MICROBIOLOGICAL INVESTIGATION OF CANINE OTITIS EXTERNA

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Otitis externa (OE) in dogs is the most frequent disease of ear canal with multi factorial etiology. Many dogs with OE remain refractory to treatment and often the condition is recurrent. Multiple etiological agents together with emerging antibiotic resistance are the primary obstacles for adopting a successful treatment regimen. In the present study, twenty eight out of thirty one (90.3%) dogs with otitis externa were found to have microbial etiology. The microbial agents isolated were *Staphylococcusspp*., *Escherichiaspp*, *Pseudomonasspp*., *Streptococcusspp*., *Malasseziaspp*., *Candidaspp*., *Aspergillusspp*., and *Rhizopusspp*. Antimicrobial sensitivity profile of the isolates revealed high resistance patterns against multiple antibiotics. The study emphasizes the importance of antibiotic susceptibility testing before imposing therapeutic regimen.

Keywords: Canine; Otitis externa; Bacteria; Fungi; Antimicrobial Resistance.

Otitis externa (OE) is one of the most common disorder of ear canal in dogs. Canine OE accounts to 10 per cent of the total casesin small animal veterinary practice (Angus, 2004; Cole, 2004; Senthil *et al*., 2010). Although, canine OE is multifactorial, microbial etiology has an important role in the genesis of the infection. Inflammation of the external auditory canal from the pinna to the tympanic membrane is referred to as OE. The affected dogs exhibit clinical signs such as erythema of ear pinna, excoriation, malodorous discharges and pruritis as evidenced by head shaking and pain. Hair in the ear canal, pendulous ears, increased humidity, moisture retention, injuries, neoplasms, stenosis of the ear canal, prolonged antibiotic treatment acts as predisposing factors that directly change the micro climate of the ear canal (Rosser, 2004; Paterson, 2016). Trauma owing to exuberant ear cleaning or from the instruments may also allow bacterial colonization. These conditions favour rapid multiplication of opportunistic bacteria and yeasts resulting in severe otitis. Bacteria such as Staphylococcus *spp*, Streptococcus *spp*, P.mirabilis, E.coli and yeasts such as Malassezia*spp* are the normal microbial flora in the ear canal (Petrov *et al.*, 2013). However, the predisposing factors favour rapid multiplication of bacteria and expression of their virulence factors (Scott *et al*., 2001).Chronic cases or repeated infections may result in chronic pain, deafness and risk of development of antimicrobial resistance. Though the ailment is not life threatening, pets and the pet owners may face a perplex situation due to its chronic and recurrent nature. Hence, the present investigation is taken up with an objective to evaluate the microbial profile in clinical cases of canine otitis externaand to determine the antimicrobial susceptibility of the isolated organisms.

Materials and Methods

Dogs presented to Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram with clinical as unilateral or bilateral drooping of ears, head shaking, pruritis, pain during palpation, erythema, swelling of ear skin or of ear canal with increased amount of cerumen were included in the study. A total of 31 clinical cases of canine otitis externa were identified that included dogs from both the genders and of different breeds from 7 months to 15 years of age. Exudates from the ear canals were collected using sterile swabs. The samples were used for bacterial and fungal cultures as well as antimicrobial susceptibility testing. For bacterial isolation, the swabs are transferred into BHI broth and incubated at 37^0 C for 24-48 hours. The bacterial isolates were identified based on Gram's staining, colony characteristics and biochemical tests as per conventional microbiological methods. The isolates were further confirmed by polymerase chain reaction employing genus specific primers targeting 16S rRNA. Fungal culture included aerobic cultivation of the samples on Sabouraud's dextrose agar (supplemented with 0.4 g/L chloramphenicol) in duplicate. One plate is incubated at 25^{0}C and the other at 37^{0}C and incubated for 2-7 days. The fungal isolates were identified based upon microscopic morphology in Lactophenol cotton blue staining and colony characteristics. Antibiotic susceptibility pattern was determined by streaking the cultures on Muller Hinton agar plates and antibiotic discs were placed as per Kirby-Bauer disk diffusion method. Behaviour of the isolates to different classes of antimicrobial agents was determined by as per the standards of Clinical Laboratory Standards Institute.

Results and Discussion

Ear exudate samples from 31 dogs with unilateral OE were investigated in the present study. The most frequent clinical signs noticed in dogs with otitis externaare purulent discharges with foul odour, head tilt, oedematous ear pinna, head shaking and pain on palpation (Figure-1).

Of the thirty one clinical specimens collected from dogs exhibiting clinical signs of otitis externa, microbial agents could be isolated from 28 samples (90.3%). From the 28 samples, 51 microbial agents could be isolated. Distribution of these microbial agents is shown in Figure-1. The predominant agent identified in the present study was coagulase- positive Staphylococcus *spp*. representing, 31% of the total organisms identified. E.coli accounts to 23%, while Pseudomonas *spp*. accounts to 16% of the microbial agents identified. Beta- haemolytic Streptococci could be isolated from a single sample. Pseudomonas *spp*. was isolated from dogs with chronic and recurrent otitis cases that failed to respond to previous antibiotic therapy. Among the fungal agents, Candida *spp* was predominant (10%), followed by Malassezia*spp* (8%), Rhizopus*spp* (6%) and Aspergillus*spp* (4%) (Figure-1).

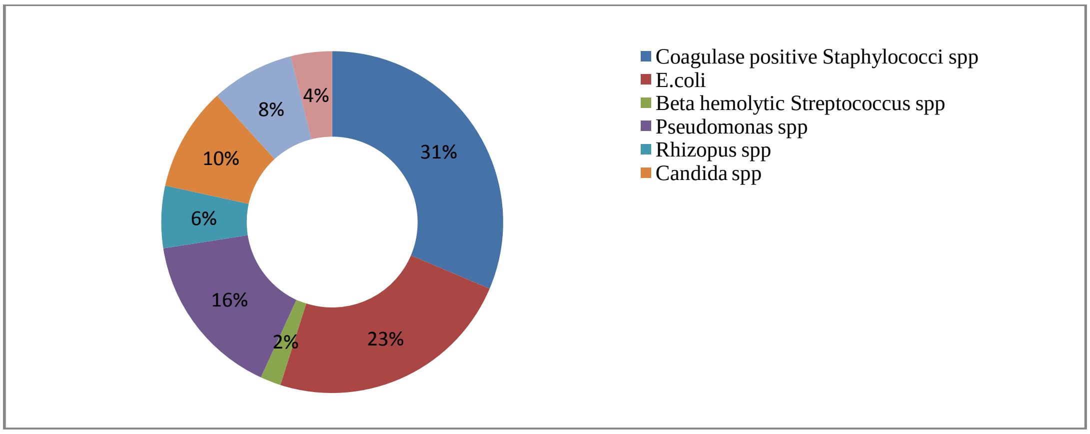

FIGURE-1. NUMBER AND DISTRIBUTION OF MICROBIAL ISOLATES (N = 51) FROM DOG EAR SECRETION SAMPLES POSITIVE FOR MICROBIOLOGICAL AGENTS (N = 28)

Among the 28 samples positive for microbial analysis, single- infections were observed in 7 (25%) samples. Among the single- infections, coagulase- positive Staphylococcus *spp*. was the frequent organism (57.1%). By contrast, E.coli was not detected as a sole agent of canine otitis. Multiple organisms were isolated from 21 (75%) samples: 15 samples with two microbial species and 6 samples with three microbial species (Table-1).Previous researchers reported a slightly higher incidence of coagulase positive Staphylococcus *spp* (54%) in dogs with OE as also reported by Borissov *et al*., 1997 and Hariharan *et al*., 2006. Lower incidence in the present investigation might be due to smaller sample size. Prevalences of other microbial agents such as E.coli and Pseudomonas spp are similar to other studies like of Oliviera *et al*., 2008; Petrov *et al*., 2013). Among the multiple infections also, coagulase- positive Staphylococcus *spp*. was highly predominant (11/21 i.e., 52.3%), followed by Pseudomonas *spp*. (8/21 i.e., 38%) and E.coli (7/21 i.e., 33.3%). The above organisms were also found to be increasingly associated with fungi like Malassezia*spp* and Candida *spp*.

Table-1. Distribution of Microbial Agents by Species in Multiple Infections

2 species	Bacterial /fungal association	Number & frequency (%)
Coagulase positive *Staphylococcus* spp.	*Rhizopus* spp.	2 (9.5)
	Candida spp.	1 (4.7)
	E.coli	4 (19.04)
Pseudomonas spp.	*Staphylococcus* spp.	1 (4.7)
	E.coli	2 (9.5)
	Malassezia spp.	2 (9.5)
E.coli	*Malassezia* spp.	1 (4.7)
	Candida spp.	1 (4.7)
	Aspergillus spp.	1 (4.7)
>2 species	**Bacterial /fungal association**	**Number & frequency (%)**
Coagulase positive *Staphylococcus* spp.	E.coli / *Pseudomonas* spp.	2 (9.5)
	Rhizopus spp./ *Candida* spp./ *Streptococcus* spp.	2 (9.5)
E.coli	*Pseudomonas* spp./*Malassezia* spp.	1 (4.7)
	Candida spp./*Aspergillus* spp.	1 (4.7)

Antimicrobial susceptibility testing revealed that most of the bacterial isolates obtained in this study were resistant to more than one antimicrobial agent. Antibiotics that are least effective against the study isolates were beta lactamase inhibitors (Penicillins and cephalosporins) and tetracyclines (Table-2). Two isolates of coagulase- positive Staphylococcus *spp*. were found to be resistant to Methicillin. Pseudomonas *spp*. isolates were found to be resistant to wide variety of antibiotics. Similar finding was reported by other research groups also asalso reported by Hariharan *et al*., 2006 and Petrov *et al*., 2013. One sample from which we could isolate gram positive and negative bacteria as well as fungi was found to be resistant to all the tested antibiotics.

TABLE-2. Antimicrobial Susceptibility Pattern of Few Bacteria Isolated in This Study

Bacteria	Senstive/ Resistant	Antibiotic
Gram positive bacteria (Staphylococcus spp. &Streptococcus spp.)	Sensitive	β-lactam combinations (amoxicillin/ clavulanic acid and ampicillin/cloxacillin) Aminoglycoside (gentamicin &amikacin)
	Resistant	Methicillin, Tetracyclines,

		Chloramphenicol &Enrofloxacin
Gram negative bacteria E.Coli	Sensitive	Cefotaxime/ Clavulanic acid, Ampicillin/Sulbactum, Gentamicin
	Resistant	Amoxycillin/Sulbactum
Gram negative bacteria Pseudomonas spp.	Sensitive	Ceftriaxone, Streptomycin, Gentamicin
	Resistant	Amikacin, Ceftazidime, Tetracycline, Amoxycillin, Ampicillin/Sulbactum, Penicillin G
		Cefotaxime, Cefazolin, Oxytetracycline, Enrofloxacin, Gentamicin
		Oxytetracycline, amoxicillin/ clavulanic acid, Ampicillin/Sulbactum, Enrofloxacin, Cefotaxime

In the present study, both bacteria and fungi could be recovered from most of the clinical samples and Staphylococcus *spp*. was the predominant one. Furthermore, microbial culture and antimicrobial susceptibility testing should be made mandatory before advocating therapy to prevent chronic and recurrent infections.

References

Angus, J.C. (2004). Otic cytology in health and disease.*Vet. Clin.Small.Anim.*, **34:** 411-424.

Borissov, I., Urumova, V., Lytzkanov, M. and Groseva, V. (1997). Clinical and microbiological studies in dogs with otitis.*Vet. Science*, **XXIX:** 365-371.

Cole, LK. (2004). Otoscopic evaluation of the ear canal.*Vet. Clin. Small Anim.*, **34**: 397-410.

Hariharan, H., Coles, M., Poole, D., Lund, L. and Page, R. (2006). Update on antimicrobial susceptibility of bacterial isolates from canine and feline otitis externa. *Can. Vet. J.*, **47**: 253-255.

Oliveira, L.C., Leite, C.A., Brilhante, R.S. and Carvalho, C.B. (2008). Comparative study of the microbial profile from bilateral canine otitis externa. *Can. Vet. J.*, **49**: 785-788.

Paterson, S. (2016). Topical ear treatment– options, indications and limitations of current therapy. *Journal of Small Animal Practice*, **7**: 67-75.

Petrov, V., Mihaylov, G., Tsachev, I., Zhelev, G., Marutsov, P. and Koev, K. (2013). Otitis externa in dogs: microbiology and antimicrobial susceptibility. *Revue Méd.Vét.*, **164**(1): 18-22.

Rosser, E.J. (2004). Causes of otitis externa. *Vet. Clin. Small Anim.*, **34:** 459-468.

Scott, D.W., Miller, W.H., Griffin, CE. (2001). Otitis, *In*: Muller and Kirk's Small Animal Dermatology. Saunders, Philadelphia, U.S. Pp. 143-148.

Senthil, K.K., Selvaraj, P., Vairamuthu, S., Mala, S. and Kadiresan, D. (2010). Antibiogram patterns of microbes isolated from otitis externa of dogs. *Tamil Nadu Journal Veterinary and Animal Sciences*, **6**(3): 145-147.