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Bacterial Isolates from Canine External Ocular Disease and their Antimicrobial Sensitivities

David Williams* MA MEd VetMD PhD DECAWBM CertVOphthal CertWEL FHEA FRCVS

Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, UK

Corresponding Author:** ***David Williams, *Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES,UK,* ***Email:*** *dlw33@cam.ac.uk*

Abstract: *Topical antibiotics are widely used in veterinary ophthalmology for cases of conjunctivitis and corneal ulceration, often without prior use of bacterial isolation, culture and sensitivity being performed. Here bacteriological samples are taken from a series of such cases and the bacteria isolated reported together with their antibiotic sensitivities. A significant number of isolates were resistant to fusidic acid, perhaps the most widely used topical antibiotic in UK veterinary practices. This study suggests that bacteriological culture and sensitivity of samples taken from cases of ocular disease would be worthwhile to ensure that optimal treatment is provided.*

Keywords: *Dog, eye, bacteria, antibiotic sensitivity.*

1. INTRODUCTION

External ocular disease involving the conjunctiva and cornea in association with bacterial agents is considered to be a common problem in small animal practice. Conditions include bacterial conjunctivitis, kerato conjunctivitis sicca, dacrocystitis, bacterial keratitis presenting as corneal ulceration, and traumatic lesions. In animals with external ocular disease, treatment is often initiated before results of the microbial culture and sensitivity are known. Therefore knowledge of the most commonly isolated pathogens is advantageous when selecting a topical therapeutic agent, if indeed infectious agents are involved.

Several studies have described the normal canine conjunctival flora and Gram-positive bacteria particularly *Staphylococcus* sp. and *Streptococcus* sp. are the most commonly isolated species (Peterson-Jones, 1997; Gerding et al., 1993; Prado et al., 2005) and these *Staphylococci* spp. are assumed to be the source of the bacteria involved in external ocular disease. Coagulase positive staphylococcal species such as *S. aureus* and the biotype *intermedius* have found to be the most common isolates along with coagulase negatives (e.g. *S. epidermidis)* and beta-haemolytic *Streptococcus* sp. in external ocular disease (Gerding et al 1988, Murphy et al 1978).

With this in mind, fusidic acid gel (Isathal, Dechra, Skipton UK) is licensed for the treatment of ocular surface infections in the dog, cat and rabbit, and in particular canine bacterial conjunctivitis. Fusidic acid inhibits bacterial protein synthesis by preventing release of elongation factor G (ER-G) from the ribosome (Bodley et al 1969). In vitro, fusidic acid is primarily active against various strains of staphylococci (all commonly implicated in infectious conjunctivitis in the dog and rabbit), including *S.aureus*, *S. intermedius* and most coagulase-negative staphylococci (Rietveld et al 2005). There is limited activity against streptococci and most Gram-negative bacteria are resistant. It is used both topically and systemically for the treatment of staphylococcal disease in humans particularly for superficial skin infections such as atopic dermatitis and impetigo, and in the Netherlands, fusidic acid gel is most frequently prescribed for acute infectious conjunctivitis (Rietveld et al, 2005). It is said to have the advantage of requiring only once daily application and it is commonly used empirically in small animal practice for treatment in conjunctivitis and ulcerative keratitis.

Other topical antimicrobials frequently used to treat canine ocular infections include chloramphenicol and gentamicin.

Chloramphenicol (Redidrops™) 1%, which is a broad-spectrum bacteriostatic agent effective against a wide variety of Gram negative and positive organisms, is indicated prophylactically

after entropion and ectropion surgery and penetrating corneal injuries, as well as superficial ocular infection. Gentamicin (Clinagel™ Vet/Tiacil Ophthalmic Solution) is a broad spectrum antimicrobial of the aminoglycoside group, and indicated in Gram-negative ocular surface infections particularly in progressive deep stromal ulcers and infection with *Pseudomonas* spp.

The purpose of this retrospective study was to evaluate microbial cultures isolated from canine ocular swabs with clinical signs of external ocular disease, establishing the range of microbial agents involved (if any) and antibiotic susceptibility, with particular focus on identifying the prevalence of any resistance to fusidic acid in theoretically sensitive staphylococcal species. Resistance to fusidic acid in *S. aureus* and other staphylococci has been well documented (Dobie et al., 2004) and can occur by the horizontal acquisition of the *fusB* (which encodes an ER-G-binding protein that protects the staphylococcal translation apparatus from inhibition by fusidic acid) (O'Neill et al., 2006) or *fusC* determinants (O'Neill et al., 2007), or by spontaneous mutation in the gene encoding EF-G (*fusA*)(O'Neill et al., 2004). Mason et al. (2003) reported a significant association in human medicine between high rates of general practice prescribing of topical fusidic acid and the emergence of fusidic acid resistance among strains of *S. aureus*, and this pattern could be relevant to veterinary medicine.

Results of a smaller study from ear isolates have been included for comparison.

2. METHODS

Between June 2014 and April 2017 the medical records were reviewed from 64 dogs (of a variety of breeds) presented to the Queens Veterinary School Hospital with clinical signs of superficial bacterial ocular infection and/or corneal ulceration in at least one eye. Before sampling, a full ophthalmic examination was carried out, using direct and indirect ophthalmoscopy and slit-lamp biomicroscopy, and Schirmer tear testing was carried out to rule out keratoconjunctivitis sicca. Commercial bacteriological swabs premoistened with sterile saline were uses to sample from the 74 clinically affected eyes. The swabs were submitted for aerobic and anaerobic bacteriological cultures and antimicrobial sensitivity tests. Isolates were identified by their colonial morphology, Gram's staining technique and by commercial biochemical identification methods. Disc diffusion sensitivity tests were carried out using antibiotic sensitivity discs containing the antibiotics of interest (fusidic acid, chloramphenicol, gentamycin and neomycin). Zones of sensitivity were assessed. To estimate Minimum Inhibitory Concentrations, E-tests on the isolates from 18 of the ocular swabs were performed.

Over the same time period, bacteriological swabs taken from 9 ears from 6 dogs were also subject to the same protocol.

3. RESULTS

3.1. Bacterial Cultures

Of the 74 eyes sampled, 30 (40.5%) yielded no growth. Of the 30 negative cultures, 4 (13.3%) were from eyes diagnosed with ulcerative keratitis and 26 (86.6%) had conjunctivitis.

The bacterial species isolated are displayed in Table 1. Positive cultures were obtained in 44 of the 74 affected eyes sampled (59.5%). Of these, 21 eyes yielded a single isolate (47.7%) and 23 yielded mixed infections with two isolates or more (53.3%). In total there were 85 isolates, of which 56.5% were Gram-positive bacteria and 44% were Gram-negative.

Table1. *Frequency of isolation of 85 bacteria from 74 eyes from 64 dogs with external ocular disease*

Organism	No. isolates	% all isolates	% of G+ve/G-ve respectively
G+ve			
Streptococcus gp G	7	8.2	14.6
Staphylococcus coagulase +ve (*S. intermedius/aureus*)	5	5.9	10.4
Staphylococcus coagulase -ve	25	29.4	52.1
Bacillus sp.	4	4.7	8.3
Corynebacterium sp.	3	3.5	6.2
Clostridium sp.	3	3.5	6.2
Micrococcus sp.	1	1.2	2.1
TOTAL	**48**	**56.5**	**100**
G-ve			

Pseudomonas sp.	13	15.3	35.1
Pasteurella sp.	9	10.6	24.3
E. coli	4	4.7	10.8
E.vulneris	1	1.2	2.7
Proteus mirabilis	4	4.7	10.8
Chryseomonas sp.	1	1.2	2.7
Klebsiella sp.	2	2.4	5.4
Pantoea	2	2.4	5.4
No ID available	1	1.2	2.7
TOTAL	**37**	**43.5**	**100**
TOTAL ISOLATES	**85**		

Staphylococcal isolates accounted for 35.3% of the total and 62.5% of all Gram-positive isolates; coagulase-negative species were the predominant *Staphylococci* isolate and were isolated almost 5 times more than coagulase-positive species (*S.intermedius* and *S.aureus*) (83.3% vs 16.7%). Other Gram-positive isolates included *Streptococci*, *Bacillus* and *Corynebacteria*. *Pseudomonas* sp. and *Pasteurella* sp. were the most prevalent Gram-negative isolates, accounting for 15.3% and 10.6% of all isolates respectively.

All eyes were treated as independent entities, as of the 10 dogs with bilateral symptoms and positive cultures, only 2 had identical cultures and sensitivities in both eyes.

3.2. Antibiotic Sensitivities

The results of the antibiotic susceptibility testing are displayed in Table 2.

Table2. *Antibiotic susceptibility of the bacterial isolates from 74 eyes from 64 dogs with external ocular disease. The antibiotics tested were four of the common topical preparations used*

	FUSIDIC ACID		**CHLORAMPHENICOL**		**GENTAMYCIN**		**NEOMYCIN**	
G+ve	% R	% S	% R	% S	% R	% S	% R	% S
Streptococcus gp G (n=7)	100	0	0	100	100	0	100	0
Staphylococcus sp. coagulase +ve (n=5)	40	60	20	80	40	60	80	20
Staphylococcus sp. coagulase –ve (n=25)	68	32	4	96	33	66	63	37
Bacillus sp (n=3)	66	33	33	66	0	100	33	66
Corynebacterium sp. (n=3)	33	66	0	100	0	100	100	0
Clostridium sp. (n=3)	na	na	0	100	na	na	na	na
Micrococcus sp. (n=1)	0	1	0	100	0	100	0	100
TOTAL (47)	**61.4**	**38.6**	**6.4**	**93.6**	**39.5**	**60.5**	**69.8**	**30.2**
G-ve								
Pseudomonas sp. (n=12)	100	0	58	42	58	42	83	17
Pasteurella sp. (n=9)	89	11	0	100	66	33	100	0
Escherichia sp. (n=5)	80	20	0	100	20	80	100	0
Proteus mirabilis (n=4)	100	0	75	25	50	50	100	0
Others (n=7)	100	0	42.9	57.1	42.9	57.4	85.7	14.3
TOTAL (36)	**91.6**	**8.4**	**33.3**	**66.6**	**52.8**	**47.2**	**91.6**	**8.4**
TOTAL ALL (83)	**72.2**	**27.8**	**18.1**	**81.9**	**43.4**	**46.6**	**74.7**	**25.3**

In Vitro Sensitivity of Gram-Positive Isolates

Fusidic acid showed greatest efficacy against the coagulase positive *Staphylococci* but in contrast, poor efficacy against the coagulase negative isolates, of which only 32% were susceptible. All the *Streptococcal* isolates were resistant. In total, more than 61% of all Gram-

positive isolates were resistant. Chloramphenicol in contrast was effective against more than 93% of the Gram-positive isolates. Only 1 out of the 5 *Staphylococcal* coagulase positive isolates and 1 out of the 25 coagulase negative isolates was resistant. All of the other Gram-positive organisms (*Bacillus, Corynebacterium* and *Micrococcus* sp) were susceptible. All *Streptococcal* isolates were resistant to gentamicin. 40% and 33% of the coagulase positive and negative isolates respectively were resistant. All other Gram-positive species were susceptible. Over 69% of the isolates were resistant to neomycin.

In Vitro Sensitivity of Gram-Negative Isolates

Over 91% of isolates were resistant to fusidic acid, with only 1 out of the 9 *Pasteurella* and 1 out of the 4 *Escherichia* spp. isolates susceptible. All *Pseudomonas* isolates were resistant. 66% of isolates were susceptible to chloramphenicol but 58% of *Pseudomonas* isolates were resistant. All *Pasteurella* spp. and *Escherichia* spp. were susceptible. 47% of isolates were susceptible to gentamicin. Again, 58% of *Pseudomonas* isolates were resistant, as were 66% of *Pasteurella* isolates. Only 3 out of the 36 isolates were susceptible to neomycin.

E-Strip results are displayed in table 5.

Table5. *Distribution of MICs (mg/L) from E-test results from the isolates of 18 eyes*

	Fusidic Acid		Chloramphenicol		Gentamicin	
Staphylococcus sp (p=coagulase-positive; n=coagulase negative)	0.25 (n) 0.75 (n) 1 (n) 1 (n) 1 (p) 1.5 (p) 2 (p) 3 (n) 4 (n) 24 (n) 24 (n) 32 (n)	S S S S S S S S R R R R	0.5 (n) 2 (n) 2 (n) 2 (n) 3 (n) 3 (n) 3 (n) 3 (n) 4 (p) 6 (n) 6 (n) 6 (p)	S S S S S S S S S S S R	0.047(n) 0.19(n) 0.25(n) 0.38(n) 0.5(p) 0.5(n) 0.5(n) 0.75(n) 0.75(n) 1(n) 1(p) 1.5(n)	S S S S S S S S S S S S
Streptococcus sp.	3 6 8 8 8	R R R R R	1.5 2 2 3 3	S S S S S	8 8 12 23 24	R R R R R
Pseudomonas sp.	>256 >256 >256	R R R	48 >256 >256	R R R	0.047 6 8	S R R
Pasteurella sp.	3 24 128	R R R	0.75 1 1.5	S S S	0.75 3 12	S S R
E. coli	>256 >256 >256	R R R	1.5 4 6 12	S S S S	1.5 1.5 2	S R R

Staphylococci isolates were resistant to fusidic acid at MICs of ≥ 4mg/L, with a range of between 0.25 – 32mg/L. *Streptococci* spp. showed a range from 3-8mg/L, and all were resistant. MICs for *E. coli* to fusidic acid were all greater than 256mg/L and the isolates were resistant.

Comment on Comparison with Ear swabs (Table 3 and 4)

Table3. *Frequency of isolation of 19 bacteria from 9 ear swabs from 6 dogs*

Organism	No. isolates	%of all isolates	% G+ve/G-ve respectively
G+ve			
Streptococcus gp G	2	10.5	15.4
Staphylococcus coagulase +ve	4	21.1	30.8
Staphylococcus coagulase -ve	5	26.3	38.5
Corynebacterium sp.	2	10.5	15.4
TOTAL	**13**	**68.4**	**100**

G-ve			
Pseudomonas sp	3	15.8	50.0
E.coli	2	10.5	33.3
Proteus mirabilis	1	5.3	16.7
TOTAL	**6**	**31.6**	**100**
TOTAL ISOLATES	**19**		

Table4. *Antibiotic susceptibility of the 19 bacterial isolates from 9 ears from 6 dogs*

ORGANISM	**R**	**S**	**R**	**S**	**R**	**S**
	FUSIDIC ACID		**GENTAMYCIN**		**NEOMYCIN**	
G+ve	(%)		(%)		(%)	
Streptococcus gp G (n=2)	1 (50)	1 (50)	2 (100)	0 (0)	2 (100)	0 (0)
Staphylococcus coag +ve (n=4)	2 (50)	2 (50)	1 (25)	3 (75)	2 (50)	2 (50)
Staphylococcus coag – ve (n=5)	3 (60)	2 (40)	2 (40)	3 (60)	1 (20)	4 (80)
Corynebacterium sp. (n=2)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)
TOTAL (13)	6 (46)	7 (54)	5 (38)	8 (62)	5 (38)	8 (62)
G-ve						
Pseudomonas sp (n=3)	3 (100)	0 (0)	1(33.3)	2(66.6)	3 (100)	0 (0)
E. coli (n=2)	2 (100)	0 (0)	0 (0)	2 (100)	2 (100)	0 (0)
Proteus mirabilis (n=1)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)
TOTAL (6)	6 (100)	0 (0)	1(16.6)	5(83.3)	5(83.3)	1(16.6)
TOTAL (19)	**12(63.2)**	**7(36.8)**	**6(31.6)**	**13(68.4)**	**10(52.6)**	**9(47.4)**

2 out of the 4 coagulase-positive *Staphylococcus* spp. and 3 out of the 5 coagulase negative isolates were resistant to fusidic acid. Almost half (6 out of 13) of the total Gram-positive and all Gram-negative isolates were resistant.

4. DISCUSSION

4.1. Bacterial Cultures

Over 40% of swabs from eyes with clinical signs yielded negative cultures and this is of note since a diagnoses of acute conjunctivitis therefore should not always warrant topical antibiotic treatment. Conjunctivitis, characterised by clinical signs such as irritation, hyperaemia and a mucopurulent ocular discharge are common presentations in small animal practice but there are numerous aetiologies. Non-infectious causes commonly include irritants (e.g. chemicals/dust), allergies and less commonly auto-immune disease such as pemphigus. Mechanical irritation from a foreign body, lid abnormalities such as entropion and ectropion, and lash or hair abnormalties such as ectopic cilia, distichiasis or trichiasis can also be a cause of the above signs. Infectious causes include viruses (such as distemper) and bacteria and often, bacterial infections are secondary to conformational abnormalities mentioned previously. Other underlying dysfunctions predisposing to ocular surface infections include keratoconjunctivitis sicca (Barnett KC et al 1987). The reported prevalence of sterile cultures here highlights the importance of identifying any underlying causes (e.g. Schirmer tear test and examining the fornix and under the third eyelid for foreign bodies such as grass awns) and not making a diagnoses of infectious conjunctivitis on the basis of signs and symptoms. However, even in human medicine this is often the case with GPs not able to differentiate between a bacterial and non-bacterial cause (Rietveld et al 2005) and more importantly their randomised controlled trial concluded that the current widespread prescription practices of fusidic acid by GPs is unjustified – cure rates in two groups of patients presenting with ‘red eye’ in the fusidic acid gel and placebo group were similar at 7 days. Furthermore, although resistance to fusidic acid was recognized as a potential problem soon after its release, clinically significant rates of resistance have been associated with the widespread and often inappropriate use of topical fusidic acid (monotherapy) ointment/cream for chronic skin conditions, and this is likely to be the case with regards to ocular infections.

Previous investigations of the bacterial types associated with ocular surface disease in dogs have shown that Gram-positive isolates predominate and Gram-negatives such as *Pseudomonas* sp and coliforms are isolated at a

lower frequency (Murphy et al 1978; Gerding et al 1988,); in contrast here we see that almost half of the isolates (44%) are Gram-negative. The empirical use of only one topical therapeutic such as fusidic acid therefore would be of no benefit in such infections.

Consistent with previous studies, *Staphylococcus* spp were the most frequently isolated organisms (35.3% of the total and 62.5% of all Gram-positive isolates) from dogs with clinical signs of external ocular disease (Murphy et al 1978; Gerding et al 1988; Lin and Petersen Jones 2007). However, contrary to these studies, coagulase-negative species were the predominant *Staphylococci* isolate here and were isolated almost 5 times more than coagulase-positive species (*S.intermedius* and *S.aureus*) (83.3% vs 16.7%). In contrast, Murphy et al 1978 found *S.aureus* to be the most frequently isolated organism and Lin et al 2007 and Gerding et al 1988 found *S.intermedius* to be the most common. This is of note because fusidic acid is particularly active against coagulase-positive *Staphylococcus* sp however if the infectious aetiology is coagulase-negative organisms in many infections, fusidic acid treatment may not be as beneficial.

Consistent with other studies (Murphy et al 1978; Gerding et al 1988; Prado et al 2005, Lin et al 2007) *Streptococcus* and *Corynebacterium* were also frequently isolated Gram-positive isolates, and *Pseudomonas* and *Escherichia* spp were frequently isolated Gram-negative species. In addition, here we also see that *Pasteurella* comprises 10.6% of all isolates and 24.3% of all Gram-negative isolates.

4.2. Antibiotic Susceptibility Test

We have found resistance to some of the commonly used ophthalmic preparations.

All 7 streptococcal isolates were resistant to fucidic acid and gentamicin but susceptible to chloramphenicol. This is reasonably consistent with a study by Lin et al (2007) where 67% of the 19 isolates were resistant to gentamicin (to be expected given it is mainly effective versus Gram-negatives) and only 10% were resistant to chloramphenicol. Gerding et al 1988 also found 10% were resistant to chloramphenicol. This highlights that the activity of fusidic acid cannot include all Gram positive bacteria and *Streptococci* sp clearly are not susceptible. Considering here these formed almost one tenth (8.2%) of isolates and in other studies over 25% of all ocular isolates in dogs with external eye disease (eg Gerding et al 1988) the use of fusidic acid treatment empirically maybe ineffective and a better choice would be chloramphenicol.

Though all coagulase-positive staphylococci were susceptible to fusidic acid, consistent with previous in-vitro studies demonstrating activity versus staphylococci only (eg Morrissey et al 2004), particularly the coagulase-positive *S. aureus* and *S. intermedius*, 68% of the 25 coagulase-negative species were resistant. As discussed previously, resistance of staphylococci to fusidic acid and the mechanisms involved have been well documented. Despite susceptibility of the coagulase-positive isolates in this study, the coagulase negative species show a high incidence of resistance. Fusidic acid resistance specifically in coagulase-negative staphylococci has been reported in bovine coagulase negative isolates (Yazdankhah, SP et al, 2006) and more recently in *S. epidermidis* (McLaws et al 2008). Given the fact that coagulase-negative organisms accounted for 29.4% of all isolates, the use of fusidic in initial treatment of external ocular disease is therefore unjustified and chloramphenicol would be a more suitable first-line treatment given 80% and 96% of all coagulase-positive and coagulase-negative were susceptible respectively. This is consistent with Lin et al (2007), reporting that the majority of coagulase-positive isolates were susceptible. Also in comparison to this study, we also find a lower rate of resistance of *Staphylococci* spp to gentamicin; 60% and 66% of coagulase positive and negative isolates respectively were susceptible. Gentamicin is reported to be efficacious against *Staphylococci* as well as Gram-positive organisms (Wagner 1986).

Pseudomonas spp (particularly *P. aeruginosa*) infection of corneal ulcers can be serious because of the release of enzymes that result in corneal stromal liquefaction and potentially rapid progression of the ulcer. All of the 12 *Pseudomonas* isolates were resistant to fusidic acid, and 7 were resistant to gentamicin. This differs from Tolar et al (2006) who found that 25 out of 25 *P.aeruginosa* isolates from canine corneal ulcers were sensitive to gentamicin however there are concerns that there is an increase in the resistance to the aminoglycosides by *P.aeruginosa* isolated from infected human eyes (Gelender et al 1984). This has important implications in the treatment of progressive corneal ulcers where gentamicin is indicated, and highlights the importance of therapeutic

selection based on culture and sensitivity results. When these are pending, a fluoroquinolone such as ciprofloxacin might be a better choice as resistance to *Pseudomonas* is reported to have a low incidence (Lin et al 2007). With regards to chloramphenicol, 7 out of the 12 isolates were resistant. Morrissey et al (2004) found it inactive against *P.aeruginosa* in human ocular isolates, as did Gerding et al (1988) and Lin et al (2007) in canine isolates.

Of the 5 *Escherichia* spp isolates, 4 were resistant to fusidic acid, all were susceptible to chloramphenicol and 4 were susceptible to gentamicin. Lin et al 2007 reports 80% of isolates were resistant to gentamicin however the study was conducted in Taiwan where gentamicin is one of the first choices of antibiotics used by practicioners so they acknowledge this could account for the higher rates of resistance. Moreover, the small number of isolates here may not give an accurate representation.

The MIC of an antimicrobial agent is a value that has been used to determine breakpoints that predict the probability of clinical success, detect resistant populations, or both (Mouton, 2002). Clinical breakpoints are dependent on the antimicrobial activity and pharmacology of the drug and are used to predict a cure, achieving clinical success with the antimicrobial agent. In contrast, microbiologic breakpoints, as used in this study, are established to identify isolates that may be categorized as susceptible when applying clinical breakpoints but harbour resistance mechanisms that result in their reduced susceptibility to the agent being tested. These microbiologic breakpoints are therefore useful in monitoring the emergence of resistance. However in this study it would have been useful to establish the clinical outcome after treatment with fusidic acid, taking bacteriology samples at 0 and 7 days and establish if any staphylococcal infection is eliminated. Results could then be compared with microbiologic resistance and breakpoints to see if the two correlated. However, also to be taken into account with this methodology is natural processes involved in infection elimination, regardless of treatment. Further work also needs to establish concentrations of the fusidic acid that remain on the ocular surface after topical treatment and make comparisons between microbiological MICs.

Agar dilution, disc diffusion and E-test MIC methods have shown to give comparable results to determine in-vitro susceptibility of fusidic acid (Skov et al 1999). Although there are no Clinical and Laboratory Standards Institute (CLSI)-defined breakpoints for fusidic acid, susceptibility is generally defined as an MIC of ≤ 0.25 or ≤ 0.5mg/L and resistance as an MIC of $\geq$ 2mg/L (Collingnon et al 1999; Turnidge 1999). However here we see that an MIC of $\geq$ 4mg/L is consistent with resistance as documented by the disc diffusion technique.

5. CONCLUSION

Negative cultures were the result of over 40% of the swabs, and we have shown similar range of organisms isolated from the canine eye in superficial ocular disease as those reported in other geographical locations, with the exception of the novel predominance of coagulase-negative staphylococcal isolates as oppose to coagulase-positive species. In addition, a great proportion of the former have demonstrated resistance to fusidic acid, and in total over 60% and 90% of Gram-negative and Gram-positive isolates respectively were resistant. Furthermore almost half of isolates were Gram-negative. This has important implications in therapeutic decisions considering the wide-scale empirical treatment with fusidic acid (indicated for *Staphylococci* spp ocular infection) for dogs presenting with conjunctivitis and superficial corneal ulcers - if indeed any infectious agents are involved; if there are that an infection is not necessarily most likely to be Gram-positive bacteria; and if they are that they would in fact be susceptible to fusidic acid. In addition, long-term application of antibiotics in the absence of actual infection may lead to antibiotic resistance or overgrowth of organisms outside the spectrum of activity of the drug. Therefore, the choice of antimicrobial therapy before obtaining microbial susceptibility results can be based on clinical signs, gram stains, and a history of previous antimicrobial treatment and response to therapy and knowledge of antibiotic susceptibility is important to provide the most efficacious antimicrobial treatment. With these results in mind, chloramphenicol appears a suitable first choice – prevalence of resistance to both Gram-positive and negative isolates was the lowest out of all the antimicrobials. In progressing corneal ulcers and infection with *Pseudomonas* spp. ciprofloxacin is likely to be an effective choice. Gentamicin and fusidic acid have shown to be least active, mainly due to inherent weaknesses in spectrum of activity and emerging fusidic acid resistance, and as it has been proposed in human medicine (for

example by Howden et al. (1996)), restriction on the use of topical fusidic acid monotherapy should be considered given the documented association of the emergence of staphylococcal resistance to this antimicrobial.

REFERENCES

[1] Barnett, K.C., Sansom, J. 1987. Diagnosis and treatment of keratoconjunctivitis sicca in the dog. Vet Rec. 120(14):340-345

[2] Bodley, J.W., Zieve, F.J., Lin, L., Zieve, S.T. 1969 Formation of the ribosome-G factor-GDP complex in the presence of fusidic acid. Biochem Biophys Res Commun. 37(3):437-43

[3] Collignon, P., Turnidge, J. 1999. Fusidic acid in vitro activity. Int J Antimicrob Agents. 12 Suppl 2:S45-58

[4] Dobie, D., Gray, J. 2004. Fusidic acid resistance in Staphylococcus aureus. Arch Dis Child. 89(1):74-7

[5] Gelender, H., Rettich, C. 1984. Gentamicin-resistant Pseudomonas aeruginosa corneal ulcers. Cornea. 3(1):21-6

[6] Lin, C.T., Petersen-Jones, S.M. 2007. Antibiotic susceptibility of bacterial isolates from corneal ulcers of dogs in Taiwan. J Small Anim Pract. 48(5):271-4

[7] Mason, B.W., Howard, A.J., Magee, J.T. 2003. Fusidic acid resistance in community isolates of methicillin-susceptible Staphylococcus aureus and fusidic acid prescribing. J Antimicrob Chemother. 51(4):1033-6

[8] McLaws, F., Chopra, I., O'Neill, A.J. 2008. High prevalence of resistance to fusidic acid in clinical isolates of Staphylococcus epidermidis. J Antimicrob Chemother. 61(5):1040-

[9] Morrissey, I., Burnett, R., Viljoen, L., Robbins, M. 2004. Surveillance of the susceptibility of ocular bacterial pathogens to the fluoroquinolone gatifloxacin and other antimicrobials in Europe during 2001/2002. J Infect. 49(2):109-14

[10] Murphy, J.M., Lavach, J.D., Severin, G.A. 1978. Survey of conjunctival flora in dogs with clinical signs of external eye disease. J Am Vet Med Assoc. 172(1):66-8

[11] O'Neill, A.J., Larsen, A.R., Henriksen, A.S., Chopra, I. 2004. A fusidic acid-resistant epidemic strain of Staphylococcus aureus carries the fusB determinant, whereas fusA mutations are prevalent in other resistant isolates. Antimicrob Agents Chemother. 48(9):3594-7

[12] O'Neill, A.J., McLaws, F., Kahlmeter, G., Henriksen, A.S., Chopra, I. 2007. Genetic basis of resistance to fusidic acid in staphylococci. Antimicrob Agents Chemother. 51(5):1737-40

[13] Peterson-Jones, S, 1997. Quantification of conjunctival sac bacteria in normal dogs and those suffering from keratoconjunctivitis sicca. Vet Comp Ophthalmol 7:29–35

[14] Prado, M.R., Rocha, M.F. Brito, E.H., Girão, M.D., Monteiro, A.J., Teixeira, M.F., Sidrim, J.J. 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(1):33-

[15] Rietveld, R.P., Rieter, G., Bindels, P.J., Bink, D., Sloos, J.H., van Weert, H.C. 2005. The treatment of acute infectious conjunctivitis with fusidic acid: a randomised controlled trial. Br J Gen Pract. 55(521):924-30

[16] Tolar, E.L., Hendrix, D.V., Rohrbach, B.W., Plummer, C.E., Brooks, D.E., Gelatt, K.N. 2006. Evaluation of clinical characteristics and bacterial isolates in dogs with bacterial keratitis: 97 cases (1993-2003). J Am Vet Med Assoc. 228(1):80-5

[17] Turnidge, J., Collignon, P. 1999. Resistance to fusidic acid. Int J Antimicrob Agents. 12 Suppl 2:S35-44

[18] Wagner, D.S., Salstrom, S.J., File, T.M., Tan, J.S. 1986. Canine model for the simultaneous measurement of antibiotic levels in tissues and bacterial killing rate. Antimicrob Agents Chemother. 29(6):965-8

[19] Yazdankhah, S.P., Asli, A.W., Sørum, H., Oppegaard, H., Sunde, M. 2006. Fusidic acid resistance, mediated by fusB, in bovine coagulase-negative staphylococci. J Antimicrob Chemother. 58(6):1254-6

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