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# Antimicrobial Sensitivity Profile of Eye Infection in Dogs

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**A B S T R A C T**

In the present study 88 number of corneal swabs were collected from 8 different breeds of dog suffering from corneal diseases. All the dogs were presented to Teaching Veterinary Clinical Complex of Odisha Veterinary College for treatment during the period from December 2018 to June 2019. The breeds consists of Non-descript (n=18), Pug (n=11), Labrador (n=10), German shepherd (n=12), Spitz (n=14), Golden retriever (n=10), Dalmatian (n=7), and Mastiff (n=6). There were no history of injury of the eye prior to infection and all most all the dogs were naturally infected with various microbial agents. In order to identify the microbial isolates and its antimicrobial susceptibility profile all the isolates were subjected to routine microbial procedure. Out of 246 number of bacterial isolates, the most commonly isolated bacteria were *Staphylococcus* spp. (35%) followed by *Streptococcus* spp (27%), *Pseudomonas* spp. (26%), and *E. Coli* (10%). The antimicrobial susceptibility test was done by disk diffusion method in which ciprofioxacin, cephalexin, neomycin and amoxycillin/clavulanic acid was sensitive to *Staphylococcus* spp., cephalexin, chloramphenicol and amoxycillin/clavulanic acid was sensitive to *Streptococcus* spp. and amikacin, gentamicin and tobramycin was found to be highly sensitive *to Pseudomonas* spp. Similarly, out of 115 fungal isolates, the most commonly fungal isolates were found to be *Aspergillus* spp. (40%) and *Candida* spp (59%), whereas mixed fungal infection were found to be prominant. Antifungals like fluconazole, voriconazole and miconazole were found to be sensitive to *Aspergillus* spp. as well as *Candida* spp.

## Introduction

Ocular infectious disease by microorganisms is a major cause of corneal opacity and loss of vision in dogs. The normal cornea prevents bacterial invasion by various anatomical, mechanical, immunological and microbiological mechanisms. Failure of these defenses may contribute to ulcerative keratitis and predispose the cornea to bacterial infection. The eye is remarkably resistant to constant bombardment of potentially pathogenic microorganisms. At the level of

cornea, the defense against bacterial invasion is determined by the integrity of anatomical barriers and the associated immune status of the host, disruption of which induces bacterial invasion. Sloughing of epithelial cells of cornea and conjuctiva and precorneal tearfilm (lysozyme, lactoferrin etc.) also plays an important role in preventing the bacterial infection.

The normal microbial population of the ocular surface is believed to interfere with invading organisms by depriving them of nutrients and secreting substances with antimicrobial properties. Destruction of normal ocular flora by long-term use of topical antimicrobials or corticosteroids can result in overgrowth of pathogenic bacteria, yeast and fungi (Franck, 2003). Fungal keratitis is reported less frequently in the dog than some other animal species but can be associated with substantial ocular morbidity and vision loss (Pucket *et al.,* 2012).

The objectives of this study is to isolate and identify the causative organism causing ocular infection in dogs and to determine antimicrobial sensitivity pattern.

## Materials and Methods

In the present study a total number of 88 samples from different breeds like Nondescript (18), Pug (11), Labrador (10), German Shepherd (12), Spitz (14), Golden Retriever (10), Dalmatian (7), and Mastiff (6) were collected from dogs presented to Teaching Veterinary Clinical Complex for treatment OUAT, Bhubaneswar during the period from December, 2018 to June, 2019. The samples were collected in sterile cotton swab moistened with 1-2 drops of sterile normal saline solution (0.9% NaCl) (Hindley *et al.,* 2016). Duplicate samples were collected from each animal.

### Microbial analysis

For bacterial isolation, the samples were inoculated in standard broth like Brain Heart Infusion (BHI) broth and incubated overnight at 37°C. Then identification of the bacteria was done by routine isolation techniques using Gram's staining and culture media. Samples were inoculated in various selective media like McConkey lactoseagar (MLA), blood agar (BA), mannitol salt agar (MSA), pseudomonas isolation agar (PIA) and incubated for 24 hr at 37°C. Then motility, colony morphology with reference to colony size, colour and nature of colony, were studied along with various biochemical tests (Stephen *et al.,* 2010) for identification.

### Mycological analysis

The samples were inoculated into sabouraud dextrose agar (SDA) and potato dextrose agar (PDA). The plates were incubated at 37°C for 3-4 days. Identification of the fungus was done by observing colony morphology with reference to colour, hyphae and nature of colony. Microscopic examination was done by Lactophenol cotton blue staining and Gram staining.

### Antibiogram study

Muller Hinton Agar (MHA) obtained from M/S Hi-Media Laboratories Ltd. Mumbai was employed for the *in-vitro* antibiotic sensitivity test of bacterial isolates as per the commonly used disk diffusion method Bauer *et al.,* (1966) and the zone of inhibition was recorded.

The antibiotic discs used for bacterial isolation were ciprofloxacin (30mcg), cephalexin (30mcg), gentamicin (10mcg), polymyxin B (300mcg), neomycin (30mcg), chloramphenicol (30mcg), tobramycin (30mcg), amikacin (30mcg),

amoxycilli/clavulanic acid (30mcg) whereas, antifungal discs used were fluconazole (10 mcg), voriconazole (1mcg) and mionazole (30mcg). Muller Hinton Agar (MHA) along with 2% glucose and 0.5mcg/ml methylene blue (CLSI) were used for antifungal sensitivity test as per guideline. All the antifungal and antibiotic discs were obtained from HiMedia, Mumbai, India. The results were interpreted according to criteria set by Clinical and Laboratory Standards Institute (CLSI) (Wayne, 2009).

## Result and Discussion

In the present study, out of 88 eye swabs collected, 246 number of bacterial isolates and 115 number of fungal isolates were obtained by routine microbial isolation. *Staphylococcus* spp. (87/246, 35%) was found as the most predominant bacterial isolate (Lin and Jones, 2007) followed by *Streptococcus* spp. (68/246, 27%), *Pseudomonas* spp. (66/246, 26%) and *E.Coli* (25/246, 10%). Similarly, *Candida* spp. (68/115, 59%) was the predominant fungal isolate followed by *Aspergillus* spp. (47/115, 40%) which shows 29% of fungal infection out of total microbial isolates. This result is similar to that of study of Samuelson *et al.*, (1984).

The distribution of various microbial isolates are depicted in Table 1. Out of total 246 number of bacterial isolates, 155/246 (63%) were found gram positive and 91/246 (36.9%) were found to be gram negative isolates. Most of the above organisms are ubiquitous and indigenous microflora of corneal and conjuctival surface (Furiani, 2011). Weak defense mechanism of the cornea may predisposes the animal to this infection. Macroscopically *Pseudomonas aeruginosa* produces flurescin pigment (greenish colour) on Pseudomonas Isolation Agar media as well as on MHA plate, *β*-hemolysis was shown by *Streptococcus* spp. on blood agar plates, pink colour colony was produced by *E.Coli* on McConkey lactose agar. Similarly, *Aspergillus* spp. produces characteristic velvety or wooly yellowish green colonies whereas *Candida* spp. produces whitish, shiny and convex colonies of 4 to 5 mm in diameter after incubation for 3-4 days on SDA plate. On lactophenol cotton blue staining, club shaped vesicle with uniseriate conidia was identified *as Aspergillus* spp. and budding yeast cells (blastoconidia) were found on gram staining. *Candida* spp. were found to be gram positive.

On analysis of antibiogram susceptibility pattern produced by various anbiotics and antifungals, it was found that *Staphylococcu* spp. was highly sensitive to cephalexin, neomycin, ciprofloxacin and amoxycillin/clavulanic acid. *Streptococcus* spp. were sensitive to cephalexin, chloramphenicol and amoxycillin/clavulanic acid.

*Pseudomonas* spp. isolates were sensitive to ciprofloxacin, gentamicin, amikacin and tobramycin whereas *E. Coli* isolates were susceptible to ciprofloxacin and amikacin. This study is in agreement with the study of Hindley *et al.*, (2016) and Lin *et al.*, (2007). Similarly, both the *Aspergillus* spp. and *Candida* spp. isolates were susceptible to fluconazole, voriconazole and miconazole (Stacy *et al.*, 2003). The detail antimicrobial profile is depicted in Table 2.

In case of *Staphylococcus* spp. antibiotics like ciprofloxacin, cephalexin, gentamicin, polymyxin B, neomycin, chloramphenicol, amoxycillin/clavulanic acid showed minimum inhibitory zone of 22, 20, 15, 8, 17, 17 and 18 mm respectively. In case of *Pseudomonas* spp. ciprofloxacin, cephalexin, gentamicin, polymyxin B, neomycin, chloramphenicol, tobramycin and amikacin showed minimum inhibitory zone of 21, 10, 16, 12, 14, 12, 15 and 17 mm respectively.

**Table.1** Shows the distribution of various micrbial isolates collected from 88 number of corneal swabs of dog

| Sl no. | Name of the dog breed | No. of corneal swab collected | *Staphylococcus* spp. | *Streptococcus* spp. | *Pseudomonas* spp. | *E. coli* | *Aspergillus* spp. | *Candida* spp. |
|---|---|---|---|---|---|---|---|---|
| **01** | Non-descript | 18 | 18 | 18 | 15 | 6 | 11 | 16 |
| **02** | Pug | 11 | 11 | 7 | 9 | 3 | 8 | 9 |
| **03** | Labrador | 10 | 10 | 8 | 8 | 3 | 7 | 6 |
| **04** | German shepeherd | 12 | 12 | 7 | 7 | 3 | 7 | 9 |
| **05** | Spitz | 14 | 14 | 10 | 10 | 5 | 5 | 8 |
| **06** | Golden Retriver | 10 | 9 | 7 | 8 | 2 | 4 | 9 |
| **07** | Dalmatian | 7 | 7 | 5 | 5 | 1 | 3 | 5 |
| **08** | Mixed | 6 | 6 | 6 | 4 | 2 | 2 | 6 |
| | **Total** | **88** | **87** | **68** | **66** | **25** | **47** | **68** |

**Table.2** Shows the antimicrobial and antifungal profile of eye infection in dogs (n=88)

| Name of microbial isolates | Number susceptible/total numer of isolates tested to antimicrobials with percentage | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CIP | CN | GEN | PB | N | C | TOB | AK | AMC | FLU | VCZ | MIC |
| ***Staphylococcus* spp. (n=87)** | 87/87 (100%) | 87/87 (100%) | 73/87 (85%) | 10/87 (12%) | 87/87 (100%) | 73/87 (85%) | - | - | 80/87 (92%) | - | - | - |
| ***Streptococcus* spp. (n=68)** | 11/68 (16%) | 68/68 (100%) | 0/68 (0%) | 0/68 (0%) | 0/68 (0%) | 68/68 (100%) | - | - | 60/68 (89%) | - | - | - |
| ***Pseudomonas* spp. (n=66)** | 66/66 (100%) | 0/66 (0%) | 66/66 (100%) | 59/66 (90%) | 14/66 (22%) | 0/66 (0%) | 66/66 (100%) | 66/66 (100%) | - | - | - | - |
| ***E.coli* (n=25)** | 12/25 (50%) | - | 8/25 (33%) | 8/25 (33%) | 4/25 (16%) | 8/25 (33%) | 8/25 (33%) | 16/25 (67%) | - | - | - | - |
| ***Aspergillus* spp. (n=47)** | - | - | - | - | - | - | - | - | - | 47/47 (100%) | 47/47 (100%) | 47/47 (100%) |
| ***Candida* spp. (n=68)** | | | | | | | | | | 68/68 (100%) | 68/68 (100%) | 68/68 (100%) |

CIP = Ciprofloxacin, CN = Cephalexin, GEN = Gentamicin, PB = Polymyxin B, N = Neomycin, C = Chloramphenicol, TOB = Tobramycin, AK = Amikacin, AMC = Amoxycillin Clavulanic acid, FLU = Fluconazole. VCZ = Voriconazole, MIC = Miconazole

*Not all antimicrobial agents were tested against each microbial isolates.

**Table.3** Shows the diameter of zone of inhibition (mm) of various antimicrobials against each microbial isolates of eye infection in dogs (n=88)

| Name of microbial isolates | Diameter of zone of inhibition in mm | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CIP | CN | GEN | PB | N | C | TOB | AK | AMC | FLU | VCZ | MIC |
| ***Staphylococcus* spp.** | 22 | 20 | 15 | 8 | 17 | 17 | - | - | 18 | - | - | - |
| ***Streptococcus* spp.** | 19 | 19 | 11 | - | 11 | 18 | - | - | 18 | - | - | - |
| ***Pseudomonas* spp.** | 21 | 10 | 16 | 12 | 14 | 12 | 15 | 17 | - | - | - | - |
| ***E.coli*** | 20 | - | 13 | 10 | 14 | 16 | 13 | 17 | - | - | - | - |
| ***Aspergillus* spp.** | - | - | - | - | - | - | - | - | - | 20 | 17 | 27 |
| ***Candida* spp.** | | | | | | | | | | 22 | 18 | 26 |

CIP = Ciprofloxacin, CN = Cephalexin, GEN = Gentamicin, PB = Polymyxin B, N = Neomycin, C = Chloramphenicol, TOB = Tobramycin, AK = Amikacin, AMC = Amoxycillin Clavulanic acid, FLU = Fluconazole. VCZ = Voriconazole, MIC = Miconazole

*Not all antimicrobials were tested against all microbial isolates.

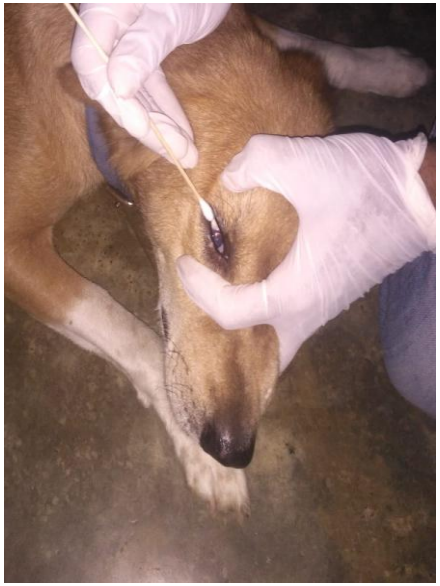

**Fig.1** Collection of sample from nondescriptive breed

**Fig.2** *Aspergillus niger* on SDA plate

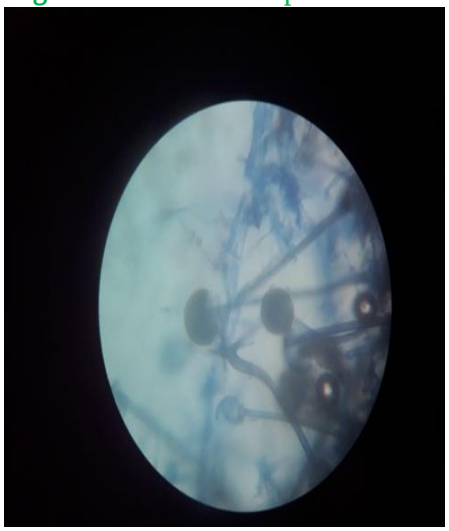

**Fig.3** *Aspergillus* spp. on Lactophenol cotton blue staining

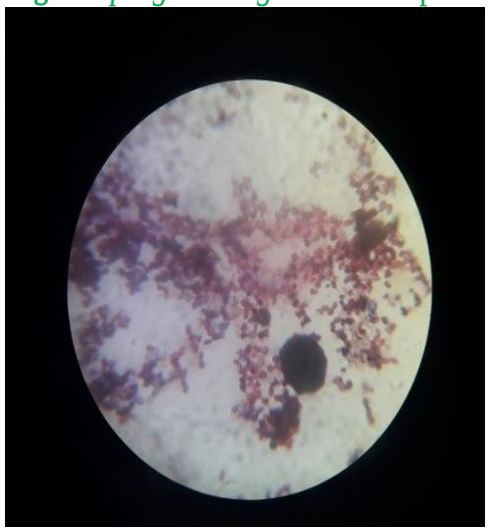

**Fig.4** *Candida* spp. on Gram staining

In case of *Streptococcus* spp. isolates antibiotics like ciprofloxacin, cephalexin, gentamicin, neomycin, chloramphenicol, amoxycillin/clavulanic acid showed inhibitory zone of 19, 19, 11, 11, 18 and 18 mm respectively. In case of *E. Coli* antibiotics like ciprofloxacin, gentamicin, polymyxin B, neomycin, chloramphenicol, tobramycin, amikacin showed inhibitory zone of 20, 13, 10, 14, 16, 13 and 17 mm respectively. Similarly, in case of *Aspergillus* spp.antifungals like fluconazole (10 mcg), voriconazole (1mcg), mionazole (30mcg) showed minimum inhibitory zone of 20, 17 and 27 mm respectively where as in case of *Candida* spp. they showed 22, 18 and 26 mm respectively. Inhinitory zone of different antimicrobials can be found in Table 3.

Corneal infection are usually treated with broad spectrum antibiotics before the culture sensitivity and antimicrobial susceptibility testing. Therefore knowledge regarding the microbial prevalence and its sensitivity profile is important for correct administration of therapy.

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