# The effects of topical chloramphenicol and ciprofloxacin on conjunctival bacterial flora of healthy dogs

**Malmasi, A.[1*], Selk Ghaffari, M.[2], Sadeghi-Hashjin, G.[3], Davoodi, M.[1], Capiau, E.[4], Sharifian Fard, M.[5], Ahadinejad, S.[1], Bahonar, A.[6]**

[1]*Department of Internal Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[2]*Department of Clinical Sciences, School of Veterinary Medicine, Islamic Azad University-Karaj Branch, Karaj, Iran*

[3]*Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[4]*Department of Ophthalmology, University of Ghent, Ghent, Belgium*

[5]*Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium*

[6]*Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

**Key words:**
chloramphenicol, ciprofloxacin, conjunctival flora, dog

**Correspondence**
Malmasi, A.
Department of Internal Diseases, Faculty of veterinary Medicine, University of Tehran, Tehran , Iran
Tel: +98(21) 66920035
Fax: +98(21) 66438327
Email: amalmasi@ut.ac.ir

Received: 29 October 2014
Accepted: 12 January 2015

## Abstract:

**BACKGROUND:** Normal bacterial flora of the conjunctiva, which inhibits growth of opportunistic organisms, has an important role in the prevention of ocular infections. If resident flora is inhibited by disease or long-term application of antibiotics, opportunistic pathogens overgrow, leading to disease. **OBJECTIVES:** The aim of this study was to investigate the effects of ophthalmic chloramphenicol and ciprofloxacin on bacterial conjunctival flora in healthy dogs. **METHODS:** A total of 16 animals were divided into 2 equal groups which received either chloramphenicol (CHL) (0.5%) or ciprofloxacin (CIP) (0.3%). In both groups, the right eye of each animal was treated with 2 drops of antibiotics every 8 and 6 hours, respectively, for 1 week and the left eye received artificial tear solution and served as control. Bacterial and fungal cultures were performed 8 hours before and after the treatment. Fisher's Exact test and SPSS software were used for statistical analyses ($p<0.05$). **RESULTS:** There were no statistically significant differences between control and test eyes and bacterial isolates in both groups. In CHL group, after treatment *Staphylococcus* spp (62.5%), *Bacillus* spp (12.5%) from the right eyes and *Staphylococcus* spp (75%) and *Bacillus* spp (12.5%) from left eyes were isolated. In CIP group, after treatment the bacterial isolates of right eyes were *Staphylococcus* spp (87.5%), *Aerococcus* spp (37.5%), *Viridans streptococcus* (25%), *Micrococcus* spp (12.5%), *Bacillus* spp (12.5%); *Staphylococcus* spp (75%), *Micrococcus* spp (25%), *Bacillus* spp (12.5 %) were isolated from left eyes of dogs after 1 week administration of artificial tear. **CONCLUSIONS:** Topically applied chloramphenicol and ciprofloxacin had no significant changes in or detrimental effects on normal bacterial flora of treated dogs.

## Introduction

The normal ocular flora is thought to play a defensive role against the pathogenic microbes by depriving them of nutrients, producing antimicrobial-like substances and occupying space on the conjunctival epithelium. However, under diseased conditions, the microbes composing the normal flora may become opportunistic pathogens, and known pathogens such as gram-negative bacteria and fungi are more frequently isolated. If resident flora is inhibited by disease or long-term application of antibiotics, opportunistic pathogens overgrow and lead to disease (Asghari and Gharachorlou, 2011). Chronic administration of topical antibiotics results in a shift from the normal predominantly gram-positive to gram-negative bacterial flora and may facilitate the emergence of resistant bacterial strains. Normal bacterial flora of the conjunctiva has been evaluated in most of the animals and human. It is believed that most of bacterial isolates in different species of animals are gram positive bacteria with *Staphylococcus* at the top (Gemensky et al., 2005). The other bacterial isolates can have a wide variation. Nowadays, ophthalmic antibiotics are frequently used for different ocular infections. Since several studies in human and small animals showed that broad spectrum antibiotics such as chloramphenicol and flouroquinolones are effective in commonly isolated bacteria from conjunctival sacs of infected eyes (Prado et al., 2006; Kowalski et al., 2003), the antibiotics are thus recommended for those animals with ocular infections waiting for culture results. Although resistance against ciprofloxacin in Staphylococcal and Streptococcal infections is common, Chloramphenicol and Ciprofloxacin are still antimicrobials of choice used in veterinary practice.

In human, it is believed that ciprofloxacin 0.3% and chloramphenicol 0.5% cause a reduction in normal bacterial flora of conjunctiva (Coskun et al., 2011; Ermis et al., 2004; Snyder-Perlmutter et al., 2000); however, there is no study about the effect of these antibiotics on normal bacterial flora of canine conjunctiva. The hypothesis is that chronic use (1 week) of antibiotics would promote a shift in bacterial flora from gram- positive to gram- negative bacteria or promote a shift from nonpathogenic flora to opportunistic pathogens or even increase fungal organisms. The aim of this study was to investigate the possible effects of chronic administration of ciprofloxacin and chloramphenicol on balance of normal, non-pathogenic conjunctival bacterial flora, and probable alteration towards pathogenic or gram-negative bacteria or even fungal organism.

## Materials and Methods

**Animals and experimental protocol:** A total of 16 healthy dogs of either sex were randomly divided into 2 groups of 8 each. The right eyes received two drops of either chloramphenicol 0.5% (Chlobiotic 0.5%, Sina Darou, Tehran, Iran ) every 8 h (CHL Group) or ciprofloxacin 0.3% (Ciplex, Sina Darou, Tehran, Iran) every 6 h (CIP Group) for 1 week. The left eyes received vehicle (artificial tear) only and served as controls. The drops were instilled into the conjunctival sacs.

**Bacteriologic and fungal cultures:** Bacteriologic and fungal cultures from conjunctival sacs were performed twice, 8 h before the first and 8 h after the last treatments. All cultures were performed in a double-blind manner and neither the person who took the specimens nor the microbiologist who evaluated bacterial growth knew the sources of the samples. Specimens were taken by rubbing sterile cotton tipped swabs to the conjunctival sacs. Afterwards, all specimens were inoculated onto 5% blood agar, chocolate agar, Mac Concky agar, thioglycolate broth, and Sabouraud's Dextrose Agar (SDA) for fungal organisms, immediate-

ly. The blood and chocolate agar plates were incubated at 35-37°c for 48 h. Thioglycolate broth were checked every day for any bacterial and SDA for fungal growth and if there were negative maintained for 5 more days. After the incubation period, colonies were stained by Gram staining and bacterial identification was accomplished based on their morphologies and differential diagnostic tests. This was performed for both pre treatment and post treatment cultures, the same.

**Data analysis:** SPSS (Version 16) was used to analyze the data. To evaluate the difference between groups, Fisher's Exact test was used and whenever the $p<0.05$, the difference was taken statistically significant.

## Results

In both groups (CHL and CIP groups), the most frequent bacterial isolates were different types of coagulase negative *Staphylococcus* and other bacterial isolates were *Enterococcus*, *Micrococcus*, *Bacillus*, *Streptococcus* and *Aerococcus*. In CHL group, pre-treatment cultures were 100% positive for right eyes and 87.5% for left eyes. This was almost the same in CIP group. After 1-week administration of ophthalmic drops, post-treatment cultures in CHL group were 75% and 87.5% positive for right and left eyes, respectively. However, in CIP group, it was 100% positive for both right and left eyes.

All bacterial isolates from right and left eyes in pre- and post- treatment cultures were gram-positive in both groups and neither gram negative bacteria nor fungi were isolated.

In CHL group, after 1 week administration of chloramphenicol 0.5%, *Staphylococcus* spp. (62.5%), *Bacillus* spp (12.5%) from the right eyes and *Staphylococcus* spp. (75%) and *Bacillus* spp (12.5%) from left eyes were isolated (Table 1).

In CIP group, after 1 week administration of ciprofloxacin 0.3%, *Staphylococcus* spp. (87.5%), *Aerococcus* spp. (37.5%), *Viridans streptococcus* (25%), *Micrococcus* spp. (12.5%) and *Bacillus* spp. (12.5%) from the right eyes and *Staphylococcus* spp. (75%), *Micrococcus* luteus (25%) and *Bacillus* spp (12.5%) from the left eyes were isolated (Table 1).

All *Staphylococci* in CHL group were coagulase negative (100%) and no statistical dif-

Table 1. Bacteria isolated from canine eyes, before and after treatment with either chloramphenicol 0.5% (2 drops, q8h, for 7 days) or ciprofloxacin 0.3% (2 drops, q6h, for 7 days) and artificial tear solution was used in control eyes.

| | **Chloramphenicol (0.5%)** | | | | **Ciprofloxacin (0.3%)** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Treated eyes n (%)** | | **Control eyes n (%)** | | **Treated eyes n (%)** | | **Control eyes n (%)** | |
| *Bacterial organisms* | Before | After | Before | After | Before | After | Before | After |
| *Staphylococcus* spp. | 5(62.5) | 5(62.5) | 5(62.5) | 6(75) | 5(62.5) | 7(87.5) | 7(87.5) | 6(75) |
| *Aerococcus* spp. | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 3(37.5) | 0(0) | 0(0) |
| *Viridans streptococcus* | 0(0) | 0(0) | 1(12.5) | 0(0) | 0(0) | 2(25) | 0(0) | 0(0) |
| *Enterococcus* spp. | 1(12.5) | 0(0) | 1(12.5) | 0(0) | 2(25) | 0(0) | 1(12.5) | 0(0) |
| *Micrococcus* spp. | 0(0) | 0(0) | 0(0) | 0(0) | 1(12.5) | 1(12.5) | 1(12.5) | 2(25) |
| *Bacillus* spp. | 1(12.5) | 1(12.5) | 1(12.5) | 1(12.5) | 1(12.5) | 1(12.5) | 0(0) | 1(12.5) |

ferences were detected with pre-treatment cultures. Most of *Staphylococci* in CIP group were coagulase negative (83.3%) and no statistical differences were detected with pre-treatment cultures, either.

## Discussion

Nowadays, different types of ophthalmic antibiotics are commonly used for treatment of ophthalmic diseases in veterinary practice. The advantages of proper administration of broad- spectrum topical antibiotics for bacterial keratitis, corneal ulcers, conjunctivitis, and etc. have been pointed in some studies (Asghari and Gharachorlou, 2011; Leibowitz, 1991). These antibiotics, despite all advantages, have some disadvantages and there is limited knowledge of their effects on normal bacterial flora of healthy conjunctiva.

Most gram-positive bacterial isolates from normal conjunctiva of dogs are *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Bacillus* and *Pseudomonas* and *E.coli* from gram negative bacteria (Gemensky et al., 2005; Haghkhah et al., 2005; Kudirkienė et al., 2006; McDonald, Watson, 1978; Prado et al., 2005). These organisms are considered as non-pathogenic bacteria and can protect conjunctival surface from invasion of pathogenic organism. In our study, *Staphylococcus*, *Enterococcus*, *Micrococcus*, and *Bacillus*, all gram-positive, were isolated with no gram-negative bacteria and fungi detection.

On the other hand, these normal bacterial flora such as *S. intermedius* along with *S. aureus* are potentially pathogenic bacteria that are responsible for some ocular infections. Coagulase negative bacteria have low pathogenicity; however, in damaged conjunctival epithelium or in immunosuppressive conditions or diabetes mellitus they may lead to disease. Other bacterial isolates were *Enterococcus faecalis* (25%), *Micrococcus* spp. (12.5%), and *Bacillus* spp. (12.5%). In our study, like the previous ones, *Staphylococcus* spp. were the most frequent bacteria isolated from conjunctival sacs of healthy dogs. This shows that *Staphylococci* cause ocular infections; however, they exist in healthy eyes as well. Furthermore, changes in bacterial biological environment can alter the pathogenicity of organisms (Gerding et al., 1988).

Antibiotic susceptibility testing results suggest that gram positive cocci and bacilli (like *Staphylococcus*, *Streptococcus*, *Bacillus*) isolated from both healthy and infected eyes are mostly sensitive to Chloramphenicol and fluoroquinolones (Kowalski et al., 2003; Prado et al., 2006; Mino et al., 2005).

In our study, antibiotic therapy using Chloramphenicol and Ciprofloxacin had no significant effects on positive culture results. In addition, no detrimental effects on conjunctival floral balance were shown in post-treatment culture which might be due to the absence of any ocular injuries and gram-negative bacterial growth. These findings are consistent with the findings of Gemensky et al. (2005) who showed that application of topical antimicrobial preparations in horses did not result in any shift towards gram-negative bacteria as seen in clinically ill patients.

However, quantitative studies conducted on normal bacterial flora of human eyes to evaluate the efficacy of Ciprofloxacin showed that this antibiotic is potent in bacterial reduction of normal flora (Ermis et al., 2004; Coskun et al., 2011; Snyder-Perlmutter et al., 2000). Thus, it is suggested to evaluate bacterial growth in a quantitative manner to determine the effects of antibiotics on reduction of normal ocular flora in dogs, in future studies.

In conclusion, it seems Chloramphenicol and Ciprofloxacin, as broad spectrum antibiotics, can be safely administered in ocular bacterial infections with no detrimental effects on the balance of conjunctival bacterial flora.

## Acknowledgments

The authors wish to express their gratitude to A. Aliari, E. Bijari and M. Asgari, staff of the Small animal teaching hospital, Faculty of Veterinary Medicine, University of Tehran for their valuable technical assistance.

## References

1. Asghari, A., Gharachorlou, A. (2011) Assessing the role of proper administration of antibiotics in the treatment of corneal ulcers in dog. Adv Environ Biol. 5: 2084-2086.
2. Coskun, M., Altintas, A.G., Anayol, M.A., Raza, S., Celikbilek, N., Simsek, S. (2011) Evaluation of efficacy of topical povidone-iodine and different types of fluoroquinolones in the sterilization of bacterial flora on the conjunctiva. J Ocular Pharmacol Ther. 27: 589-592.
3. Ermis, S.S., Aktepe, O.C., Inan, U.U., Ozturk, F., Altindis, M. (2004) Effect of topical dexamethasone and ciprofloxacin on bacterial flora of healthy conjunctiva. Eye. 18: 249-252.
4. Gemensky-Metzler, A.J., Wilkie, D.A., Kowalski, J.J., Schmall, L.M., Willis, A.M., Yamagata, M. (2005) Changes in bacterial and fungal ocular flora of clinically normal horses following experimental application of topical antimicrobial or antimicrobial- corticosteroid ophthalmic preparations. Am J Vet Res. 66: 800-811.
5. Gerding, P.A., Mc Laughlin, S.A., Thoop, M.W. (1988) Pathogenic bacteria and fungi associated with external ocular diseases in dogs: 131 cases (1981-1986). J Am Vet Med Assoc. 193: 242-244.
6. Haghkhah, M., Sarchahi, A.A., Molazem, M. (2005) Conjunctival flora in normal dogs. Online J Vet Res. 9: 79-83.
7. Kowalski, R.P., Dhaliwal, D.K., Karenchak, L.M., Romanowski, E.G., Mah, F.S., Ritterband, D.C., Gordon, J.Y. (2003) Gatifloxacin and Moxifloxacin: An in vitro susceptibility comparison to Levofloxacin, Ciprofloxacin, and Ofloxacin using bacterial keratitis isolates. Am J Ophthalmol. 136: 500-505.
8. Kudirkiene, E., Žilinskas, H., Šiugždaite, J. (2006) Microbial flora of the dog eyes. Veterinarija Ir Zootechnika. 34: 18-21.
9. Leibowitz, H. (1991) Clinical evaluation of Ciprofloxacin 0.3% ophthalmic solution for treatment of bacterial keratitis. Am J Ophthalmol. 112: 34S–47S.
10. McDonald, P.J., Watson, A.D.J. (1978) Microbial flora of normal canine conjunctivae. J Small Anim Pract. 17: 809-812.
11. Mino de Kaspar, H., Koss, M.J., He, L., Blumenkranz, M.S., Ta, C.N. (2005) Antibiotic Susceptibility of Preoperative Normal Conjunctival Bacteria. Am J Ophthalmol. 139: 730-733.
12. Prado, M.R., Rocha, M.F., Brito, E.H., Girão, M.D., Monteiro, A.J., Teixeira, M.F., Sidrim J.J.C. (2005) Survey of bacterial microorganisms in the conjunctival sac of clinically normal dogs and dogs with ulcerative keratitis in Fortaleza, Ceará, Brazil. Vet Ophthalmol. 8: 33-37.
13. Prado, M.R., Brito, E.H.S., Girao, M.D., Sidrim, J.J.C., Rocha, M.F.G. (2006) Identification and antimicrobial susceptibility of bacteria isolated from corneal ulcers of dogs. Arq Bras Med Vet Zootec. 58: 1024-1029.
14. Snyder-Perlmutter, L.S., Katz, H.R., Melia, M. (2000) Effect of topical ciprofloxacin 0.3% and ofloxacin 0.3% on the reduction of bacterial flora on the human conjunctiva. J Cataract Refract Surg. 26: 1620-1625.