

Antibiotic Susceptibility of Staphylococci Isolates from Patients with Chronic Conjunctivitis: Including Associated Factors and Clinical Evaluation

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Abstract

Purpose: To determine species of staphylococci in chronic conjunctivitis, their antibiotic susceptibility pattern, patient treatments, clinical course, and clinical conditions.

Methods: In this prospective study, 243 conjunctival cultures were taken from 191 patients with chronic conjunctivitis, we obtained staphylococci susceptibility patterns with E-test, and they were analyzed in coagulase-positive and negative. The minimum inhibitory concentration for 90% of isolates (MIC90) was determined for *Staphylococcus aureus* and *Staphylococcus epidermidis*. Additionally, clinical follow-up and associated factors of all patients were analyzed depending on methicillin resistance (MR) or susceptibility (MS) bacterial state.

Results: One hundred and eight (44%) cultures were positive; 81 positive cultures were Gram-positive of which, 77 were staphylococci, 29 coagulase-positive with *S. aureus* as the most prevalent, 89% MS, and 11% MR. And 48 were coagulase-negative with *S. epidermidis* as the most isolated with 36% of MS and 64% of MR. Poor susceptibility was found in the staphylococcus coagulase-negative/MR group. Moxifloxacin and vancomycin show the best *in vitro* activity for all isolates. The MIC90 of moxifloxacin and vancomycin were 0.064/1.5, 0.64/3.0, and 1/3.0 for *S. aureus*-MS, *S. epidermidis*-MS, and *S. epidermidis*-MR, respectively. The most frequently associated factors found in patients with positive culture for staphylococcus were exposure to the health care system 23 (29.87%) of 77 patients and dry eye 23 (29.87%) of 77 patients. Both with a proportion of 3 in 10.

Conclusion: Coagulase-negative staphylococci were the most frequently isolated from the conjunctiva with 58.33% of MR; even though multiresistance was detected, their susceptibility to a fourth-generation fluorquinolone, commonly used, such as moxifloxacin, was preserved.

Introduction

CONJUNCTIVITIS IS ONE of the most frequent infections of the ocular surface, corresponding to 2% to 5% of the diagnosis in general medical practice and to one third of the eye problems.¹ Depending on the duration of the signs and symptoms, it may be hyperacute, acute, or chronic. The problem of conjunctivitis lies in the chronic type, which lasts for more than 3 weeks, is difficult to treat, and can be associated to blepharitis, infection of the lacrimal system, and tear film dysfunction.^{2,3} In Colombia, the prevalence of these infections is not known, nor their pathogens and antibiotic susceptibility.

Worldwide, the majority of publications relate to acute conjunctivitis in which, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* are the most common pathogens.⁴ Coagulase-negative *Staphylococcus* and *S. aureus*

are the germs most frequently isolated in chronic conjunctivitis, with a tendency for an increased antibiotic resistance in recent years.^{5,6} Oliveira et al. reported that coagulase-negative methicillin-resistant *Staphylococcus* had *in vitro* resistance to multiple antibiotics used in ophthalmology.⁷ Bacteria susceptibility to antimicrobials varies according to the socioeconomic factors at the individual and national levels either in developing or industrialized countries.⁸ Emergence of resistant *Staphylococcus* from the conjunctiva is of great concern, because its virulence is related to the clinical outcome of ocular infections like keratitis or endophthalmitis.^{9,10}

The purpose of this study was to identify the germs that cause chronic conjunctivitis, their antimicrobial susceptibility pattern to antibiotics generally used in ophthalmology (oxacillin, cefoxitin, vancomycin, moxifloxacin, ciprofloxacin, clindamycin, erythromycin, tobramycin, and cefazolin), determining the minimum inhibitory concentration for 90% of

TABLE 1. TOTAL BACTERIA ISOLATED FROM 108 CONJUNCTIVAL CULTURES

Gram-positive bacteria	n (%)	Gram-negative bacteria	n (%)	p
<i>Staphylococcus epidermidis</i>	44	<i>Klebsiella pneumonia</i>	3	0.0001
<i>Staphylococcus aureus</i>	28	<i>Pseudomonas fluorescens</i>	2	
<i>Staphylococcus warneri</i>	2	<i>Escherichia coli</i>	2	
<i>Staphylococcus lugdunensis</i>	2	<i>Pseudomonas aeruginosa</i>	2	
<i>Staphylococcus haemolyticus</i>	1	<i>Morganella morganii</i>	9	
<i>Streptococcus mitis</i>	3	<i>Ralstonia pickettii</i>	1	
<i>Streptococcus salivarius</i>	1	<i>Proteus mirabilis</i>	2	
		<i>Pseudomonas stutzeri</i>	6	
Total Gram-positive	81 (75)	Total Gram-negative	27 (25)	

isolates (MIC90), and evaluate the treatment, clinical course, and the conditions related to the host.

Methods

Upon approval of the research ethics committee, a prospective study with a nonprobability sample of sequential allocation was conducted, according to the selection and exclusion criteria for the study population. The research adhered to the tenets of the Declaration of Helsinki. Between January 2010 and January 2011, outpatients of the Clínica de Oftalmología de Cali, Colombia, with a mean age of 51 ± 10 years and diagnosis of chronic conjunctivitis, were included.

Inclusion criteria

Inclusion criteria required a history of symptoms over a period of at least 3 weeks and presence of the following clinical signs: red eye, daily discharge, subtarsal papillae or follicles, minimal mucoid or mucopurulent secretion, thickening of the conjunctiva, and positive cultures. Patients with unilateral or bilateral illness were included. If the patient was being treated with any antibiotic at recruitment, it had to be suspended for 48 h before taking the samples for culture and the incubation time was longer.

We excluded patients who did not sign informed consent.

Through a questionnaire, we sought information about the following.

1. Demographic data.
2. Exposure-associated factors such as being a health worker, having home health care, living with a health worker, and having been hospitalized up to a year before the culture.
3. The use of medical devices: probes or contact lenses.
4. The presence of systemic diseases: rheumatoid arthritis and diabetes.
5. Ophthalmological diseases associated: blepharitis, glaucoma, dry eye, and ocular allergy.

The empirical treatment begins with quinolones or fluor-quinolones according to institutional protocol for chronic conjunctivitis. In cases without clinical improvement, with this treatment is recommend the use of vancomycin. In addition, there was clinical follow-up of all the cases.

Cultures

The technique for obtaining cultures from the conjunctival fornix was standardized, using topical anesthesia, eye speculum, and sterile swabs.

Culture methods for identification of Gram-negative and Gram-positive organisms

Conjunctival specimens were inoculated on culture media: blood agar, chocolate agar, and thioglycolate broth, and were immediately sent to the reference laboratory. Once isolates were identified, they were coded and frozen at -70°C for later analysis of antibiotic susceptibility.

Microorganism identification

The identification of the bacteria was performed by an automated method of MicroScan Walk Away 96 (Dade Behring).

Antibiotic susceptibility testing

The antimicrobial susceptibility tests were performed by the E-Test (bioMerieux, Marcy l'Etoile, France). The antibiotics tested for coagulase-positive and -negative staphylococcus were oxacillin, cefoxitin, vancomycin, moxifloxacin, ciprofloxacin, clindamycin, erythromycin, tobramycin, and ceftazolin.

The MIC were determined as the lowest concentration of an antibiotic that inhibits the growth of 90% of a microorganism. MIC results were reported as a quantitative value in terms of $\mu\text{g}/\text{mL}$. The bacterial isolates were considered susceptible or resistant by comparing the MIC of each with the Clinical and Laboratory Standards Institute breakpoints.¹¹ The standards are based on the safe achievable concentrations of antibiotics in the serum. There are no standards for topical ocular therapy that represent the concentrations of antibiotics in the ocular tissue.

Data analysis

The percentages of susceptibility to different antibiotics and the associated factors for each patient with positive

TABLE 2. SPECIES OF *STAPHYLOCOCCUS* ISOLATES

Staphylococci (n) 77						
Coagulase-positive n=29 (37.66%)			Coagulase-negative n=48 (62.33%)			P=0.06
MS MR		MS MR				
<i>S. aureus</i>	25	3	<i>S. Epidermidis</i>	16	28	
<i>S. haemolyticus</i>	0	1	<i>S. lugdunensis</i>	2		
			<i>S. warneri</i>	2		
Total	25	4	Total	20	28	

MR, methicillin resistance; MS, methicillin susceptibility.

TABLE 3. *IN VITRO* SUSCEPTIBILITY PROFILE WITH E-TEST FOR COAGULASE-POSITIVE AND -NEGATIVE ISOLATED FROM CONJUNCTIVA

Antibiotics	CPS-MS (n=25) % Susceptibility	CPS-MR (n=4) % Susceptibility	CNS-MS (n=20) % Susceptibility	CNS-MR (n=28) % Susceptibility	Total CPS (n=29) % Susceptibility	Total CNS (n=48) % Susceptibility
Cephalothin	100	25	100	46.43	89.66	68.75
Cefoxitin	96	0	n/e	n/e	82.76	n/e
Ciprofloxacin	100	100	70	71.43	100	70.84
Clindamycin	92	100	80	39.29	93.2	56.25
Erythromycin	84	100	60	28.58	86.3	41.67
Moxifloxacin	100	100	90	89.3	100	89.59
Oxacillin	100	0	100	0	86.3	41.67
Tobramycin	96	75	90	28.58	93.2	54.17
Vancomycin	100	100	100	100	100	100

CPS-MS, coagulase-positive staphylococcus/methicillin susceptible; CPS-MR coagulase-positive staphylococcus/methicillin resistance; CNS-MS, coagulase-negative staphylococcus/methicillin susceptible; CNS-MR, coagulase-negative staphylococcus/methicillin resistance.

culture were obtained with a descriptive analysis. The comparison of proportions was determined with the chi-square test, when the *P* value is <0.05, the two proportions are significantly different. The lowest drug concentrations preventing the growth of 50% and 90% of the bacterial strains tested were recorded as the MIC50 and MIC90, respectively, and were analyzed nonparametrically using the Kruskal–Wallis test as reported by Kowalski et al.¹² The analysis was performed using Med Calc software.

Results

Two hundred and forty three conjunctival cultures were taken, of which, 108 (44%) were positive and 135 (56%) were negative; 81 positive cultures were Gram-positive and 27 were Gram-negative (Table 1).

Of the Gram-positive, 77 were staphylococci, 29 coagulase-positive with *S. aureus* as the most prevalent, 89% methicillin susceptibility (MS), and 11% methicillin resistance (MR). And 48 were coagulase-negative with *Staphylococcus epidermidis* as the most isolated with 36% of MS and 64% of MR (Table 2).

From the total of staphylococci, there was found an MR of 13.7% for coagulase-positive and 58.33% for coagulase-negative staphylococci.

The antibiotic susceptibility for different antibiotics were obtained, and the results were separated in coagulase-positive and coagulase-negative for each one, according to the MR or the MS, and was found that vancomycin was the only antibiotic to which the bacteria showed 0% of resistance (Table 3).

The most frequently associated factors found in patients with chronic conjunctivitis and positive culture for staphylococcus were exposure to the health care system and eye diseases such as dry eye, although the comparison between MR and MS does not show statistically significant difference (*P*>0.05) in any associated factor (Table 4).

Sixty-one patients were treated with fluoroquinolones, of which, 83.60% received moxifloxacin; two of these patients were being treated before inclusion in the study with gentamicin and neomycin–polymyxin–dexamethasone (Table 5).

Twelve patients who were being treated with moxifloxacin at the beginning of the study with partial clinical

TABLE 4. ASSOCIATED FACTORS IN PATIENTS WITH POSITIVE CULTURES

	Patient factors associated with positive culture for staphylococci						
	CPS-MS n (%)	CPS-MR n (%)	P	CNS-MS n (%)	CNS-MR n (%)	P	n (%)
Total cultures (n)	25	4		20	28		77
Exposure categories	8 (32)	3 (75)	0.27	8 (40)	4 (14.28)	0.09	23 (29.87)
Health worker	0	0		2	0		2
Home health care	0	0		3	0		3
Living with health worker	3	2		0	2		7
Hospitalization up to a year before the culture	5	1		3	2		11
Use of medical devices	4 (16)	1 (25)	0.78	0 (0)	1 (3.57)	0.86	6 (7.79)
Probes	1	0		0	0		1
Use of contact lenses	3	1		0	1		5
Systemic diseases	0	0		0 (0)	2 (7.14)	0.62	2 (2.59)
Rheumatoid arthritis	0	0		0	2		2
Ophthalmological diseases associated	8 (32)	1 (25)	0.76	9 (45)	10 (35.7)	0.72	28 (36.36)
Blepharitis	0	0		3	0		3
Glaucoma	0	0		1	0		1
Dry eye	7	1		5	10		23
Ocular allergy	1	0		0	0		1

TABLE 5. MEDICAL TREATMENT RECEIVED BY PATIENTS AND ITS RELATION TO THE ISOLATED BACTERIA AND THE METHICILLIN-RESISTANCE PROFILE

Antibiotics	CPS-MS	CPS-MR	CNS-MS	CNS-MR	n (%)
	n	n	n	n	
Moxifloxacin	11	4	17	19	51 (68)
Gatifloxacin	5	0	0	3	8 (10.6)
Levofloxacin	2	0	0	0	2 (2.6)
Ciprofloxacin	2	0	1	1	4 (5.3)
Vancomycin	0	0	2	5	7 (9.3)
Oral antibiotic	3	0	0	0	3 (4.0)
Total	23	4	20	28	75

improvement were added a lubricant, for the culture purpose, the moxifloxacin was stopped for 48 h and then was restarted.

About 9.3% of the cases that were treated with vancomycin had previously received multiple antibiotic treatments (tobramycin, clindamycin, ciprofloxacin, moxifloxacin, levofloxacin, and neomycin-polymyxin-dexamethasone) without clinical improvement, and cultures were reported positive for coagulase-negative methicillin-resistant staphylococci.

Seventy five of the 77 eyes with positive staphylococci cultures were followed up. One patient died before completing the follow-up and another one did not return. Of the 75 patients followed up, all showed improvement in their symptoms.

Tables 6–8 present the descriptive statistics of the median, MIC50, MIC90, and susceptibility of *S. aureus* and *S. epidermidis* to tested antibiotics.

S. aureus/MS shows good susceptibility for all antibiotics and *S. epidermidis*/MS low susceptibility to ciprofloxacin and erythromycin.

S. epidermidis/MR was only susceptible to moxifloxacin and vancomycin.

Discussion

The coagulase-negative staphylococci infections have gained importance in recent decades; previously, they were not taken into account as pathogens because of their ubiquity in humans and were considered as normal flora, but the latest publications on endophthalmitis, keratitis, and conjunctivitis show an increase in their prevalence.^{7,13,14}

The MR in frequently isolated staphylococci from ocular infections is a serious problem due to the difficulty in making appropriate treatment with antibiotics commonly used in ophthalmology. And also because associated with the MR is the cross-resistance to all beta-lactams and to other antibiotics, such as cephalosporins, quinolones, and aminoglycosides.

In our health facility, the *S. epidermidis* was the most frequently isolated from the conjunctiva with 58.33% of MR; even susceptible, though multiresistance was detected, the susceptibility to moxifloxacin was preserved with MIC90 1.0, which was the same when we add *S. epidermidis* MS cases to *S. epidermidis* MR. Similar results were found by Yamada et al., who analyzed the conjunctiva of healthy patients, and they obtained MIC90 0.78 to moxifloxacin from *S. epidermidis* MR and this result was confirmed with the genetic expression of staphylococcus mutation.¹⁵

In contrast, the other study of Harper et al., the MIC90 of coagulase-negative staphylococcus isolated from endophthalmitis cases was ≥ 32 .¹⁴ This high value is perhaps correlated to the virulence of strains in severe infections.

In our study, the *S. epidermidis* MS shows resistance to ciprofloxacin MIC90 >32 and susceptibility to moxifloxacin MIC90 0.64, similar to results published by Ana Luisa Höfling-Lima et al.; the best results for the treatment of coagulase-negative staphylococci conjunctivitis and keratitis, were obtained with moxifloxacin and gatifloxacin, compared with quinolones of the previous generation.¹⁶

In our data, *S. aureus* was the second frequent bacteria isolated with 10% of MR, similar to the results published by Freidlin et al., with 16.7%,¹⁷ unlike reports presented by

TABLE 6. DESCRIPTIVE STATISTICS OF MINIMUM INHIBITORY CONCENTRATIONS ($\mu\text{g/mL}$) FOR *S. AUREUS*/METHICILLIN-SUSCEPTIBLE ISOLATES OF CHRONIC CONJUNCTIVITIS TO 9 ANTIBIOTICS

<i>S. aureus</i> -MS n=25						
Antibiotics	Median	MIC50	MIC90	Mode	Min MIC to max MIC	% Susceptibility
Cephalothin	0.75	0.75	4.0	1.0	0.023 to 8	100
Cefoxitin	3.0	3.0	6.0	6.0	0.19 to 6.0	96
Ciprofloxacin	0.19	0.19	0.5	0.38	0.025 to 0.75	100
Clindamycin	0.094	0.094	0.47	0.125	0.016 to >256	92
Erythromycin	0.194	0.194	0.38	0.125	0.023 to >256	84
Moxifloxacin	0.047	0.047	0.064	0.047	0.008 to 0.064	100
Oxacillin	0.50	0.50	1.0	0.50	0.064 to 1.0	100
Tobramycin	0.75	0.75	3	1	0.25 to 48	96
Vancomycin	1.5	1.5	1.5	1.5	0.38 to 2.0	100

MIC, minimum inhibitory concentration.

TABLE 7. DESCRIPTIVE STATISTICS OF MINIMUM INHIBITORY CONCENTRATIONS ($\mu\text{g/mL}$) FOR *STAPHYLOCOCCUS EPIDERMIDIS*/METHICILLIN-SUSCEPTIBLE ISOLATES OF CHRONIC CONJUNCTIVITIS TO 8 ANTIBIOTICS

S. epidermidis-MS n=16						
Antibiotics	Median	MIC50	MIC90	Mode	Min MIC to max MIC	% Susceptibility
Cephalothin	0.19	0.19	1.5	0.19	0.023 to 1.5	100
Ciprofloxacin	0.125	0.125	>32	0.064–0.19	0.038 to >32	75
Clindamycin	0.094	0.094	>256	0.094	0.023 to >256	81.25
Erythromycin	0.38	0.38	>256	>256	0.064 to >256	62.5
Moxifloxacin	0.064	0.064	0.64	0.064	0.012 to 2.0	87.5
Oxacillin	0.19	0.19	0.75	0.19	0.016 to 0.75	100
Tobramycin	0.25	0.25	>256	0.125–0.25	0.016 to >256	87.5
Vancomycin	1.5	1.5	3.0	2.0	1.0 to 3.0	100

TABLE 8. DESCRIPTIVE STATISTICS OF MINIMUM INHIBITORY CONCENTRATIONS ($\mu\text{g/mL}$) FOR *STAPHYLOCOCCUS EPIDERMIDIS*/METHICILLIN-RESISTANT ISOLATES OF CHRONIC CONJUNCTIVITIS TO 8 ANTIBIOTICS

S. epidermidis-MR n=28						
Antibiotics	Median	MIC50	MIC90	Mode	Min MIC to max MIC	% Susceptibility
Cephalothin	1.25	1.0	>256	1.0	0.38 to >256	46.43
Ciprofloxacin	0.69	0.64	>32	0.19	0.064 to >32	71.43
Clindamycin	96	96	>256	>256	0.047 to >264	39.29
Erythromycin	>256	>256	>256	>256	0.19 to >264	28.58
Moxifloxacin	0.15	0.125	1.0	1.0	0.016 to 2.0	89.3
Oxacillin	4.0	4.0	>256	4.0	1.0 to >256	0
Tobramycin	160	64	>256	>256	0.047 to >256	28.58
Vancomycin	2.0	2.0	3.0	2.0	1.0 to 3.0	100

Bascom Palmer in 2008, in which, a high incidence of *S. aureus* MR (42, 9%) is described.⁴ Furthermore, we found that these *S. aureus* were not resistant to the other antibiotics tested.

In this study, the most frequent association to conjunctivitis was dry eye, which, as it is already known,¹⁸ predisposed to infection; this disease is considered an extreme condition of the ocular surface, where the bacterial virulence can increase. We consider that it is important to discard chronic bacterial conjunctivitis, when there is poor response to treatment in patients with moderate or severe dry eye. Other associations were found, such as living with a health care worker and to have been previously hospitalized. We performed a comparison of proportions between MR and MS for each association evaluated, and we did not find any difference ($P > 0.05$ for each).

It would have been important to know whether these microcolonies of methicillin-resistant staphylococcus prone to multidrug resistance were biofilm producers, which is known to be an important factor of virulence and multi-resistance.^{19–21} In this study, the MR was obtained phenotypically by oxacillin and ceftioxin tests; in another study, it could be possible to confirm the presence of the *mecA* gene by genotypification, which is the gold standard test for MR.²² It would also be important to consider testing for the presence of biofilm in the coagulase-negative staphylococcus to correlate it with the chronicity of the infection and also with the virulence of the microorganism.^{20,21}

Ideally, we would like to have other fluoroquinolones tested, like gatifloxacin and besifloxacin, but they were not available in the E-test in Colombia.

In conclusion, this study demonstrated the importance of coagulase-negative staphylococcus as a cause of chronic conjunctivitis and the tendency to develop multiple antibiotic resistance of methicillin-resistant strains, with a conserved susceptibility to moxifloxacin and vancomycin. It is very important to obtain cultures to guide treatment with an antibiogram, but in cases with negative results of isolation, our findings suggest to treat empirically with moxifloxacin as a first-line antibiotic.

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