Original article

In vitro antibiotic susceptibility to fluoroquinolones

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ABSTRACT

Objective: To determine the antibiotic susceptibility of bacteria recovered from cultures of ocular infections in the Fundación Oftalmológica de Santander–Clínica Carlos Ardila Lulle (FOSCAL).

Materials and methods: Retrospective descriptive study of a series of registries of cultures of samples from ocular surfaces and intraocular fluids from the OCULAB-FOSCAL laboratory in Floridablanca (Colombia) made between January and December of 2007. Antibiotic sensitivity screening by the method of Kirby–Bauer with impregnated Sensi-Discs™ of determined antibiotic concentrations was performed.

Results: A total of 352 samples were studied: 160 from conjunctiva, 150 from cornea and 42 from intraocular fluids. Of the total of the samples more than one microorganism was recovered 45.65% of the samples. Gram-positive and gram-negative bacteria were identified in 78.7 and 18.4%, respectively. Resistance to gatifloxacin, moxifloxacin, ciprofloxacin and levofloxacin was observed in 6.3, 8.9, 33.2 and 35.6%, respectively, of gram-positive bacteria.

Resistance to gatifloxacin, moxifloxacin, ciprofloxacin and levofloxacin was also observed in 7.4, 16.7, 16.7% and 25.9%, respectively, of gram-negative bacteria. The overall bacterial resistance (gram-positive and gram-negative) to moxifloxacin was 10.15%, and to gatifloxacin it was 6.46%, being which showed a statistically significant difference (p < 0.05).

Conclusions: In our study the development of bacterial resistance to fourth generation fluoroquinolones was demonstrated in ocular samples. However, lower levels of resistance to fourth generation fluoroquinolones compared with that of third and second generation were found, particularly to gram-positive. Gatifloxacin showed lower resistance levels than moxifloxin. Nevertheless, interpretation of this superiority must be made with caution in the clinical field, since other factors, such as tissue penetration and in vivo activity, must be taken into account.

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### Resumen

**Objetivo:** Determinar la susceptibilidad antibiótica de las bacterias obtenidas en cultivos de infecciones oculares en la Fundación Oftalmológica de Santander - Clínica Carlos Ardila Lulle (FOSCAL).

**Materiales y métodos:** Estudio descriptivo retrospectivo de una serie de registros de cultivos de muestras de superficie ocular y líquidos intraoculares del laboratorio OCULAB-FOSCAL en Floridablanca (Colombia) realizados entreeroño y diciembre de 2007. Se realizó antibiograma por el método de Kirby-Bauer con sensídicos impregnados de concentraciones determinadas de antibiótico.

**Resultados:** Se recogieron un total de 352 muestras de los cuales 160 fueron de conjuntiva, 150 fueron de córnea y 42 de líquidos intraoculares. Se recuperó más de un microorganismo en el 45,65% del total de las muestras. El 78,7 y el 18,4% de las bacterias identificadas correspondieron a Gram positivos y a Gram negativos, respectivamente. El 63, 8,9, 33,2 y 35,6% de las bacterias Gram positivas fueron resistentes a gatifloxacino, moxifloxacino, ciprofloxacino y levofloxacino, respectivamente. El 7,4, 16,7, 16,7 y 25,9% de las bacterias Gram negativas fueron resistentes a gatifloxacino, moxifloxacino, ciprofloxacino y levofloxacino, respectivamente. La resistencia bacteriana global (tanto Gram positivos como Gram negativos) a moxifloxacino fue del 10,15% y a gatifloxacino del 6,46%, siendo esta diferencia estadísticamente significativa (p < 0.05).

**Conclusiones:** En nuestro estudio, se evidenció el desarrollo de resistencia bacteriana en muestras oculares incluso con las fluoroquinolonas de cuarta generación. Sin embargo se encontraron menores niveles de resistencia para las fluoroquinolonas de cuarta generación para las de tercera y segunda generación, especialmente entre Gram positivos. Gatifloxacino mostró menores niveles de resistencia que la moxifloxacino. La interpretación de esta superioridad debe, sin embargo, hacerse con cuidado en el campo clínico, ya que se deben tener en cuenta otros factores como la penetración tisular y la actividad in vivo.

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### Introducción

El introducción de antimicrobianos agentes fue one of the most significant developments in modern medicine, although it was soon found that microorganisms were able to develop resistance to said agents. In order to counteract the development and dissemination of resistant organisms, a rational use of antibiotics has been proposed as well as a call to discover and develop new antimicrobial agents.

The ophthalmologist must confront various types of infections, the most common of which are superficial infections such as conjunctivitis which fortunately do not lead to severe consequences. On the other hand, corneal or posterior segment infections such as endophthalmitis and retinitis can produce severe visual sequels. Endophthalmitis has been associated to severe visual loss in 20% of patients and frequently requires posterior vitrectomy and the application of intravitreal antibiotics. Due to the severity of this type of infection prevention is the most appropriate approach and prophylactic presurgery antibiotic treatments have become the norm.

Fluoroquinolones are bactericidal agents frequently used in ophthalmology due to their high effectiveness, broad range and specific activity on gram-negative pathogens. Fluoroquinolones prevent the synthesis of bacterial deoxyribonucleic acid (DNA) due to the inhibition of topoisomerases II and IV. According to their activity, they are divided in second generation fluoroquinolones such as ciprofloxacin; third-generation fluoroquinolones such as levofloxacin; and fourth generation such as moxifloxacino and gatifloxicino.

In general, fluoroquinolones are considered to be very good options for treating and preventing various ocular infections. This explains their increasing use in ophthalmology. However, improper use of either systemic or topical antibiotics can lead to the appearance of bacterial resistance. In fact, some recent studies suggest that this resistance could be appearing even with fourth generation fluoroquinolones. Accordingly, the aim of this paper is to analyze in vitro antibiotic susceptibility of the ocular pathogen flora in ocular infection cultures at the Ophthalmological Foundation of Santander–Carlos Ardila Lulle Clinic (FOSCAL), Floridablanca (Colombia). The collected information could be useful for an empirical selection of antibiotics in addition to providing a true picture of microbial resistance in our environment.

### Materiales y métodos

A descriptive and retrospective study of a series of records of ocular surface sample cultures (conical or conjunctival) and intraocular liquids of the OCULAB-FOSCAL Lab (Floridablanca-Santander, Colombia), between January and December 2007.
The conjunctival surface samples were taken with a sterile cotton or calcium alginate cotton swab. For the corneal samples, a sterile Kimura spatula was used and additionally with clockmaker’s tweezers for sampling tissue remains in the compromised area. In some cases, the corneal tissue obtained during the penetrating keratoplasty surgery was studied. Intraocular liquid was sampled with vitreal puncture (vitreous humor) or with paracentesis (aqueous humor).

The direct study was made carrying out an extension in the glass plate, performing Gram and 10% potassium hydroxide (KOH) analysis. After doing the extension for the direct analysis, a sample was taken to place it in the culture and in a second step it was seeded with bacteriological matter in chocolate agar with CO₂, blood agar and McConkey agar. If anaerobics were suspected, the culture was made in phenylethyl alcohol, meat-liver agar, blood agar and chocolate agar in anaerobiosis.

Several culture media were utilized to provide various sample sources for agar plate culture. The media included standard low concentration nutritive culture (3 g/L yeast extract, Clna 6 g/L, 15 g/L peptone base, 1 g/L glucose) and triptose medium (20 g/L triptose, 1 g/L glucose, 5 g/L Clna, 0.005/L thiamin dichloride). After placing the sample in the culture at 37 °C, 24 hours were allowed to lapse before taking samples for a new evaluation under direct analysis for the solid agar cultures.

The cultures were checked at 24, 48 and 72 hours, reporting the microbial growth in each culture. In the absence of growth, the bacterial culture media were discarded at day 5.

Antibiogram was performed only for the positive culture bacteria, taking a sample of the culture over the agar and placing it in a brain—heart culture for approximately 12 hours. Subsequently, it was extended in a Mueller Hinton agar for antibiogram, seeding 3 times the entire surface of the plate rotating it 60° after each seeding to obtain uniform inoculation. The lid was left slightly open 5 min in order to absorb surface humidity, after which the antibiotic-impregnated discs were placed (Becton Dickinson-BBL™ Sensi-Disc™ Susceptibility Test Discs). Said discs were specific for gram-positive or gram-negative (Kirby–Bauer method).

The antibiotic sensidiscs had specific concentrations. The antibiograms were performed applying conventional diffusion methodology in Mueller Hinton agar. The agar plates were incubated 24 hours at 35 °C, studying the bacterial growth therein. The diameter of the inhibition area formed around each disc was measured. With this reference we were able to report whether the microorganism had sensitivity (S), intermediate sensitivity (I) or resistance (R) to each of the antibiotics assayed in the plates.

The variables were taken with an individual collection card for each sample, following the rules of the Helsinki Declaration and transferred to a Microsoft Excel database. The variables were analyzed with STATA and Epi Info 6.

**Results**

Overall, 352 samples of conjunctival surface cultures were collected (conjunctival secretion), corneal surface (keratitis and corneal ulcers, corneal button sample, LASIK interface sample) and intraocular liquids (aqueous humor and vitreous humor samples). Of all the samples, 160 were of the conjunctiva (45.46%), 150 of the cornea (42.61%) and 42 of intraocular liquids (11.93%).

Overall, the distribution was of 203 males and 149 females. A greater and statistically significant prevalence of corneal and intraocular liquids samples was found in males (p = 0.10), matching the reports published by Serrano-Calderon et al. in our institution, possibly related to the higher risk of ocular trauma in males.

Of all the samples, 322 (91.42%) had positive cultures. More than one microorganism was recovered (polymicrobial infection) in 147 cultures (45.65% of the samples). This figure is significantly higher than those reported in other studies (21% found by Yeh et al.). In order to determine whether the microorganisms present in the polymicrobial infections had different resistance than monomicrobial infections, analysis was made with cross infection tables with single microorganisms against polymicrobial infection both for gram-positive and gram-negative separately, without finding statistically significant differences.

The total number of identified bacteria was of 385: 303 gram-positive (78.7%), 71 gram-negative (18.4%), 1 anaerobic (0.3%), 2 Mycobacterium (0.5%) and in 8 cases Chlamydia trachomatis was suspected due to the presence of suggestive cytoplasmatic inclusions (2.1%); the remarkable predominance of gram-positive infections matches the findings of previous studies (Kunimoto et al. reported 71% of gram-positive germs in keratitis cultures). The total number of fungi was of 67 in all of the samples, with a predominance of Aspergillus sp. (46.3%), Fusarium sp. (26.9%) and Candida sp. (20.9%). The overall number of parasites was of 39, of which 94.9% were free-living amoebae of the Acanthamoeba sp. family.

Within the general group of gram-positive cultures, the most common were Staphylococcus nsp. (227 cases) including: 170 Staphylococcus coagulase-negative (including Staphylococcus epidermidis) and 57 Staphylococcus aureus coagulase positive, followed by siguieron Corynebacterium sp. (59 cases), non-hemolytic Streptococcus type B (7 cases), Bacillus sp. (6 cases), Streptococcus viridans (2 cases) and Enterococcus sp. (2 cases).

Within the gram-negatives, the most common were Haemophilus sp. (24 cases), followed by Klebsiella enterobacter (18 cases), Pseudomonas sp. (11 cases), Escherichia coli (7 cases), Enterobacter sp. (3 cases), Acinetobacter sp. (2 cases), Acinetobacter (2 cases); Serratia marcescens, Proteus mirabilis, Salmonella typhi and Aeromonas hydrophilla (1 case each).

In the conjunctive, 207 germs were identified, the most frequent being Staphylococcus sp. (mostly coagulase negative including S. epidermidis) and Corynebacterium sp. (Fig. 1), matching previous studies. Also gram-negative bacteria were evidenced but in a considerably lower percentage.

In what concerns the cornea, the main isolated bacteria were Staphylococcus sp. (mostly coagulase-negative including S. epidermidis), Acanthamoeba sp., Aspergillus sp., Corynebacterium sp. and Fusarium sp. (Fig. 2).

From intraocular liquids the main bacteria were Staphylococcus coagulase-negative (including S. epidermidis) and S. aureus coagulase-positive (Fig. 3).
The antibiogram was performed in the cultures with positive sample for bacteria, placing sensidiscs with moxifloxacin (Table 1), gatifloxacin (Table 2), levofloxacin (Table 3) and ciprofloxacin (Table 4).

Comparison between resistance to fluoroquinolones

The microorganisms exhibited lower resistance against fourth generation fluoroquinolones than against second and third-generation ones, with this difference being statistically significant (p < 0.05).

The overall bacterial resistance (gram-positive and gram-negative of any anatomic origin) to moxifloxacin was of 10.15% (33 out of 325) and gatifloxacin was of 6.46% (21 out of 325), with this difference being statistically significant (p < 0.05) by $\chi^2$ in SPSS 10 (Fig. 4). The overall bacterial resistance to levofloxacin was of 30.77% (100 out of 325) and to ciprofloxacin of 32.0% (104 out of 325) (Fig. 4).

Bacterial resistance to fluoroquinolones for gram-positive bacteria, in growing order of resistance, was as follows: gatifloxacin 6.3% (17 out of 271), moxifloxacin 8.9% (24 out of 271), ciprofloxacin 33.2% (90 out of 271), levofloxacin 35.6% (91 out of 271) (Fig. 5).

In turn, resistance to fluoroquinolones for gram-negative in growing order of resistance was as follows: gatifloxacin 7.4% (4 out of 54), moxifloxacin 16.7% (9 out of 54), levofloxacin 38.9% (21 out of 54) and ciprofloxacin 45.5% (24 out of 54) (Fig. 5).
16.7% (9 out of 54), ciprofloxacin 25.9% (14 out of 54) (Fig. 6).

The greater resistance to moxifloxacin than to gatifloxacin exhibited a statistically significant difference for positive as well as negative coagulase S. aureus (p = 0.038 and p < 0.005 respectively); although it was not statistically significant for Pseudomonas sp. (p = 0.737), probably due to the small amount of samples of these microorganisms.

Discussion

As described above, fluoroquinolones act on gyrase DNA (topoisomerase II) and on topoisomerase IV, with fourth generation fluoroquinolones exhibiting the highest action and the lowest probability of bacterial resistance as well as greater range of action because they act simultaneously on both enzymes. In order to develop resistance to second generation fluoroquinolones (ciprofloxacin, ofloxacin) bacteria only need one mutation whereas the newer fluoroquinolones such as moxifloxacin and gatifloxacin were specifically designed to be less affected by spontaneous mutations as generally two bacterial mutations are required to generate resistance.17 However, resistance to older generation fluoroquinolones has increased significantly in the last decade, and this may facilitate microorganisms developing resistance to fourth generation fluoroquinolones through mutation. The development of resistance to fourth generation fluoroquinolones has been

| Table 1 – Antibiotic sensitivity to moxifloxacin. |
|---|---|---|---|
| | Gram-positive | Gram-negative |
| | S | I | R | T | S | I | R | T |
| Conjuntiva | 68 (52.7%) | 9 (3.8%) | 12 (9.3%) | 129 (100%) | 16 (53.3%) | 9 (30.0%) | 5 (16.7%) | 30 (100%) |
| Cornea | 58 (52.3%) | 43 (38.7%) | 10 (9.0%) | 111 (100%) | 8 (53.3%) | 4 (26.7%) | 2 (20.0%) | 15 (100%) |
| Intraocular liquids | 11 (35.5%) | 18 (58.1%) | 2 (6.4%) | 31 (100%) | 4 (44.4%) | 4 (44.4%) | 1 (11.2%) | 9 (100%) |

Sensitivity to moxifloxacin for bacteria from corneal, conjunctival and intraocular liquid samples. I: intermediate sensitivity; R: resistant; S: sensitive; T: total.

| Table 2 – Antibiotic sensitivity to gatifloxacin. |
|---|---|---|---|
| | Gram-positives | Gram-negatives |
| | S | I | R | T | S | I | R | T |
| Conjuntiva | 80 (62.0%) | 41 (31.8%) | 8 (6.2%) | 129 (100%) | 21 (70.0%) | 8 (26.7%) | 1 (3.3%) | 30 (100%) |
| Cornea | 65 (58.6%) | 37 (33.3%) | 9 (8.1%) | 111 (100%) | 9 (60.0%) | 4 (26.7%) | 2 (13.3%) | 15 (100%) |
| Intraocular liquids | 18 (58.1%) | 13 (41.9%) | 0 (0.0%) | 31 (100%) | 5 (55.6%) | 3 (33.3%) | 1 (11.1%) | 9 (100%) |

Sensitivity to gatifloxacin for bacteria from corneal, conjunctival and intraocular liquid samples. I: intermediate sensitivity; R: resistant; S: sensitive; T: total.

| Table 3 – Antibiotic sensitivity to levofloxacin. |
|---|---|---|---|
| | Gram-positives | Gram-negatives |
| | S | I | R | T | S | I | R | T |
| Conjuntiva | 38 (29.5%) | 48 (37.2%) | 43 (33.3%) | 129 (100%) | 13 (43.3%) | 11 (36.7%) | 6 (20.0%) | 30 (100%) |
| Cornea | 27 (24.3%) | 47 (42.4%) | 37 (33.3%) | 120 (100%) | 5 (33.3%) | 9 (60.0%) | 1 (6.7%) | 20 (100%) |
| Intraocular liquids | 5 (16.1%) | 15 (48.4%) | 11 (35.5%) | 34 (100%) | 3 (33.3%) | 4 (44.5%) | 2 (22.2%) | 12 (100%) |

Sensitivity to levofloxacin for bacteria from corneal, conjunctival and intraocular liquid samples. I: intermediate sensitivity; R: resistant; S: sensitive; T: total.
bacterial keratitis, with higher percentages for gram-positive bacteria along the lines of our results (in cornea samples, a level of 27% was found for all the bacteria: 28.8% for gram-positives and 13.3% for gram-negatives), with increasing resistance in recent years. In 2002, Mather et al. reported in vitro superiority of fourth generation fluoroquinolones over ciprofloxacin, ofloxacin and levofloxacin, particularly over gram-positive bacteria. The efficacy of these fluoroquinolones is very similar with gram-negative bacteria, as confirmed in recent in vitro bacterial susceptibility studies which evidenced an increase of microorganism resistance to second and third generation fluoroquinolones (levofloxacin and tosufloxacin) in normal conjunctival flora. Said studies also warned of some Staphylococcus sp. strains with possible resistance mechanisms to fluoroquinolones, which is highly relevant for clinical practice. It has also been published that dry eye patients exhibit higher resistance to fluoroquinolones. 

In this study we have observed increased resistance in comparison to previous studies. A striking finding which is specific to fourth generation fluoroquinolones is that the bacterial resistance levels were higher in gram negative germs than in gram-positive germs. This tendency would justify a switch to antimicrobials with lower microbial resistance levels such as fourth generation fluoroquinolones, particularly in situations where the antibacterial action is critical such as in the treatment of corneal or intraocular infections and endophthalmitis prophylactic treatment.

As regards specific pathogens, Pseudomonas aeruginosa continues to be a highly resistant bacterium. However, in our study and due to the small sample size it was difficult to determine the antibiotic sensitivity of this pathogen. Staphylococcus sp. exhibited low resistance to these 2 important antibiotics. However, resistance is on the increase when compared to previous studies, which means that caution must be exercised in the management of these antibiotics.

Our study evidenced an important amount of infections by Acanthamoebas sp. (37 out of 231 samples). This is an infrequent microorganism (under 1% of corneal ulcers) and is generally related to the use of contact lenses. Our study correlates with the high prevalence described in a previous study carried out in our institution which evidenced that 25% of infectious keratitis patients were infected by Acanthamoebas sp., although unrelated to the use of contact lenses. A prospective study is being arranged to determine risk factors for this type of amoeba keratitis in our environment.

The development of bacterial resistance to all fluoroquinolones, including fourth-generation ones, is a fact. However, the resistance percentage for the fourth-generation fluoroquinolones is statistically significantly lower compared to third and second generation ones, particularly for gram-positive bacteria.

When comparing gatifloxacin and moxifloxacin, the former exhibited lower in vitro microbial resistance levels in our study, with statistically significant differences. Even so, the interpretation of this superiority must be very prudent in the clinical field because other factors must be taken into account such as the tissue penetration of these 2 antibiotics (higher for moxifloxacin), which produces a greater intraocular concentration thereof.
To conclude, we found that second and third generation fluoroquinolones exhibit high microbial resistance levels in our environment. Fourth generation fluoroquinolones still exhibit low bacterial resistance level and for this reason their clinical effectiveness in treatment as well as it prophylaxis must be higher. We consider that indiscriminate use or poor dosage of these antibiotics can enhance the appearance of microbial resistance and therefore they must be reserved for treating resistant conjunctival infections, corneal infections and endophthalmitis.

Conflicts of interest

The authors have not declared any conflicts of interest.

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