritation in their sockets[1]. These annoyances lead to the frequent use of antibiotic drops, which predisposes to increase the resistance of the conjunctival flora and select the species with greater pathogenic potential[2].

Several studies[3-8] have investigated the conjunctival flora in anophthalmic sockets, although most of them were published in the 1990s or earlier. These reports have generally shown higher rates of pathogens in sockets compared to the normal conjunctival flora. Moreover, some authors have suggested that the flora of the anophthalmic socket could affect the healthy eye flora[4-5,8-9]. If pathogens from the socket persist in a surgical field, the risk of developing an intraocular infection in the fellow eye is increased[10]. Therefore, these authors recommended maximizing the anti-infective prophylaxis of the fellow eye during intraocular surgery. However, the antibiotic sensitivity of the isolated microorganisms has not been analyzed in these studies.

The purpose of this paper was to characterize the conjunctival microbiota of anophthalmic patients, in both their socket and their healthy eye, and determine and compare the antibiotic susceptibility of the isolated species from the two microbial communities.

SUBJECTS AND METHODS

Ethical Approval This study protocol was approved by the Ethics Committee of the University Hospital of León (Spain) in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects before participation.

The microorganisms isolated in this research were obtained from samples taken from 60 anophthalmic patients in a previous investigation[9]. That study was designed to explore the relationship between conjunctival flora and comfort of the socket in anophthalmic patients. Inclusion criteria, exclusion criteria and the technique to obtain the specimens were thoroughly described in that publication[9]. Basically, selected subjects were unilateral anophtalmic patients with daily wearing of artificial eye, and with stable symptomatology in the last month. Patients that regularly used self-prescribed antibiotic eyedrops in their sockets were also included. Subjects with acute conjunctivitis, conjunctival cyst, orbital implant exposure, or wearing a poorly fitting prosthesis were excluded. Regard the technique to collect the samples, topical anesthetic was not used to avoid damaging the conjunctival microorganisms[9]. Sterile rayon swabs (Copan Diagnostics Inc., Murrieta, CA, USA) previously moistened with sterile brain heart infusion (BHI) culture medium were used to take three microbiological specimens of each patient: the lower conjunctival sac of 1) the healthy eye; 2) the anophthalmic socket before removing the ocular prosthesis (pre-prosthesis sample); and 3) the socket after removing the artificial eye using a suction cup (retro-prosthesis sample). Each swab was cut off and placed in a tube (Becton, Dickinson & Company, Franklin Lakes, NJ, USA) with 4 mL of sterile BHI, and the tube was closed. Samples were taken to the clinical microbiology laboratory of our hospital within an hour after collection.

The 180 tubes with the samples were incubated at 37℃ in an aerobic atmosphere[9]. They were assessed 24h after collection and then daily for 10d before they were classified as negative-cultures. If growth was noted, the broth was subcultured to different media for microorganism identification. Culture media for fungi and aerobic, facultative anaerobic and strict anaerobic bacteria were used.

Microbial identification was founded on growth in selective media, colonies morphology, and the utilization of MicroScan (Siemens, Munich, Germany) or API (bioMérieux, Marcy l'Etoile, France) systems. *Staphylococci* were identified by using panel 31 of the MicroScan system, *Enterococci* by panel 32, fermentative Gram-negative bacteria by panel 53, and non-fermentative Gram-negative bacteria by panel 54. The other microorganisms were identified by API galleries.

The antibiotic sensitivity was determined by MicroScan panels (Siemens, Munich, Germany) in those bacteria suitable for their use (*Staphylococci*, *Enterococci* and Gram-negative bacteria), and by diffusion discs in plates (Oxoid, Basingstoke, UK) in the other microorganisms. Antifungal sensitivity was not tested. The selection of antibiotics, their minimum inhibitory concentration (MIC), and inhibition zones were established according to the guidelines from the Clinical and Laboratory Standards Institute (CLSI) of 2010 and 2011. Thus, isolates were initially classified as sensitive, intermediate, or resistant for each antibiotic in accordance with CLSI break points. Subsequently, the intermediate sensitivity was considered resistant[11].

The isolated bacterial species were clustered to perform the antibiotic susceptibility analysis. Groups that settled initially were *Staphylococci*, *Streptococci*, *Enterococci*, *Micrococcus spp.*, coryneform bacteria[12], and Gram-negative bacteria. Since *Staphylococci* were the most isolated bacteria from the conjunctival flora, they were classified in 3 new groups: coagulase-positive *Staphylococci* (CPS), coagulase-negative *Staphylococci* (CNS) excluding *Staphylococcus epidermidis* (*S. epidermidis*), and *S. epidermidis* which formed its own group. Moreover, microorganisms were catalogued as pathogens or saprophytes. CNS (*S. epidermidis* and other species), *Micrococcus spp.*, and *Coryneform* bacteria were classified as saprophytic microorganisms. Other groups were considered pathogens.

Statistical Analysis Descriptive statistics were performed. The "percent susceptible" was calculated as the number of susceptible isolates over the total number of isolates times 100[13]. Group comparisons of 2 by 2 cross tables were conducted with the two-tailed Fisher's exact test, while the Chi-square test (with Yate's correction when appropriate) was used in the other cases. Analysis were run using SPSS for Windows version 22.0 (SPSS Inc., Chicago, IL, USA). The statistical significance level was set as P value less than 0.05.

RESULTS

A total of 251 isolates were cultured from 60 anophthalmic patients (62 isolates from healthy eye conjunctiva, 93 from pre-prosthesis space of the socket, and 96 from retro-prosthesis space). The patients were 36 male subjects and 24 female subjects. The average age of the patients was 61.9 (range 25-89y). The results of the microbiological identification are shown in Table 1.

A total of 14 different microbial species were isolated in the healthy eyes, while 35 different species were cultured from the sockets. Of the 60 samples obtained from healthy eyes, there were 9 that showed no growth. Likewise, 3 specimens had no growth among pre-prosthesis samples. On contrary, all of the specimens from retro-prosthesis space had a positive culture. Therefore, the positive culture rate was 85.0%, 95.0%, and 100.0% for healthy eye, pre-prosthesis and retro-prosthesis samples, respectively.

Table 1 Isolated microorganisms from anophthalmic patients *n* (%)

Isolated microorganisms	No. isolates		
	Healthy eye	Pre-prosthesis	Retro-prosthesis
Gram-positive	60 (96.8)	82 (88.2)	83 (86.5)
Coagulase-negative *Staphylococci*	48 (77.4)	52 (55.9)	55 (57.3)
Staphylococcus epidermidis	40 (64.5)	44 (47.3)	42 (43.8)
Staphylococcus warneri	4 (6.5)	2 (2.2)	5 (5.2)
Staphylococcus capitis	2 (3.2)	2 (2.2)	4 (4.2)
Staphylococcus lugdunensis	2 (3.2)	1 (1.1)	2 (2.1)
Staphylococcus simulans	-	1 (1.1)	1 (1.1)
Staphylococcus hominis	-	-	1 (1.1)
Staphylococcus lentus	-	1 (1.1)	-
Staphylococcus schleiferi	-	1 (1.1)	-
Coagulase-positive *Staphylococci*	5 (8.1)	11 (11.8)	12 (12.5)
Staphylococcus aureus	5 (8.1)	11 (11.8)	11 (11.5)
Staphylococcus intermedius	-	-	1 (1.1)
Coryneform bacteria	3 (4.8)	2 (2.2)	2 (2.2)
Corynebacterium macginleyi	1 (1.6)	1 (1.1)	-
Corynebacterium amycolatum	1 (1.6)	-	-
Corynebacterium striatum	1 (1.6)	-	-
Corynebacterium pseudodiphtheriticum	-	-	1 (1.1)
Cellulomonas spp.	-	1 (1.1)	-
Dermabacter hominis	-	-	1 (1.1)
Enterococci	1 (1.6)	2 (2.2)	1 (1.0)
Enterococcus faecalis	1 (1.6)	1 (1.1)	1 (1.1)
Enterococcus faecium	-	1 (1.1)	-
Micrococci	1 (1.6)	1 (1.1)	-
Micrococcus luteus	1 (1.6)	1 (1.1)	-
Streptococci	2 (3.2)	14 (15.1)	13 (13.5)
Streptococcus oralis	1 (1.6)	6 (6.5)	5 (5.2)
Streptococcus mitis	-	3 (3.2)	3 (3.1)
Streptococcus acidominimus	-	1 (1.1)	-
Other viridans group *Streptococci*	1 (1.6)	3 (3.2)	4 (4.2)
Streptococcus pneumoniae	-	1 (1.1)	1 (1.1)
Gram-negative	2 (3.2)	10 (10.8)	11 (11.5)
Klebsiella oxytoca	1 (1.6)	1 (1.1)	2 (2.1)
Klebsiella pneumoniae	-	1 (1.1)	1 (1.1)
Pseudomonas aeruginosa	-	2 (2.2)	2 (2.1)
Pseudomonas fluorescens	-	1 (1.1)	-
Stenotrophomonas maltophilia	-	2 (2.2)	2 (2.1)
Serratia liquefaciens	-	1 (1.1)	1 (1.1)
Alcaligenes spp.	-	1 (1.1)	1 (1.1)
Moraxella spp.	1 (1.6)	-	1 (1.1)
Veillonella parvula	-	1 (1.1)	-
Enterobacter intermedium	-	-	1 (1.1)
Fungi	-	1 (1.1)	2 (2.1)
Candida parapsilosis	-	1 (1.1)	1 (1.1)
Saccharomyces cerevisae	-	-	1 (1.1)
Total	62 (100)	93 (100)	96 (100)

Table 2 Antibiotic sensitivities of *S. epidermidis* according to the area of isolation[a]

Antibiotic	Susceptible isolates of *S. epidermidis*			
	Healthy eye	Pre-prosthesis	Retro-prosthesis	P^b
Fluoroquinolones				
Ciprofloxacin	28	32	28	0.829
Levofloxacin	28	32	28	0.829
Aminoglycosides				
Gentamicin	37	35	33	0.169
Tobramycin	30	32	28	0.686
Amikacin	36	36	30	0.099
Beta lactams				
Penicillin	1	3	3	0.592
Amoxicillin-clavulanate	27	32	27	0.697
Oxacillin	27	33	28	0.650
Others				
Fusidic acid	33	37	36	0.924
Erythromycin	16	18	19	0.875
Tetracycline	32	37	31	0.496
Clindamycin	27	34	28	0.486
Trimethoprim-sulfamethoxazole	39	42	40	0.846
Rifampin	40	44	42	-
Vancomycin	40	44	42	-
Daptomicina	40	44	42	-
Linezolid	40	44	42	-

[a]*S. epidermidis* isolates were 40, 44, and 42 from healthy eyes, pre-prosthesis and retro-prosthesis spaces, respectively. All isolates of *S. epidermidis* were tested for all antibiotics. [b]Pearson's Chi-square test.

Statistical analysis was performed after isolates were grouped. There were not statistical differences between isolates from pre-prosthesis and retro-prosthesis space, so the strains from the socket were assessed together. The most commonly isolated species, in both healthy eyes and sockets, was *S. epidermidis*, followed by *S. aureus* (Table 1). CNS group was the main in both healthy eyes (77.4%) and sockets (56.6%). CPS accounted for 8.1% of isolates in healthy eyes and 12.1% in sockets. The remaining groups were less than 5% of cultures, except *Streptococci* and Gram-negative bacteria in the socket (14.3% and 11.1%, respectively). The proportion of pathogenic species in isolates from socket (40.7%; 77/189) was significantly higher than from healthy eye (16.1%; 10/62; $P<0.001$). When this analysis was carried out in groups, only *Streptococci* were significantly increased in the socket ($P=0.020$), but not the Gram-negative bacteria ($P=0.076$) and the other pathogenic groups.

The antibiotic sensitivities of each microbiological group (*S. epidermidis*, other CNS, CPS, *Coryneform* bacteria, *Enterococci*, *Micrococci*, *Streptococci*, and Gram-negative bacteria) were compared according to the site of isolation (healthy eye, pre-prosthesis or retro-prosthesis space). The results of isolated *S. epidermidis* were exposed in Table 2. There were no significant differences in antibiotic sensitivities between strains of *S. epidermidis* from healthy eyes and sockets (Table 2). The same analysis was performed on the other bacterial groups (with the antibiotics tested in each group), and no differences regarding to the isolation site were demonstrated in any case. Based on these results and according to the microbiological group, the isolates from sockets and healthy eyes were joined to perform the following antibiotic sensitivities.

The rates of susceptible staphylococcal cultures were showed in Table 3. All isolates of *Staphylococcus* genus were tested for the same antibiotics, as all of them were identified by using the panel 31 of the MicroScan system. The three groups of *Staphylococci* (*S. epidermidis*, other CNS, and CPS) were poorly susceptible to penicillin (5.6%, 24.1%, and 10.7%, respectively) and erythromycin (42.1%, 75.9%, and 78.6%, respectively). CNS isolates different of *S. epidermidis* exhibited high susceptibilities to most other antibiotics, except for tetracycline (65.5%). In contrast, the resistance rates of *S. epidermidis* and CPS to different antibiotic groups such as aminoglycosides, fluoroquinolones, and beta lactams were much higher. There were no significant differences in aminoglycosides susceptibility between

Table 3 Antibiotic sensitivities of *Staphylococcal* isolates[a]

Antibiotic	Percent susceptible[b] (%)		
	S. epidermidis	Other CNS	CPS
Fluoroquinolones			
Ciprofloxacin	69.8	96.6	85.7
Levofloxacin	69.8	96.6	89.3
Aminoglycosides			
Gentamicin	83.3	96.6	82.1
Tobramycin	71.4	96.6	75.0
Amikacin	81.0	96.6	75.0
Beta lactams			
Penicillin	5.6	24.1	10.7
Amoxicillin-clavulanate	68.3	96.6	75.0
Oxacillin	69.8	96.6	75.0
Others			
Fusidic acid	84.1	89.7	96.4
Erythromycin	42.1	75.9	78.6
Tetracycline	79.4	65.5	82.1
Clindamycin	70.6	89.7	71.4
Trimethoprim-sulfamethoxazole	96.0	100	100
Rifampin	100	100	100
Vancomycin	100	100	100
Daptomycin	100	100	100
Linezolid	100	100	100

[a]Cultured *Staphylococci* from healthy eyes and sockets were 126 isolates of *S. epidermidis*, 29 of other CNS, and 28 of CPS. All staphylococcal isolates were tested for all antibiotics; [b]For each microbiological group, the number of susceptible isolates over the total number of isolates times 100.

S. epidermidis and CPS. Nevertheless, there was a higher susceptibility to levofloxacin of CPS (89.3%; 25/28) compared to *S. epidermidis* (69.8%; 88/126; *P*=0.036). Ciprofloxacin susceptibility rates were comparable to levofloxacin (85.7% for CPS and 69.8% for *S. epidermidis*), although in this case the statistical significance was not reached (*P*=0.104). The oxacillin resistant rate for *S. epidermidis* (30.2%; 38/126) and CPS (25.0%; 7/28) were similar (*P*=0.653), while there was a significantly lower susceptibility to erythromycin of *S. epidermidis* (42.1%; 53/126) compared to CPS (78.6%; 22/28; *P*=0.001).

Gram positive non-staphylococcal isolates (29 *Streptococci*, 7 *Coryneform* bacteria, 4 *Enterococci* and 2 *Micrococci*) showed high susceptibility to ampicillin (94.7%) and cefotaxime (97.2%; Table 4). Gentamicin was only tested in isolates of *Coryneform* bacteria and *Micrococci*, and all of them were susceptible. Oxacillin was tested in 19 isolates of viridans group *Streptococci*, of which 9 were resistant (47.4%). There was not any resistant Gram-positive (neither *Staphylococcus* nor no-*Staphylococcus*) to rifampin, vancomycin, daptomycin or linezolid (Tables 3 and 4).

Table 4 Antibiotic sensitivities of non-*Staphylococcal* Gram-positive isolates[a]

Antibiotic	No. susceptible/ No. total tested	Percent susceptible (%)
Fluoroquinolones		
Levofloxacin	35/41[b]	85.4
Aminoglycosides		
Gentamicin	9/9	100
Beta lactams		
Penicillin	36/42	85.7
Oxacillin	10/19	52.6
Ampicillin	36/38	94.7
Cefotaxime	35/36	97.2
Others		
Chloramphenicol	32/36	88.9
Erythromycin	26/42	61.9
Tetracycline	31/41	75.6
Clindamycin	28/36	77.8
Trimethoprim-sulfamethoxazole	20/29	69.0
Rifampin	37/37	100
Vancomycin	42/42	100
Daptomycin	11/11	100
Linezolid	42/42	100

[a]Total non-*Staphylococcal* Gram-positive isolates=42 (29 *Streptococci*; 7 *Coryneform* bacteria; 4 *Enterococci*; and 2 *Micrococci*); [b]In this case, levofloxacin sensitivities were performed on 41 of the 42 non-*Staphylococcal* Gram-positive isolates (levofloxacin sensitivity was not performed on 1 *Coryneform* bacterium).

Regarding the Gram-negative isolates, they were mainly susceptible to beta lactams, specifically to cephalosporins (Table 5). All the tested strains to cefoxitin, cefotaxime, and cefepime were susceptible. Although these isolates did not include *Pseudomonas spp.* in the case of cefoxitin and cefotaxime, cefepime was tested in 6 isolates of *Klebsiella spp.*, 5 *Pseudomonas spp.*, 2 *Serratia liquefaciens*, and 1 *Alcaligenes spp*. In contrast, the antibiotic susceptibility rates to cefuroxime (88.9%) and ceftazidime (78.9%) were lower (Table 5).

Nine patients recognized to use self-prescribed antibiotic eye drops in their socket during the last month (6 patients use tobramycin, 2 ofloxacin, and 1 terramycin). However, a total of 36 isolates (17 from the healthy eye, 14 from the pre-prosthesis space, and 15 from the retro-prosthesis area) were recovered from these subjects. These isolates were 31 Gram-positive microorganisms (26 *Staphylococci* isolates: 20 *S. epidermidis*, 5 *S. aureus*, 1 *S. capitis*; 3 viridans group *Streptococci*; and 1 isolate each of *Enterococcus faecalis* and *Dermabacter hominis*); 2 Gram-negative bacteria (both *Klebsiella pneumoniae* from the pre-prosthesis and retro-prosthesis space of the socket of the patient No.39); and 3 fungi (2 *Candida*

Table 5 Antibiotic sensitivities of Gram-negative isolates[a]

Antibiotic	No. susceptible/ No. total tested	Percent susceptible (%)
Fluoroquinolones		
Ciprofloxacin	12/16[b]	75.0
Levofloxacin	12/14	85.7
Aminoglycosides		
Gentamicin	16/20	80.0
Tobramycin	16/20	80.0
Amikacin	16/20	80.0
Beta lactams		
Amoxicillin-clavulanate	10/10	100
Piperacillin/tazobactam	17/21	81.0
Cefoxitin	7/7	100
Cefuroxime	8/9	88.9
Cefotaxime	9/9	100
Ceftazidime	15/19	78.9
Cefepime	14/14	100
Others		
Trimethoprim-sulfamethoxazole	18/22	81.8
Imipenem	16/21	76.2
Meropenem	8/12	66.7
Aztreonam	13/15	86.7

[a]Total Gram-negative isolates=23 (6 *Klebsiella spp.*, 5 *Pseudomonas spp.*, 4 *Stenotrophomonas maltophilia*, 2 isolates each of *Serratia liquefaciens*, *Alcaligenes spp.*, and *Moraxella spp.*, and 1 isolate each of *Enterobacter intermedium* and *Veillonella parvula*); [b]In this case, ciprofloxacin sensitivities were performed on 16 of the 23 Gram-negative isolates (ciprofloxacin sensitivities were not performed on 4 *Stenotrophomonas maltophilia*, 2 *Moraxella spp.*, and 1 isolate of *Veillonella parvula*).

parapsilosis and 1 *Saccharomyces cerevisae*, all from the socket of patient No.48). Gram-positive microorganisms from patients that used antibiotic eyedrops in their socket showed higher resistance to aminoglycosides (gentamicin, tobramycin, and amikacin) compared to Gram-positive isolates from patients who did not use antibiotics. This situation was observed in Gram-positive strains from both healthy eyes and sockets (Table 6). In addition, Gram-positive bacteria from healthy eyes of patients with antibiotic self-prescription were more resistant to tetracycline (P=0.034), although this difference was not found in socket isolates (P=0.790).

DISCUSSION

This study has determined the microbiological spectrum and antibiotic sensitivity of conjunctival flora in anophthalmic patients. Overall positive culture rates in both healthy eyes (85%) and sockets (100%) were similar to reported by other authors[3-6]. The main difference found between healthy eyes and sockets flora was the greater isolation of pathogenic microorganisms in the sockets. The most commonly isolated bacterial organisms in our study were CNS, particularly *S. epidermidis*. CNS accounted for 77.4% (48/62) in healthy eyes and 56.6% (107/189) in sockets. Similar rates of CNS have been described in the literature. Studies of conjunctival flora in healthy adults have showed an isolation rate of CNS from 48.3% to 100%[14-16]. In sockets of anophthalmic patients, CNS have ranged from 25.8% to 59.1%[4-5]. This lower CNS rate in the socket corresponded to a greater isolation of pathogens. Previous reports have described an increase in the socket flora of CPS, *Streptococci*, and Gram-negative bacteria compared to the healthy eye flora[3-4,6]. Although our results only showed a significantly increase of *Streptococci* (P=0.020), classic aerobic pathogens (CPS, *Streptococci* and Gram-negative bacteria) accounted for less than 15% (9/62) of isolates from healthy eyes and more than 35% (71/189) of isolates from sockets (P=0.001).

This dysbiosis in the socket flora can be explained by the drastic changes in conjunctival epithelium after an enucleation or evisceration surgery. The lack of globe and the use of an artificial eye produce several modifications in the biomechanics of the ocular surface. First, the bulbar conjunctiva is no longer swept by the eyelids. Second, the artificial eye is a relatively large foreign body that can produce frictional irritation of the conjunctiva when the prosthesis moves[17]. Third, the dead space between the posterior surface of the prosthesis and the anterior surface of the socket allows the accumulation of conjunctival debris, even in custom fitted prostheses[7]. These circumstances promote the increase of mucus in the socket which in turn favors the growth of pathogenic microorganisms. Despite this remarkable disparity between the conjunctival flora of sockets and healthy eyes, we did not find differences in antibiotic sensitivities of organisms from both microbial communities. Therefore, antibiotic sensitivity analysis was performed by joining the isolates of sockets and healthy eyes. Isolated organisms (Gram-positive bacteria and Gram-negative bacteria) showed antibiotic susceptibilities comparable to previous studies on healthy conjunctival flora[14,18]. All Gram-positive isolates were susceptible to vancomycin, as well as all Gram-negative isolates were susceptible to cefepime. Although the topical use of cefepime is currently limited, its stability in aqueous eyedrops has been proven[19].

As with other reports, *S. epidermidis* (50.2%; 126/251) and *S. aureus* (10.8%; 27/251) were the most frequently isolated species and exhibited a rate of oxacillin resistance of 30.2% and 22.2%, respectively. Oxacillin has replaced methicillin to identify staphylococcal isolates resistant to all beta lactam antibiotics and possibly more virulent infectious courses[13]. In the present study, the percentages of oxacillin resistant *S. epidermidis* (ORSE) and oxacillin resistant *S. aureus*

Table 6 Antibiotic sensitivities of Gram-positive isolates according to previous use of antibiotic drops[a]

Antibiotic	Isolates from healthy eyes		P^a	Isolates from sockets		P^b
	No history of recent use of antibiotics	Use of antibiotics in the socket		No history of recent use of antibiotics	Use of antibiotics in the socket	
	NS/NT (%)	NS/NT (%)		NS/NT (%)	NS/NT (%)	
Fluoroquinolones						
Ciprofloxacin	36/47 (76.6)	4/7 (57.1)	0.358	87/113 (77.0)	13/20 (65.0)	0.268
Levofloxacin	41/53 (77.4)	4/7 (57.1)	0.351	115/141 (81.6)	16/23 (69.6)	0.259
Aminoglycosides						
Gentamicin	49/51 (96.1)	3/6 (50.0)	0.006	105/114 (92.1)	8/21 (38.1)	0.000
Tobramycin	39/47 (83.0)	2/6 (33.3)	0.019	92/111 (82.9)	7/20 (35.0)	0.000
Amikacin	44/47 (93.6)	3/6 (50.0)	0.015	98/111 (88.3)	7/20 (35.0)	0.000
Beta Lactams						
Penicillin	9/53 (17.0)	1/7 (14.3)	1.000	39/141 (27.7)	4/24 (16.7)	0.321
Amoxicillin-clavulanate	36/48 (75.0)	3/6 (50.0)	0.331	86/111 (77.5)	12/20 (60.0)	0.159
Oxacillin	36/49 (73.5)	3/6 (50.0)	0.342	95/124 (76.6)	13/23 (56.5)	0.069
Others						
Fusidic acid	40/50 (80.0)	6/6 (100)	0.578	100/113 (88.5)	19/20 (95.0)	0.693
Erythromycin	25/53 (47.2)	2/7 (28.6)	0.442	83/141 (58.9)	13/24 (54.2)	0.662
Tetracycline	44/53 (83.0)	3/7 (42.9)	0.034	109/141 (77.3)	17/23 (73.9)	0.790
Clindamycin	36/53 (67.9)	4/7 (57.1)	0.676	107/136 (78.7)	16/23 (69.6)	0.418
Trimethoprim-sulfamethoxazole	49/53 (92.5)	6/7 (85.7)	0.475	123/129 (95.3)	20/23 (87.0)	0.138

[a]Gram-positive isolates from healthy eyes and sockets of 51 patients without recent use of antibiotic drops were 53 and 141, respectively; and 7 and 24 isolates, respectively, of 9 patients with use of antibiotic drops in their sockets during the last month. [b]Two-tailed Fisher's exact test. NS: Number of susceptible isolates; NT: Total number of tested isolates; %: Percent susceptible.

(ORSA) were relatively lower compared to other reports on conjunctival flora of patients undergoing cataract surgery[18,20]. However, percentages of ORSE and ORSA are very variable according to age, geographical area, and antibiotic use[20]. Different studies have placed the rate of ORSE from 4% to 47%; and the rate of ORSA from 0 to 64%[20-21].

Regarding other antibiotic groups, *S. epidermidis* and CPS had a similar susceptibility to aminoglycosides (gentamicin, tobramycin, and amikacin). However, CPS (27 isolates of *S. aureus* and 1 isolate of *S. intermedius*) were slightly more susceptible to fluoroquinolones. In addition, antibiotic sensitivity to erythromycin of *S. epidermidis* (42.1%) compared to CPS (78.6%) was clearly lower (P=0.001). These differences in the antibiotic sensitivity between *S. epidermidis* and *S. aureus* may suggest the possible use of some antibiotics (such as macrolides and fluoroquinolones) to modify the flora in the anophthalmic sockets. In this sense, Dave *et al*[2] have reported a significant increase in the percentage of *S. epidermidis* isolated from the conjunctiva at the expense of *S. aureus* after repeated exposure to macrolides (azithromycin), and they have also demonstrated an increase of *S. epidermidis* compared to Gram-negative bacteria after exposure of conjunctiva to fluoroquinolone antibiotics (ofloxacin, gatifloxacin, and moxifloxacin). Therefore, fluoroquinolones (ciprofloxacin and levofloxacin) and macrolides (erythromycin) may be better than aminoglycosides for treating socket conjunctivitis, since they decrease pathogenic species and increase the rate of *S. epidermidis*.

One of the most interesting findings of this study is the clear correlation between antibiotic sensitivities of the socket flora exposed to antibiotic drops and the healthy eye flora (Table 6). Nine patients recognized that they routinely treated their socket with self-prescribed antibiotic drops to control the discharge. Tobramycin was the most commonly used antibiotic (6/9). The average rates of Gram-positive strains susceptible to aminoglycosides were 36% (gentamicin: 38%, 8/21; tobramycin: 35%, 7/20; and amikacin: 35%, 7/20) for socket cultures exposed to antibiotic and 44% for cultures from the fellow eye. On the other hand, aminoglycoside susceptibilities of cultures in patients without antibiotic use were 88% for Gram-positive organisms of the socket and 91% for healthy eye isolates. Organisms exposure to subinhibitory concentrations of tobramycin increase the resistances[22], as it happens with other antibiotics[2]. However, in patients undergoing intravitreal

injections, antibiotics has been related to increase the resistant rate of organisms in the conjunctiva exposed to antibiotics[16,23], but not in the conjunctiva of the fellow eye[23].

The explanation of the change in antibiotic susceptibility in healthy eye flora of anophthalmic patients, when antibiotic drops are only used in the socket, would be a transfer of organisms from the socket to the healthy eye. Organisms cultured from the conjunctival sac are thought to reach the conjunctiva from the palpebral skin[24], so colonization of the ocular surface and surrounding tissues is a dynamic process[2]. The higher number of isolates from the socket compared to the fellow eye described in this study and by other reports[3-5] indirectly indicates a greater amount of organisms in the socket. Therefore, the socket would act as a reservoir of bacteria, which could be transferred to the conjunctiva of the healthy eye. A similar situation has been reported in fellow eyes of patients with unilateral nasolacrimal duct obstruction[25]. Although some authors have suggested the possible influence of socket organisms on the healthy eye[4-5,8,10], to our knowledge this is the first report of changes in the antibiotic sensitivity of the fellow eye flora in relation to the antibiotic treatment used in the socket.

This finding, if confirmed by other prospective studies, has a considerable clinical implication. As the main complaint of anophthalmic patients is discharge and crusting in the socket[1], antibiotics are commonly used to control these annoyances. However, antibiotic treatment does not achieve a long-term resolution[7] and favors the emergence of resistant flora in the socket. Some of the many resistant organisms in the socket can reach the conjunctiva of the fellow eye, by rubbing the eyes or manipulating the prosthesis. These organisms seriously increase the infectious risk of intraocular surgery on the healthy eye in anophthalmic patients.

There are a few limitations to our study. Our isolation technique failed in obtaining anaerobes. The initial pre-culture in conventional BHI under aerobic conditions probably prevented their further isolation. The data of MIC were not collected so bacteria were only classified as resistant or susceptible. In addition, some interesting antibiotics in the treatment of ocular infections such as azithromycin, gatifloxacin or moxifloxacin were not evaluated. Finally, the observational design of our study prevented an accurate evaluation of conjunctival flora changes based on the use of antibiotics. In this sense, a prospective, longitudinal study in anophthalmic patients is needed to describe how flora of sockets and healthy eyes change over time.

The outcomes of this study describe the presence of a pathogenic flora in sockets compared to healthy eyes in anophthalmic patients. This pathogenic flora of the socket can proliferate causing discharge and annoyances, and leading to use antibiotic eyedrops to control these symptoms. In this study, socket exposition to antibiotic eyedrops has been related to a higher resistant flora not only in the socket but also in the healthy eye. This situation is a risk factor for developing a severe infection in the healthy eye of anophthalmic patients.

Based on our results, quinolones (ciprofloxacin and levofloxacin) and macrolides (erythromycin) may be better therapeutic options than aminoglycosides for treating uncomplicated infections (conjunctivitis) of anophthalmic sockets, since they are more effective in eliminating pathogenic species such as *S. aureus* and less active against *S. epidermidis*.

On the other hand, in the case of a severe infection (corneal ulcer or endophthalmitis) in the healthy eye of an anophthalmic patient, vancomycin for Gram-positive organisms and cefepime for Gram-negative bacteria are the most useful antibiotics, since no resistant strain to these antibiotics has been identified in our series.

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