Persistence of conjunctival colonization by coagulase-negative staphylococci in patients undergoing cataract surgery

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Abstract

Purpose

To establish the conjunctival contamination source by coagulase-negative staphylococci (CNS) during cataract surgery and discuss the implications of this colonization as a potential risk of acquiring endophthalmitis.

Methods

Conjunctiva samples for culture from 59 patients undergoing cataract surgery were taken of the fellow eye at baseline (T1) and from the eye to be operated before (T0) and immediately after (T2) irrigation with povidone-iodine IOP5%, and at the end of surgery (T3). Genes responsible for virulence (*mecA*, *ica* and *atlE*) and antibiotic profile were determined; strain clonality of persistent colonizing *S. epidermidis* strains was established by the Multi-locus sequence typing (MLST).

Results

The frequency of CNS was significantly reduced in T2 (13.6%) from 81.4% in T0 and 86.4% in T1. The frequency of *mecA*, *ica* and *atlE* genes was 35.9%, 41.7% and 67.2%, respectively; and methicillin phenotypic resistance was 35.4%. *S. epidermidis* was the most frequent species isolated in every time point. MLST revealed in 7 patients 100% coincidence of the 7 alleles of the *S. epidermidis* isolated previous to povidone-iodine 5% disinfection and at the end of the surgery. CNS isolates from T2 or T3 corresponded to the same species, antibiotic and virulence profile as those isolates from T1 or T0.

Conclusion

Pre-surgical prophylactic scheme significantly reduced conjunctival contamination; in those that persisted, the source of contamination was mostly the patient's microbiota confirmed by the MLST system.

Introduction

Although acute postoperative endophthalmitis associated with cataract surgery is rare, it is associated with a high frequency of irreversible vision loss; even with proper treatment [1]. Various prophylactic measures have been adopted in order to reduce the incidence of this devastating complication. It is presumed that the source of infection is the patient's own conjunctival and palpebral microbiota [2]. Prophylactic measures are aimed at eliminating contamination from various sources of infection, especially the conjunctiva and eyelids of the patient [3].

Gram-positive cocci are the most frequently isolated microorganisms in acute postoperative endophthalmitis, such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus sp* [4]. It has been shown that conjunctival microbiota contaminates the anterior chamber during cataract surgery between 13% and 42% with a predominance of gram-positive cocci (*S. epidermidis* and *S. aureus*) [5–7].

Disinfection with povidone iodine (PVI) is so far the only measure considered capable of reducing the incidence of endophthalmitis [8–10]. Our group has shown in previous studies a high contamination rate of the conjunctiva before and after irrigation with 5% PVI and at the end of the surgery [11, 12]; as well as the aqueous humor [7]. In addition, a high frequency of virulence factors in strains isolated from the conjunctiva in patients undergoing cataract surgery [13], and from the health personnel [14] were reported. In none of these studies, the source of colonization was established. The objective of this study is to identify the source of conjunctival contamination by comparing the virulence profile of coagulase-negative staphylococci that remained colonizing the conjunctiva after irrigation with 5% PVI in patients undergoing cataract surgery by both phenotypic and genotypic methods and discuss the implications of this colonization as a potential risk of acquiring endophthalmitis during cataract surgery.

Methods

After approval by the Institutional Review Board (IRB) had been obtained, patients presenting for cataract surgery were recruited for this study from October 2016 to May 2017 at the Department of Ophthalmology, Medical School of the National University of Asunción, Paraguay. Cataract surgery is routinely performed at this institution using clear-cornea incision phacoemulsification technique. Those patients with decompensation or pathological clinical condition prior to surgery were excluded.

Conjunctival samples and patient data

The ophthalmologist responsible for the surgery was in charge of collecting the samples and completing the patient's data sheet. Four conjunctival samples were collected from upper/lower fornix of each patient using individual sterile cotton swabs moistened with thioglycolate broth; one sample at each of the following times: T0: sample taken from the eye to be operated before irrigation with 5% povidone iodine solution (5% PVI), T1: from the fellow eye, T2: from the eye to be operated after 5% PVI disinfection and T3: of the operated eye at the end of the surgery.

The patient data sheet included general characteristics (sex, age, medical history, side of the operated eye, duration of surgery). In addition, contamination by Coagulase-negative Staphylococci (CNS) species, antibiotic resistance / susceptibility profile, the presence of *mecA*, *ica* y *atE* genes and MLTS profile were registered.

Microbiological study

Each sample was inoculated directly into blood agar / chocolate agar. Subsequently, the swab was placed in a tube of thioglycolate broth. The primary plates of blood agar / chocolate agar were incubated at 35 ° C in an atmosphere of 5% CO₂ for 72 h and every 24 h were observed in order to detect bacterial development, and in positive case the colony count was performed. The thioglycolate broth was incubated at 35 ° C for 5 days observing every 24 h the bacterial development by degree of turbidity and recorded in crosses: +, ++ and +++ according to the presence of light, moderate or intense turbidity. Bacteria were identified and tested for antibiotic susceptibility by the Kirby Bauer method against the following drugs: Cefoxitin 30 ug, Erythromycin 15 ug, Clindamycin 2 ug, Penicillin 6 ug, Chloramphenicol 30 ug, Trimethoprim / Sulfamethoxazole 1.25 / 23.75 ug, Ciprofloxacin 5 ug, Gentamicin 10 ug, Tobramycin 10 ug, Moxifloxacin 5 ug, Tetracycline 30 ug, Novobiocin 5 ug, Polymyxin B 100 IU (Biorad, USA) in accordance with Clinical Laboratory Standards Institute guidelines [15].

DNA extraction

DNA extraction was performed according to a modified lysis method described by Zhang et al. 2004 [16], one to five bacterial colonies obtained from 24-48 hours culture in blood agar were suspended in 200 µl sterile distilled water and heated at 100°C for 15 min, frozen for 5 min, and then centrifuged at 13.000 rpm for 1min, supernatant was stored at – 20°C until use.

Amplification by Multiplex PCR

Multiplex PCR for identifying genes: *mecA*, *ica*, and *atE* was performed according to the method described by Sharma et al. 19[26]. Some changes were introduced to the original protocol. 25 uL master mix 1.5 mM MgCl₂, 0.4 mM de dNTPs, 1 mM of each primer, 5 U/ul Taq polimerase and 5 µl of the extracted DNA was added as template.

The oligonucleotide primers used in this study were described by Sharma et al. [17]. The sequence of forward and reverse primers (Bangalore Genei, Bengaluru, Karnataka, India) were as follows: gen *ica*: Forward *ica*-TTATCAATGCCGAGTTGTC e Inverse *ica*-GTTTAACGCGAGTGCGCTAT, (product size: 546 pb); gen *mecA*: *mecA1*- GTAGAAATGACTGAACGTCCGATAA and *mecA2*-CCAATTCCACATTGTTTCGGTCTAA, (product size: 310 pb), and gen *AtE*: Forward *atE*-CAACTGCTCAACCGAGAACA and inverse *AtE*-TTTGTAGATGTTGTGCCCCA, (product size: 682 pb).

Staphylococcus aureus ATCC 43300 was used as positive control for *mecA* gen. A strong biofilm *S. epidermidis* producer isolated from a patient was used as positive control for *atE*, *ica* genes and double distilled water as negative control.

PCR was performed in a thermocycler PTC-100 (MJ Research. MA, USA) under the following conditions: 30 cycles of denaturation at 94°C for 1 min, followed by annealing at 55°C for 1 min, and extension at 72°C for 2 min. PCR products and 100 pb molecular marker (BIORON) were run at 100 volts for 1 hour in 1.5 % agarose gel and TAE 1X buffer (Tris-Acetate-EDTA) and visualized by staining with 5% SYBR® Safe DNA gel stain (Invitrogen, USA).

Clonality

The single enhanced MLST scheme described by Thomas et al. [18] was used, which develops an MLST scheme consisting of the seven most discriminatory alleles of three schemes. The PCR involved an initial denaturation of 95 ° C for 3 minutes; 34 cycles of 95 ° C for 30 s, 50 ° C for 1 minute and 72 ° C for 1 minute; and a final extension of 72 ° C for 10 min. The amplicons were further purified and sequenced. The alleles at each locus were distinguished by using Sequence Output software (available from <http://www.mlst.net>). Lineages were analyzed by using the eBURST algorithm [19] available at <http://eBURST.mlst.net>.

Modified primer sequences for an improved *S. epidermidis* MLST scheme

Gen	Primer	Sequence 5'→3'
<i>gtr</i>	gtr-F gtr-R	CAGCCAATTCTTTTATGACTTTT GTGATTAAAGGTATTGATTGAAT
<i>mutS</i>	mutS-F3 mutS-R3	GATATAAGAATAAGGGTTGTGAA GTAATCGTCTCAGTTATCATGTT
<i>tpi</i>	tpi-F2 tpi-R2	ATCCAATTAGACGCTTTAGTAAC TTAATGATGCGCCACCTACA
<i>yqiL</i>	yqiL-F2 yqiL-R2	CACGCATAGTATTAGCTGAAG CTAATGCCTTCATCTTGAGAAATAA
<i>pyrR</i>	pyr-F2 pyr-R4	GTTACTAATACTTTTGCTGTGTTT GTAGAATGTAAAGAGACTAAAATGAA
<i>arcC</i>	arcC-F arcC-R	TGTGATGAGCACGCTACCGTTAG TCCAAGTAAACCCATCGGTCTG
<i>aroE</i>	aroE-F aroE-R	CATTGGATTACCTCTTTGTTTCAGC CAAGCGAAATCTGTTGGGG

The new gene sequences reported in this study have been deposited in the GenBank (access numbers DQ991011 to DQ991028).

The data were analyzed by descriptive statistics, using the Epi info 3.5.1 program (CDC, Atlanta). Results are expressed as an absolute and relative percentage frequency distribution.

Results

Fifty-nine patients, 37 women and 22 men between 23 and 89 years of age (mean age $\pm$ standard deviation: 66.9 $\pm$ 12.6 years) were studied. The most frequent pre-existing condition was arterial hypertension. Duration of surgery ranged from 14 to 55 minutes with a mean duration of 26.2 $\pm$ 8.6 minutes. Table 1.

Table 1
Characteristics of patients undergoing cataract surgery. n= 59

Characteristics	Frecuency	Percentage
Sex		
F	37	62.7
M	22	37.3
Age (average $\pm$ SD, range)		
<40	3	5.1
40-59	12	20.3
60-69	14	23.7
≥ 70	30	50.9
Underlying conditions		
Arterial hypertension (AHT)	23	39.0
Diabetes mellitus - AHT	8	13.6
Diabetes mellitus	4	6.8
Other (hypothyroidism, HIV)	2	3.4
Operated eye		
Right /left	32/27	54.2/45.8
Duration of surgery (minutes)		
(Average $\pm$ SD, range)	26.2 $\pm$ 8.6 (14-55)	
<20	6	10.2
20-30	42	71.1
>30	11	18.7

A significant decrease in ocular microbiota was obtained after disinfection with 5% povidone-iodine (T2) and an increase in positivity of cultures at the end of surgery (T3). Figure 1

Table 2 shows the results of the microbiological cultures of the samples taken at the three time points, T0: before surgery of the eye to be operated; T1: from the unoperated eye as a control, T2: immediately after receiving standard disinfection with 5% povidone-iodine. T3: at the end of the surgery. The frequency of positive culture for CNS was 42.4% in the initial plate in T1 and in the broth 62.7%. At T0 it was 35.6% in the plaque and 61.0% in broth; in T2 it was 6.8% and 16.9%, respectively. In T3 it was 6.8% and 28.8% and 33.9%, respectively. The number of colonies recovered in the cultures was generally low; approximately 50% with less than 5 colonies before disinfection; in T2 less than 5 colonies were obtained in all positive cases and in T3 less than 20 colonies in all positive cases. Also, the degree of turbidity in the thioglycolate broths at different time points is shown.

Table 2
Primary Plate Colony Count and Broth Turbidity

	T1	T0	T2	T3
	n=25 (42.7)	n=21 (35.6)	n=4 (6.8)	n=4 (6.8)
Colony counts in primary plaques				
<5	7	14	4	1
5 a 20	6	2	-	3
>20	12	5	-	-
Turbidity in thioglycolate broth				
	n=37 (62.7)	n=36 (61.0)	n=10 (16.9)	n=17 (28.8)
1+	20	19	6	8
2+	12	12	2	6
3+	5	2	-	1
Traces	-	3	2	2
T0: before surgery from the eye to be operated				
T1: from the fellow eye as control				
T2: immediately after standard 5%PVI disinfection				
T3: at the end of the surgery				

From the 59 patients, 192 CNS isolates were obtained; 75 at T0; 76 in T1; 14 in T2 and 27 in T3. Table 3 shows the frequency of the different CNS species obtained, *S. epidermidis* was the most frequent species, followed by *S. hominis*.

Table 3
Negative coagulase staphylococci isolates

Bacteria species	Frequency	Percentage
<i>S. epidermidis</i>	116	60.4
<i>S. hominis subesp hominis</i>	27	14.1
<i>S. lugdunensis</i>	12	6.3
<i>S. capitis subesp capitis</i>	11	5.7
<i>S. haemolyticus</i>	7	3.6
<i>S. schleiferi subesp schleiferi</i>	5	2.6
<i>S. caprae</i>	8	4.2
<i>S. simulans</i>	3	1.6
<i>S. warneri</i>	2	1.0
<i>S. caprae/simulans</i>	1	1.0
Total	192	100.0

Table 4 presents the resistance profile of the CNS isolates. Methicillin resistance was 35.4%.

Table 4
Antibiotic-resistance profile in CNS strains from ocular microbiota. n=192

ATB	Resistant	Intermediate	Sensible
FOX	68 (35.4%)	-	124 (64.6%)
ERY	48 (25.0%)	6 (3.1%)	138 (71.9%)
CLI	16 (8.3%)	2 (1.0%)	174 (90.6%)
CLO	37(19.3%)	2 (1.0%)	153 (79.7%)
MOX	13 (6.8%)	16 (8.3%)	163 (84.9%)
CIP	39 (20.3%)	9 (4.7%)	144 (75.0%)
PEN	133 (69.3)	-	59 (30.7%)
TMS	21 (10.9%)	1 (0.5%)	170 (88.5%)
TET	23 (12.0%)	-	169 (88.0%)
GEN	31 (16.1%)	11 (5.7%)	150 (78.1%)
TOB	19 (9.9%)	8 (4.2%)	165 (85.9%)
FOX Cefoxitin, ERI Erythromycin, CLI Clindamycin, PEN Penicillin, CLO Chloramphenicol, TMS Trimethoprim / Sulfamethoxazole, CIP Ciprofloxacin, GEN Gentamycin, TOB Tobramycin, MOX Moxifloxacin, TET Tetracycline			

The results of gene amplification by multiplex PCR are shown in Table 5. At the different time points, the *atE* gene was the most frequently found (61.4%).

Table 5
Frequency of genes encoding virulence factors in *CNS* isolates in ocular microbiota. n= 192

Virulence factor gene	T0 (n=75)	T1 (n= 76)	T2 (n=14)	T3 (n=27)	Total (n=192)
<i>mecA</i>	31 (41.3%)	28 (36.9%)	5 (35.7%)	2 (7.4%)	66 (34.4%)
<i>ica</i>	29 (38.7%)	32 (42.1%)	4 (28.6%)	7 (25.9%)	72 (37.5%)
<i>atE</i>	46 (61.3%)	55 (72.4%)	6 (42.8%)	11 (40.7%)	118 (61.4%)
none	21 (28%)	18 (23.7%)	2 (14.3%)	8 (29.6%)	49 (25.5%)
T0: before surgery from the eye to be operated					
T1: from the fellow eye as control					
T2: immediately after standard 5%PVI disinfection					
T3: at the end of the surgery					

Table 6

Characteristics of CNS strains recovered after disinfection with 5% PVI and at the end of surgery in patients undergoing cataract surgery

Patient code	Time	FOX	ERY	CLI	CLO	MOX	CIP	PEN	TMS	TET	GEN	TOB	Genes	
7	T1	R	S	S	S	S	S	R	S	S	S	S	atlE+ica+mecA	<i>S. epidermidis</i>
	T0													
	T3													
15	T0	R	S	S	S	I	R	R	S	S	S	S	atlE+mecA	
	T2													
16	T1	S	S	S	S	S	S	R	S	R	S	S	None	
	T3													
22	T0	S	S	S	S	S	S	S	S	S	S	S	None	
	T3													
23	T0	S	S	S	S	S	S	R	S	S	S	S	atlE	
	T3													
26	T1	S	S	S	S	S	S	S	S	S	S	S	atlE	
	T3													
40	T1	R	R	S	R	R	R	R	S	R	S	S	atlE+mecA	
	T0													
	T2													
46	T1	S	S	S	S	S	S	R	S	S	S	S	atlE+ica	
	T3													
48	T0	S	S	S	I	S	S	R	S	S	I	S	atlE+ica	
	T3													
55	T1	R	R	R	R	S	I	R	R	S	R	R	atlE+ica+mecA	
	T2													
	T3													
57	T0	R	S	S	R	S	S	R	S	S	R	I	atlE+ica+mecA	
	T2													
	T3													
58	T0	S	S	S	S	S	S	R	S	S	S	S	altE+ica	
	T2													
	T0	S	S	S	S	S	S	S	S	S	S	S	atlE+ica	
	T3													
1	T1	S	S	S	R	S	S	R	S	S	S	S	None	<i>S. hominis</i> <i>vb hominis</i>
T0: before surgery from the eye to be operated														
T1: from the fellow eye as a control,														
T2: immediately after standard 5%PVI disinfection														
T3: at the end of the surgery.														

Patient code	Time	FOX	ERY	CLI	CLO	MOX	CIP	PEN	TMS	TET	GEN	TOB	Genes	
	T2													
2	T0	S	S	S	S	S	S	R	S	S	S	S	atlE+ica	<i>S. capitis vb capitis</i>
	T2													
	T0	S	S	S	S	S	S	S	S	S	S	S	atlE+ica	<i>S. schleiferi vb schleiferi</i>
	T3													
14	T0	S	S	S	S	S	S	S	S	S	S	S	None	<i>S. lugdunensis</i>
	T3													
28	T1	S	S	S	S	S	S	R	S	S	S	S	None	
	T0													
	T2													
	T3													
34	T1	S	S	S	S	S	S	S	S	S	S	S	None	
	T0													
	T3													
T0: before surgery from the eye to be operated														
T1: from the fellow eye as a control,														
T2: immediately after standard 5%PVI disinfection														
T3: at the end of the surgery.														

In 17 patients (28.8%), persistence of CNS strains was observed after 5% povidone-iodine or at the end of the surgery for CNS with the same virulence profile. In 12 patients, the species that remained colonizing the conjunctiva was *S. epidermidis*. In four of them, the species that was colonizing the eye to be operated was recovered in T2 and the rest was recovered in the operated eye at the end of the surgery. In five patients, the strains were different from *S. epidermidis*. Table 7

Table 7
MLST profile of *S. epidermidis* isolated from patient's conjunctiva at three-point times

Patient	Strain	gtr	mutS	tpi	yqil	pyr	arc	aroE	ST sequence
7	7a	2	1	1	1	2	1	1	89
	7b	2	1	1	1	2	1	1	89
	7c	2	1	1	1	2	1	1	89
15	15a	2	2	1	1	2	1	1	6
	15d	2	2	1	1	2	1	1	6
40	40a	1	1	1	1	2	2	1	59
	40b	1	1	1	1	2	2	1	59
	40c	1	1	1	1	2	2	1	59
55	55a	6	1	1	10	2	1	6	173
	55b	6	1	1	10	2	1	6	173
	55c	6	1	1	10	2	1	6	173
57	57a	2	1	1	1	2	1	1	89
	57b	2	1	1	1	2	1	1	89
	57c	2	1	1	1	2	1	1	89
58	58a	6	4	*	13	29	*	22	*
	58c	2	4	6	9	9	*	2	*
46	46a	9	5	1	1	2	1	1	284
	46b	9	5	1	1	2	1	1	284
48	48a	2	1	1	3	1	1	2	488
	48b	2	1	1	3	1	1	2	488
Note. The numbers correspond to the number assigned to each allele by the MLST website.									

S. epidermidis strains that remained colonizing the conjunctiva of 8 patients were analyzed by the MLST system; in seven patients, *S. epidermidis* isolates corresponded to the same clone. Overall, six different STs were detected, ST89 was found in two patients. In the remaining 5 patients, different ST were identified. The MLST profile of the *S. epidermidis* strains is shown in Table 7.

Discussion

In this study, we used the MLST system to explore the relationship between *S. epidermidis* strains recovered previous irrigation with 5% PVI and at the end of the surgery in patients undergoing cataract surgery; 7 out of 8 strains of *S. epidermidis* isolated at both time-points had the same ST profile, indicating that they corresponded to the same clone.

Overall, 6 different STs were detected, ST89 was found in two patients. In the remaining 5 patients, different ST were identified. In the case of *S. epidermidis* ST59 isolates, there are several reports worldwide regarding their ability to carry multiple antibiotic resistance genes and their ability to form biofilm in isolates obtained from both the environment and from health personnel [20, 21]. Likewise, a study focused on the molecular characterization of *S. epidermidis* isolates that cause ocular infections, pointed to clone ST59 as one of the most frequent, as well as to other members of the clonal complex CC2 [22]. The members of this clonal complex are characterized by being strong biofilm formers, in many cases independently of the *ica* genes, and this characteristic could be responsible for the persistence of these isolates even after the decontamination procedure with povidone iodine.

Information on *S. epidermidis* isolates of the ST89 sequence is scarce; however, its detection is important because it shows the clonal diversity of *S. epidermidis* isolates, reinforcing the hypothesis that they came from the ocular microbiota of patients and not from the

hospital. Although the molecular biological technique used is very sensitive, they are not definitive. Definitive proof of the genetic identity of two organisms would require sequencing and comparison of the entire genome, which is not practical.

The pre-surgical prophylactic scheme significantly reduced conjunctival contamination, which coincides with results published by Li et al. [23, 24], and Nentwich et al. [25] who found that IOP prophylaxis is the most effective prophylactic measure and does not carry the risk of selecting resistant strains. Therefore, very potent topical antibiotics should be reserved for infectious ones and not be used as a mere prophylactic measure to reduce the risk of creating new resistance.

The multiplex PCR used in this study could be useful for the detection of virulence and for the differentiation of more virulent strains of CNS. The genes involved in the production of biofilm have been suggested by some authors such as Ishak et al. [26] and Galdbart et al. [27] as clinically significant markers of CNS strains; however, Araujo et al. [28] found a large percentage of methicillin-resistant commensal CNSs carrying the *ica* and *atfE* genes. The results of the present study are in agreement with previous work carried out by our group in which a high percentage of the ocular microbiota of patients undergoing cataract surgery carries encoding virulence factor genes [11–13]. In addition, we have shown a significant percentage of patients showed conjunctival isolates before surgery that recovered again after disinfection with 5% povidone-iodine or at the end of surgery. These strains, apart from being of the same species, showed the same profile of virulence, antibiotype and clonality factors. This highlights the potential danger of the ocular microbiota for post-surgical complications.

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Figures

Figure 1

Comparison of the percentage of positive culture for coagulase negative staphylococci in the three times. T0: before the eye surgery to be operated. T1: from the fellow eye as control. T2: immediately after standard 5% PVI disinfection. T3: at the end of the surgery.