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# Bacterial Investigation and Antibiogram in Corneal Ulcers in Dogs

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**ABSTRACT**

Corneal ulcers in dogs usually have a traumatic origin. They cause a break in the continuity of underlying corneal stroma and become rapidly contaminated with bacteria. Twenty eight clinical samples were obtained from corneal ulcer affected dogs for bacterial isolation and anti-microbial susceptibility. Bacterial growth was observed in 100% of the samples (n=28) and *Staphylococcus* spp. was isolated and characterized by culture, gram staining and various biochemical tests. Antibiogram pattern revealed that gatifloxacin and moxifloxacin antibiotics were found to be highly effective in the treatment of corneal ulcers in dogs. Corneal culture and sensitivity testing provided useful information for the diagnosis, determination of appropriate treatment and antimicrobial therapy for corneal diseases in dogs.

**Keywords:** Corneal ulcers, microbial culture, *Staphylococcus* spp.

Ulcerative keratitis in dogs is one of the most commonly encountered ocular diseases in veterinary ophthalmology (Ward, 1999; Maggs, 2008). Fortunately, this condition is also among the most treatable of various ophthalmic disorders that can threaten canine vision (Gilger, 2007). Uncomplicated superficial ulcers heal at a faster pace but traumatic origin corneal ulcers may become rapidly contaminated with bacteria (Slatter, 2001). Corneal infection is usually enhanced by disruption of the epithelium, which enables invasion of bacteria (Whitley, 2000; Ollivier, 2003). Conjunctival sac commensal microbiota have a role in maintenance of normal ocular health by preventing overgrowth of potentially pathogenic agents (Andrade *et al*., 2002). However, normal flora microorganisms can become potentially pathogenic when corneal tissue gets damaged, or if the body's infection resistance has decreased (Winn *et al*., 2006). Therefore, treatment with antibiotics must be initiated when corneal ulcers are diagnosed. Murphy *et al.* (1978) reported that coagulase positive *Staphylococcus aureus* to be the most frequently isolated organism (68%) when both eyes were affected. But when only one eye was clinically affected, *Staphylococcus aureus* could be a potential invader of normal eyes aswell. Other organisms recovered from the eyes were coagulase negative *Staphylococcus epidermidis* (27%), beta-hemolytic S*treptococcus* (19%), alpha-hemolytic *Streptococcus* (17%), *Escherichia coli* (10%), *Bacillus* spp. (11%) and *Proteus* spp. (7%).

Miller (2001) has observed that the selection of proper medical therapy may be based on culture and sensitivity results but these can be unreliable, therefore, treatment should not be delayed until the results are obtained. Tolar *et al.* (2006) suggested that the administration of ciprofloxacin or a combination of a first-generation cephalosporin and tobramycin may be used in the treatment of bacterial keratitis while awaiting results of bacterial culture and susceptibility testing. Corneal culture and sensitivity testing provides useful information for the diagnosis, determination of appropriate surgery and antimicrobial therapy in corneal diseases in dogs. Therefore, the objective of the present study was to find

out the potential invading bacteria found in corneal ulcers in dogs and compare the antimicrobial sensitivity test results with the commercially available eye drops for medicinal therapy.

## MATERIALS AND METHODS

Twenty eight samples taken from 28 dogs of different breeds affected with corneal ulcers which were brought to the Teaching Veterinary Clinic Complex, FVSc & AH, R.S. Pura, Jammu were included in the study for microbial evaluation. Sterile swabs moistened with 0.9% normal saline solution (NSS) were gently rolled over the damaged cornea and the swabs were streaked on Blood agar and Mac Conkey agar on the day of presentation. These agar plates were incubated at 37°C for 24 hours for bacterial growth. The cultural characteristics of colonies like size, shape, colour, odour, elevation and edges were studied. The growth was observed and restreaking was done until pure colonies were obtained. Typical colonies were picked and bacteria were identified by standard laboratory procedure (CLSI, 2014). The pure bacterial colonies were stained with Gram's stain and examined under binocular microscope at 100X. The pure isolates were subjected to preliminary biochemical tests which included catalase test and oxidase test and finally the results were compared with standard literature (Quinn *et al.,* 2004).

Each isolate was analyzed for antibiogram pattern with 13 antibiotics namely, Bacitracin (10µg), Chloramphenicol (10µg), Ciprofloxacin (10µg), Gatifloxacin (30µg), Gentamicin (30µg), Moxifloxacin (5µg), Neomycin (30µg), Levofloxacin (5µg), Lincomycin (10µg), Ofloxacin (2µg), Polymyxin B (300µg), Tobramycin (30µg) and Vancomycin (10µg) (HiMedia, Mumbai) by disc diffusion technique (Bauer *et al.* 1966). The confirmed isolates were inoculated in sterile Mueller Hinton broth and incubated at 37°C till light to moderate turbidity (0.5 McFarland) developed. The plate of Mueller Hinton agar was seeded with about 100 µl of inoculum using sterile cotton swab. The inoculated plates were allowed to absorb the contents. Antibiotic discs were placed on inoculated agar surface about 2 cm from one another and then incubated at 37°C for 24-48 hrs. The diameters of zone of inhibition (in millimetres) were measured using HiMedia antibiotic zone scale and interpreted as sensitive, intermediate or resistant as per criterion (CLSI, 2014) (Fig. 2).

## RESULTS AND DISCUSSION

Microbial culture examination, biochemical tests and gram staining results are presented in Table 1. Bacterial growth was recovered from all of the twenty eight samples (100%) taken from corneal ulcers on Blood agar (Fig 1a); however, there was no growth of any sample on Mac Conkey agar. Double zone of beta haemolysis around a growth of central colony characteristic of S*taphylococci* spp. was seen in all the samples.

Catalase and oxidase tests performed for the pure isolates showed prompt effervescence (positive) and no colour change (negative), respectively, in 100% of the tested

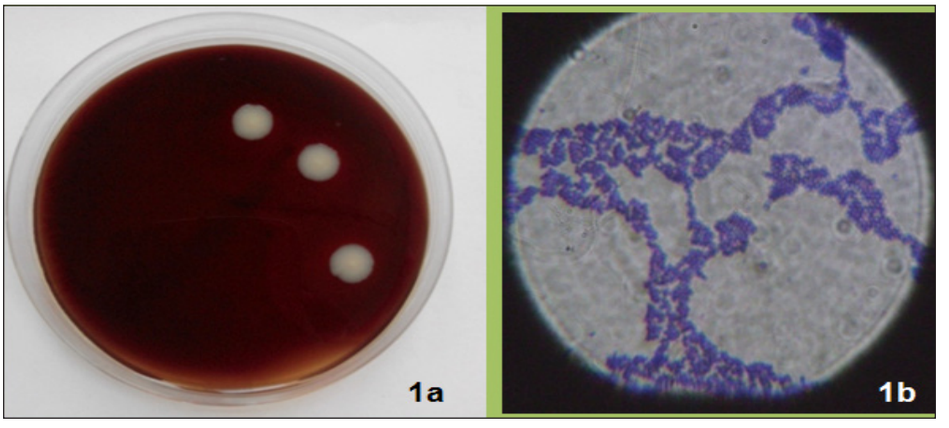


**Fig. 1:** Microbial culture of corneal ulcer swabs revealed growth of colonies on Blood agar (1a) and Gram positive organisms appearing in grape like clusters typical of *Staphylococcus spp.* on gram staining (100X) (1b)

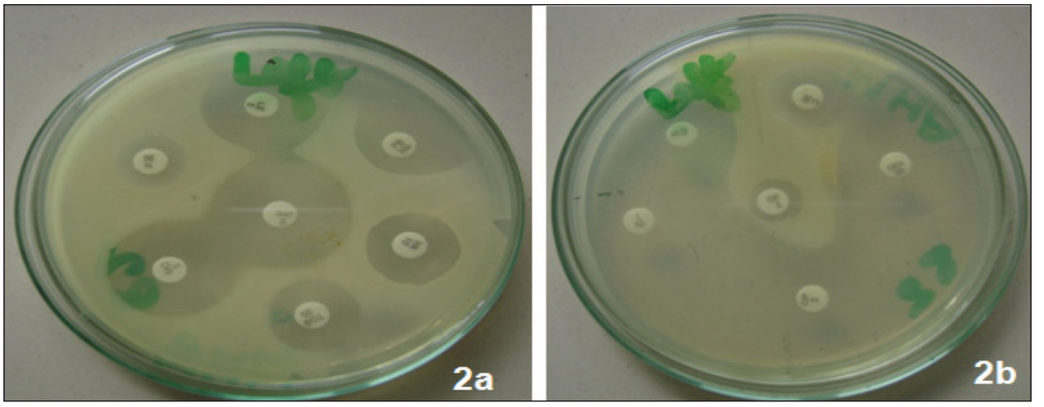


**Fig. 2:** Antibiogram pattern of 13 antibiotics by disc diffusion on Mueller Hinton agar revealed higher sensitivity to Gatifloxacin (30µg), Moxifloxacin (5µg) and near resistance to Neomycin (30µg) antibiotics (2a) whereas Bacitracin (10µg) Ciprofloxacin (5µg), Gentamicin (30µg) and Levofloxacin (5µg) were moderately sensitive

**Table 1:** Bacteriological study performed on pre-treatment day 0

| No. of cases (n) | Growth on media (%) | | | | Biochemical Tests | | | | Gram Staining (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | McConkey Agar | | Blood Agar | | Catalase (%) | | Oxidase (%) | | | |
| | Growth | No Growth | Growth* | No Growth | + | - | + | - | Gram (+ve) | G Gram (-ve) |
| n=28 | - | 100 (n=28) | 100 (n=28) | - | 100 (n=28) | — | - | 100 (n=28) | 100 (n=28) | — |

* Double zone of haemolysis characteristic of *Staphylococcus* spp.

samples. The oxidase test is positive early in almost all pseudomonas infections and can be helpful for rapid identification. The pure bacterial colonies stained with Gram's stain revealed Gram positive organisms in 100% of the samples, appearing round (cocci) in grape like clusters (Fig 1b). *Staphylococcus spp.* was identified with the help of morphology and staining. Similar results have also been obtained by earlier researchers (Gerding Jr *et al.,* 1988; Salisbury *et al.,*1995; Teixeira *et al.,* 2002) in various isolates where gram-positive microorganisms predominated over Gram-negative bacteria and *Staphylococcus* spp. was the most common isolated organism.

Different researchers have observed *Staphylococcus aureus* (Murphy *et al.,* 1978) *Staphylococcus* spp., *Streptococcus* spp. *and Enterococcus* spp. (Moore and Nasisse 1999), *Staphylococcus spp*., Streptococcus *spp.*, *Escherichia coli*, *Corynebacterium* spp. and *Klebsiella* (Ollivier, 2003), *Staphylococcus* spp., *Escherichia coli* and *Bacillus* spp. (Ramani *et al.,* 2013) as the most commonly isolated bacteria in ocular disease. In the present study, *Staphylococci* spp. was the only isolated bacteria, which is a normal commensal of eye aswell and since, these are opportunistic bacteria, they can make ocular infection more severe as they produce a number of enzymes and enterotoxins which enhance the progression of the infection and cause destruction of the cornea.

Interpretation of antibiogram pattern of thirteen antibiotics is represented in Table 2. Gatifloxacin (30µg) and Moxifloxacin (5µg) were used as topical drugs in the present study as the isolates were comparatively more sensitive to them (Fig 2a and 2b). Prado *et al.* (2006) recommended gentamicin, ciprofloxacin, chloramphenicol and tobramycin antibiotics, which had a high efficacy against all of the isolated bacteria which were mostly Gram positive cocci (80.7%) and Gram positive bacilli. Ramani *et al.* (2013) observed that the most effective antibiotics based on antibiotic sensitivity test (AST) were cefotaxime (68%) followed by enrofloxacin (52%), tetracyclin (47%), gentamicin (36%), azithromycin (26%)

**Table 2:** Antibiogram Pattern interpretation performed pre-treatment on day 0

| Antibiotic Group | Bacitracin[10] (%) | | | Chloramphenicol[10] (%) | | | Ciprofloxacin[10] (%) | | | Gatifloxacin[30] (%) | | | Gentamicin[30] (%) | | | Moxifloxacin[5] (%) | | | Neomycin[30] (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R |
| Total (n=28) | 7.14 (n=2) | 28.57 (n=8) | 64.29 (n=18) | 3.57 (n=9) | 28.57 (n=8) | 39.29 (n=11) | 25 (n=7) | 10.71 (n=3) | 64.29 (n=18) | 64.29 (n=18) | 35.71 (n=10) | - | 39.29 (n=11) | 35.71 (n=10) | 25 (n=7) | 17.86 (n=5) | 64.29 (n=18) | 17.86 (n=5) | - | - | 100 (n=28) |

| Antibiotic Group | Levofloxacin[5] (%) | | | Lincomycin[10] (%) | | | Ofloxacin[2] (%) | | | Polymyxin B[300] (%) | | | Tobramycin[30] (%) | | | Vancomycin[10] (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R |
| Total (n=28) | 10.71 (n=3) | 14.29 (n=4) | 75 (n=21) | 7.14 (n=2) | 17.86 (n=5) | 75 (n=21) | 21.43 (n=6) | 21.43 (n=6) | 57.14 (n=16) | 14.29 (n=4) | 32.14 (n=9) | 53.57 (n=15) | 35.71 (n=10) | 42.86 (n=12) | 21.43 (n=6) | 25 (n=7) | 3.57 (n=1) | 71.43 (n=20) |

and amoxicillin (21%). However, Shah *et al.* (2010) observed that moxifloxacin and gatifloxacin both have improved potency and impede growth of organisms resistant to the second and third generation antibiotics. According to Gerding Jr *et al.* (1988), the most effective antibiotics for *Staphylococcus* spp. were bacitracin, gentamicin and tobramycin; while chloramphenicol and erythromycin for *Streptococcus* spp. The antibiotic therapy should be selected based on the culture and susceptibility of the isolated bacteria and the incidence of pathogens at a location (Moore *et al.,* 1988; Gelatt, 2000). In the present study, gatifloxacin and moxifloxacin proved to be highly effective for the treatment of corneal ulcers. Fourth generation fluoroquinolones have been proved to be ideal in combating both gram postitive and gram negative microbes due to their capability of low bacterial resistance (Junejo *et al.,* 2013).

## CONCLUSION

The results of this study concluded that microbial evaluation was useful in identification of organisms from corneal ulcers and for successful medicinal treatment. It is suggested that the drug choices should be based on culture and susceptibility testing. However, treatment should not be delayed until the results are obtained. Antibiotic therapy should be used according to the incidence of pathogens at a particular location and have suggested use of moxifloxacin and gatifloxacin antimicrobials whenever ulcerative keratitis is present.

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