BACTERIAL ISOLATION AND ITS CLINICAL CORRELATION IN CHRONIC OTITIS EXTERNA IN DOGS*

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Abstract

The ear discharges from 18 dogs with chronic otitis externa was evaluated. The colour ranged from yellow to black and consistency from watery to waxy. The most common bacterial isolate obtained was Staphylococcus spp. Most of the bacteria were susceptible to fluoroquinolones. The nature of discharge and bacterial isolate were correlated.

Keywords: *canine chronic otitis externa, otic discharge, resistance, sensitivity*

Chronic otitis externa is a persistent and frustrating problem faced in canine practice. The very common perpetuating factor for otitis is bacteria and it has been fruitlessly addressed during the course of treatment due to the inappropriate use of antimicrobials and the development of resistance among the bacteria. In such situations, culture and sensitivity of otic discharge will aid in selection of the antibiotics to be given systemically and will help to avoid the use of ineffective antibacterials.

Materials and Methods

Dogs of different age, breed and sex suffering from chronic otitis externa presented to the University Veterinary Hospital, Kokkalai and Mannuthy, Kerala Veterinary and Animal Sciences University were selected for the study. The otic discharge from these dogs were collected using sterile swab after clipping the hair around the external acoustic meatus and cleaning the ear pinna with spirit. The colour and consistency of the discharge was noted. The samples were subjected to direct microscopic examination after staining with Leishman's stain and samples from eighteen dogs with a yeast count of less than five yeast cells per field and more than 25 bacteria per field were selected for microbiological study.

The swabs were incubated in peptone water at 37°C for four hours and then cultured by streak method in either nutrient agar or Brain Heart infusion agar plates and incubated at 37° C over night. Individual colonies were selected as a representative sample and streaked on nutrient agar to get pure culture. By observing the colony characteristics, morphology and responses of isolated bacteria to Gram's staining and biochemical tests like oxidase test, coagulase test, catalase test and urease test, the bacteria were identified upto the generic level as per Barrow and Feltham (1993).

The isolated pure colonies were subjected to antibiotic sensitivity test using the Disc Diffusion Technique as per Bauer *et*

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al. (1996). The antibiotic discs used included cephalexin, enrofloxacin, ciprofloxacin, levofloxacin, gentamicin, amoxicillin-clavulanic acid, penicillin G, tetracycline and streptomycin. The test results were recorded based on the zone of inhibition created.

Results and Discussion

The colour of otic discharge was yellowish brown in two dogs (11.11 %), yellow in seven (38.88 %), brown in five (27.77 %), yellowish green in two (11.11 %) and black in two dogs (11.11 %). In addition to this, blood-tinge was seen in four dogs (22.22 %)(Table 1).This was found similar to the findings of Rycroft and Saben (1977) who also obtained a higher occurrence of yellowish exudates (53 %) followed by black/brown exudates (36.5 %).

The consistency of the discharge was creamy in nine dogs (50 %), watery in five dogs (27.77 %) and waxy in four dogs (22.22 %) (Table 1).

Direct microscopic examination of cerumen of all the selected samples under the study revealed the presence of more than 25 bacteria per field and also presence of red blood corpuscles. Angus (2004) stated that otic cytology in dogs could differentiate overgrowth of *Malassezia* or bacteria from normal colonization or infection in canine *otitis externa*. It was also recommended that, more than 25 bacteria per field and more than five yeasts per field warranted therapeutic intervention.

Of the 19 microorganisms cultured from the otic discharge of 18 dogs, 63.16 % were Gram negative and 36.84 % were Gram positive. Harvey *et al*. (2001) stated that Gram negative bacteria would be predominantin chronic otitis externa in dogs.

The bacteria observed were *Staphylococcus* spp. (7/19), *Proteus* spp. (6/19), *Escherichia coli* (3/19) and *Pseudomonas* spp. (3/19). In one sample two microorganisms could be isolated *viz., Staphylococcus* spp. and *Escherichia coli.* (Table 1). Cole *et al.* (2009) stated that organisms isolated from canine chronic otitis externa mainly included *Pseudomonas* spp*., Staphylococcus* spp*., Proteus* spp*., Streptococcus* spp*., Corynebacterium* spp., *Escherichia coli* and *Enterococcus* spp*.*

The colour and consistency of otic discharge were correlated with the infecting organisms. The yellow coloured to yellowish brown or blood tinged discharge was found associated with *Staphylococcus* spp*.* (5/7) and *Escherichia coli* (2/3), the colour brown to black was found associated with *Proteus* spp. (5/6) and the colour yellowish green was found associated with *Pseudomonas* spp. (2/3). Rycroft and Saben (1977) obtained the results in a similar study as yellow or light brown was related to *Staphylococcus* spp., pale or light yellow discharge was associated with *Pseudomonas* spp. and *Proteus* spp. Chickering (1988) also stated that dark yellow to light brown otic discharge was seen in Gram positive cocci, pale yellow was associated with *Pseudomonas* spp., *Proteus* spp. and *Pasturella* spp. and dark brown with *Malassezia* spp. This study was in accordance with Degi *et al.* (2010), who stated that *Pseudomonas* spp. was associated with greenish yellow otic discharge in dogs. Creamy exudate was found associated with *Staphylococcus* spp. (4/7), *Escherichia coli* (2/3) and *Pseudomonas* spp. (3/3) and waxy was found to be associated with *Proteus* spp. (3/6). Chickering (1988) also stated that creamy discharge in dogs was associated with Gram positive cocci and thick discharge with *Pseudomonas* spp. and *Proteus* spp*.*

Out of the seven *Staphylococcus* spp. isolates obtained, all were found sensitive to enrofloxacin and levofloxacin, five isolates to ciprofloxacin and cephalexin and one to gentamicin. All the three isolates of *Escherichia coli* were sensitive to levofloxacin, two were sensitive to enrofloxacin and one each to ciprofloxacin and gentamicin. Out of six isolates of *Proteus* spp. all were sensitive to levofloxacin, four each to ciprofloxacin and gentamicin and two to enrofloxacin. Three isolates of *Pseudomonas* spp. were found sensitive to enrofloxacin and levofloxacin and of which two were found sensitive to ciprofloxacin.

In general, out of the 19 isolates obtained, all were susceptible to levofloxacin (100 %), 18 to enrofloxacin (94.7 %), 14 to ciprofloxacin (73.68 %), seven to cephalexin (36.84 %) and six to gentamicin (33.33 %) (Table 2). Pederson *et al*. (2007) who cultured the otic discharge from dogs observed that *Staphylococcus* spp. were generally susceptible

to antibiotics like enrofloxacin, cephalothin, *Proteus* spp. isolates were susceptible to gentamicin and ciprofloxacin and *Escherichia coli* were susceptible to fluoroquinolones and gentamicin. Degi *et al.* (2010) observed that among *Pseudomonas* spp. strains isolated from the dogs with chronic otitis externa 81.25 % was most sensitive to ciprofloxacin, 62.5% to enrofloxacin and 43.75% to gentamicin, lincomycin and spectinomycin. Barrasa *et al.* (2000) however mentioned that the most effective antibiotics against *Pseudomonas* spp. isolated from such dogs affected with chronic otitis externa were tobramycin (100%), marbofloxacin (91.3%), ceftazidime (91.3%), ticarcillin (86%) and gentamicin (65.2%) with less susceptibility to enrofloxacin (52.1%), probably due to its indiscriminate use.

All the bacteria isolated showed resistance towards penicillin G, streptomycin, amoxicillin-clavulanic acid and tetracycline. Out of the seven isolates of *Staphylococcus* spp., two were resistant to ciprofloxacin, two to cephalexin and six to gentamicin. Among the three isolates of *Escherichia coli*, all were found resistant to cephalexin, two each to gentamicin and ciprofloxacin and one to enrofloxacin. Of the *Proteus* spp. isolated, all were resistant to cephalexin, two each to ciprofloxacin and gentamicin, four to enrofloxacin. All the three isolates of *Pseudomonas* spp. obtained were resistant to gentamicin and cephalexin (Table 2). This finding was similar to the observations made by Penderson *et al.*(2007), who observed that *Escherichia coli* isolates were resistant to streptomycin, penicillin and tetracycline; *Proteus* spp. isolates to tetracycline and *Staphylococcus* spp. isolates to penicillins, macrolides and lincosamines in dogs. Degi *et al.* (2010) observed that in dogs out of 17 *Pseudomonas* spp. strains

Table 1. Observations on the colour, consistency and bacterial isolate/s obtained

Animal No	Colour of otic discharge	Consistency of cerumen	Isolate of the bacteria
1	Yellowish brown	Creamy	*Staphylococcus*spp.
2	Yellowish	Creamy	*Escherichia coli*
3	Yellowish brown	Creamy	*Staphylococcus* spp.
4	Brown	Waxy	*Staphylococcus* spp.
5	Reddish, blood tinged	Watery	*Staphylococcus* spp.
6	Yellow	Creamy	*Pseudomonas* spp.
7	Brown	Watery	*Proteus* spp.
8	Brown blood tinged	Waxy	*Proteus* spp.
9	Yellow	Creamy	*Staphylococcus* spp. and *Escherichia coli*
10	Brownish-blood tinged	Waxy	*Proteus* spp.
11	Yellow	Creamy	*Staphylococcus* spp.
12	Yellowish green	Watery	*Pseudomonas* spp.
13	Yellow	Creamy	*Proteus* spp.
14	Yellowish green	Creamy	*Pseudomonas* spp.

Table 2. Sensitivity and Resistance of Bacteria

Isolate	(AMC^{30}) %		(P^{10}) %		(S^{10}) %		(TE^{30}) %		(CN^{30}) %		(GEN^{30}) %		(LE^{5}) %		(CIP^{10}) %		(EX^{10}) %	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Proteus spp. (6)	-	100	-	100	-	100	-	100	-	100	66.7	33.3	100	-	66.7	33.3	33.3	66.7
Staphylococcus spp.(7)	-	100	-	100	-	100	-	100	71.4	28.6	14.3	85.7	100	-	71.4	28.6	100	-
Escherichia coli (3)	-	100	-	100	-	100	-	100	-	100	33.3	66.7	100	-	33.3	66.7	66.7	33.3
Pseudomonas spp. (3)	-	100	-	100	-	100	-	100	-	100	-	100	100	-	66.7	33.3	100	-

S – Sensitive R – Resistant
AMC- Amoxycillin - clavulanic acid, P-Penicillin G, S- Streptomycin, TE- Tetracycline, CN- Cephalexin, GEN- Gentamicin, LE- Levofloxacin, CIP- Ciprofloxacin, EX- Enrofloxacin

64.7 % were resistant to amoxicillin clavulanic acid and cephalexin, 8.5% were resistant to gentamicin, 4.11% were resistant to tetracycline, 2.35 % were resistant to enrofloxacin and 0.5% were resistant to ciprofloxacin.

In general all the 19 isolates obtained were resistant to penicillin G, streptomycin, amoxicillin - clavulanic acid and tetracycline. Six isolates were resistant to ciprofloxacin, eight to cephalexin, eleven to gentamicin and five to enrofloxacin.

The correct antibacterial has to be chosen by culture and sensitivity tests of otic discharge for an effective treatment of chronic canine otitis externa. A correlation between the nature of otic discharge and the presence of bacterial isolates provides the practitioner a clue for selection of antibacterial drugs.

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