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BACTERIOLOGICAL STUDIES ON OTITIS EXTERNA IN DOGS AND ANTIBIOTIC SUSCEPTIBILITY TESTING OF THE RECOVERED ISOLATES

By

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ABSTRACT

A number of 44 samples were examined for bacterial pathogens encountered in external ear inflammation in dogs of different ages and breeds. On bacteriological culture, *Staphylococcus aureus,Staphylococcus epidermidis,Pseudomonas aeuroginosa,Escherichiacoli* and *Klebsiella pneumonia,* represented the bacterial isolates. All isolates were subjected to antimicrobial sensitivity testing using antibacterial agents. The obtained results indicated that *S. aureus* was the most common isolated bacterial species.The antibiogram assays showed that allbacterial isolateswere sensitive to piperacillin/tazobactum and imipenem/meropenenum. *Staphylococcus* spp. isolates were sensitive to Linezolid and Vancomycin.

In conclusion: To control otitis externa (OE), susceptibility testing is be applied on isolates to guide the veterinarians to choose the most effective therapeutic regimen.

Keywords:

Dogs, otitis externa, susceptibility testing, bacteria.

INTRODUCTION

OE is one of the most common multifactorial diseases in dogs and defined as acute or chronic inflammation of the external ear canal with 5-20% prevalence**(Fernandez *et al.*,2006; Kumar *et al.*, 2010; Boda *et al.*, 2011)**. Clinical signs, such as exudate, erythema, oedema, offensive odour and pruritus are obviously seen, one or both ears could be affected, and signs can be sudden or long-term.**(De Martino *et al.*, 2016).**

The causes could be either primary (Foreign bodies, ectoparasites) or secondary following complications from ear canal stenosis, drooping ears, excessive hair in the ear canal, water in

the ear canal, obstructions, atopic dermatitis, allergic skin reactions to food, metabolic diseases, abnormal keratinization,autoimmune diseases, injury during manipulations **(Terziev and Urumova, 2018).**

The bacteria associated with OE are only opportunists and not the primary pathogens **(Rosser, 2004).** The most often isolated bacteria include *Staphylococcus* spp., *Corynebacterium* spp., *Streptococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp. **(Petrov *et al.*, 2019)**. Acute and uncomplicated OE can be treated successfully, but chronic is more challenging. Effective treatment of OE includes treatment of infection and inflammatory changes as well as determination of the factors leading to the disease development. Topical therapy is the mainstay treatment for OE although systemic use of anti-inflammatory therapy and/or antimicrobial therapy may be indicated for individual patients. Most dogs with otitis, irrespective of its cause, will benefit from anti-inflammatory therapy **(Bajwa, 2019).**

The aim of the present study was to detect and identify bacterial pathogens associated with OE in dogs with different ages with testing the antimicrobial sensitivity of the isolates.

MATERIAL AND METHODS

Sample preparation:

The present study was conducted under the regulations of IACUC of Faculty of Veterinary Medicine, Cairo University.

Forty-four dogs suffering from OE, of different breeds {Golden Retriever (8), German shepherd (5), Cocker spaniel (11), Siberian husky(3) , Labrador retriever (7), Rottweiler (4), Pekinese (3), and Griffon(3) } and ages (6 months to 13 years) were enrolled in the study. Cases represented dogs recruited to the teaching clinics of the Faculty of Veterinary Medicine, Cairo University and some private veterinary clinics in Giza Government. Debris from the affected ears was collected by rotating or rubbing sterile cotton swabs (BD Diagnostics®) with 5ml sterile saline into the leve of the vertical and horizontal portion of the external ear canal. The collected samples were immediately transported, while cold, to the laboratory of the Microbiology Department, Faculty of Veterinary Medicine, and Cairo University for bacteriological examination.

Bacteriological Examination:

The collected ear swabs were cultured onto sheep blood, MacConkey, mannitol salt and eosin methylene blue agar plates (Oxoid®, UK). The inoculated plates were incubated aerobically at 37^0C for 24-48 hours. The plates were examined for bacterial growth, and the morphological characteristics of bacterial colonies were noted. The bacterial isolates were identified using Gram's staining, presence of hemolysis as well as sugar fermentation, indole, methyl-red, Voges-Proskauer and citrate utilization tests **(Doshi *et al*., 2021).**

Susceptibility testing of bacterial isolates was carried out using the disk diffusion method on Mueller-Hinton agar (MHA; Oxoid®). The inoculum suspension was adjusted to match the turbidity of McFarland tube No. 0.5 and 20 antibiotic different disks were used. Susceptibility patterns of the bacterial isolates were interpreted according to the **Clinical and Laboratory Standards Institute (2021).**

Statistical analysis of the results was performed by the One-way ANOVA test and *P* values of less than 0.05 were regarded as statistically significant.

RESULTS

All the tested animals were presented with OE acute clinical signs including inflammation, soreness, pain,abnormal odor, exudates, excessive scratching,fever and vigorous head shaking. No chronic or recurrent cases were reported in the present study.

The overall isolation rate was the highest from dogs aging 1-5 years (47.05%) followed by those less than 1-year-old (39.7%) while dogs older than 5 years showed the least isolation rate (13.2%). It is of interest to note that, Golden Retriever and German shepherd dogs showed the highest incidence of bacterial OE followed by Cocker spaniel, Siberian husky, Labrador Retriever, Rottweiler, Pekinese, and Griffon breeds (Table1).

Gram-positive cocci belonged to *Staphylococcus* species grew on Mannitol salt agar including coagulase positive *S. aureus* and *S. epidermidis* isolates. Gram-negative bacilli, *P. aeruginosa*, *E. coli* and *K. pnemonae* were isolated and identified on biochemical bases.

S. aureus (44.11%) was the most common bacteria followed by *S. epidermidis, K. pneumonia, E. coli,* and *P. aeruginosa* in percentages of 22.05%, 16.17%, 14.07%, 2.9%, respectively.

Looking at the susceptibility results (Table 2), all staphylococci isolates were susceptible for vancomycin, piperacillin and imipenem/meropenem. *S. aureus* isolates showed resistance

percentages of 26%, 26%, 23%, 20%, 20%, 17%, 17%, 13% and 10% to ceftazidine, chloramphenicol, gentamycin, ampicillin, ciprofloxacin, azithromycin, clarithiomycin, levofloxacin and sulphamethoxazole/trimethoprim, respectively.

E. coli isolates showed resistance percentages of 50%, 50%, 40%, 30%, 30%, 20% and 10% to erythromycin,sulphamethoxazole/trimethoprim,chloramphenicol,azithromycin, ceftazidime, cefoxitin and cefotaxime, respectively. Of *K. pnemonae* isolates, 55%, 55%, 36%, 36%, 36%, 27%, 18%, 18%, 9% and 9% were resistant to clarithiomycin, sulphamethoxazole/ trimethoprim, cefotaxime, cefotrxione, ceftazidime, chloramphenicol, ampicillin, cefoxitin, azithromycin and ciprofloxacin, respectively. Concerning *P. aeruginosa* isolates, 100%, 100%, 50% and 50% were resistant to chloramphenicol, trimethoprim/ sulphamethoxazole, ampicillin and cefoxitin, respectively.

Table (1): Incidence of bacteria and yeasts associated with otitis externa in dogs in relation to age

No of samples Age	**Number and percentages of isolates**						**Total No of isolates**
		S. aureus	***S. epidermidis***	***E. coli***	***K. pnemonae***	***P. aeuroginosa***	
year< 1	**15**	**12**	**6**	**3**	**5**	**1**	**27**
		44.4%	**22.2%**	**11.1%**	**18.7%**	**3.7%**	**35.07**
1-5 years	**20**	**14**	**6**	**6**	**5**	**1**	**41**
		33.6%	**14.4%**	**14.4%**	**12.0%**	**2.4%**	**53.24**
> 5years	**9**	**4**	**3**	**1**	**1**	**-**	**9**
		44.4%	**33.3%**	**11.1%**	**11.1%**	**-**	**11.69**
Total	**44**	**30**	**15**	**10**	**11**	**2**	**77**
		38.9%	**19.5%**	**12.9%**	**14.3%**	**2.6%**	
***P*-value**		**0.197 (*FET*)**	**0.825 (χ^2)**	**0.650 (*FET*)**	**0.538 (*FET*)**	**1.000 (*FET*)**	

The percentages were calculated according to the total number of isolates in each category

*** Superscript indicates significance at $P < 0.05$; *FET*: Fisher's exact test; χ^2: Chi-square.**

Table (2): The susceptibility testing of bacterial isolates recovered from inflamed external ears of dogs.

Antimicrobial groups and related agents	*S. aureus*			*S. epidermidis*			*E. Coli*				*K. penumonia*		*P. aeruginoa*		
	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R
Beta-lactam															
Amoxicillin Clavulanate (AMC30mg)	17	7	6	8	4	3	7	3	-	6	3	2	1	-	1
Piperacillin/tazobactum (TZP,100mg)	30	-	-	15	-	-	10	-	-	11	-	-	2	-	-
Imipenem/Meropenenum (MRP,10mg)	30	-	-	15	-	-	10	-	-	11	-	-	2	-	-
Macrolides															
Azithromycin (AZM,15mg)	19	7	4	9	4	2	4	3	3	6	4	1	1	1	-
Erythromycin (E,15mg)	12	7	11	7	5	3	-	-	-	-	-	-	-	-	-
Clarithromycin (CLR,15mg)	13	13	4	6	6	3	1	4	5	1	4	6	-	2	-
Oxazolidinones															
Linezolid (LNZ,30mg)	30	-	-	15	-	-	-	-	-	-	-	-	-	-	-
Glycopeptide															
Vancomycin (VA, 30mg)	30	-	-	15	-	-	-	-	-	-	-	-	-	-	-
Cephalosporins															
Cefoxitin (FOX,30mg)	22	8	-	10	5	-	6	2	2	6	3	2	1	-	1
Ceftriaxone (CRO,30mg)	22	8	-	10	5	-	6	3	1	5	4	2	1	1	-
Cefotaxime (CTX,30mg)	22	8	-	10	5	-	6	3	1	5	4	2	1	1	-
Ceftazidime (CAZ,30mg)	10	11	9	7	4	4	4	3	3	1	6	4	-	2	-
Fluoroquinolones															
Ofloxacin (OFX,5mg)	22	8	-	11	4	-	8	2	-	9	2	-	2	-	-
Levofloxacin (LEV,5mg)	22	4	4	11	4	-	8	2	-	8	2	-	1	1	-
Ciprofloxacin (CIP,5mg)	15	11	4	7	5	3	6	4	-	8	2	1	1	1	0
Miscellaneous antibiotics															
Chloramphenicol (C,30mg)	10	11	9	5	6	4	3	3	4	5	3	3	-	-	2
Sulfonamides															
Trimethoprim/sulfamethoxazole (SXT,25mg)	18	9	3	9	5	1	2	3	5	2	3	6	-	-	2
Aminoglycosides															
Amikacin (AK,30mg)	21	9	-	10	15	-	9	1	-	11	-	-	2	-	-
Gentamycin (CN,10mg)	15	27	23	11	3	1	6	4	-	6	3	2	2	-	-
Lincomycine															
Clindamycin (CD,2mg)	15	8	7	10	3	2	-	-	-	-	-	-	-	-	-

S: susceptible M: moderately susceptible R: resistant

DISCUSSION

External ear inflammation is believed to be one of the most common conditions present in dogs and cats, and the infections can be caused by both bacterial and fungal origin. Pathogens of the skin for many veterinary species are present in up to 40 % of canine otitis cases **(Bugden, 2013).**Otitis externa is not evenly distributed across all canine breeds.Factors that predispose dogs to developing otitis externa include shapes of the ear canal and pinna, excessive moisture in the ear canal, and trauma to the ear due to excessive cleaning **(Hayes *et al.*, 1987; Zur *et al.*, 2011).**

Dog breeds with pendulous ears were associated with a higher frequency of otitis externa relative to breeds with erect ears, although ear hairiness was not. These ear traits may increase disease risk by affecting air flow, heat radiation, and convection from the ear canal relative to erect, hair-free ears, and providing conditions ideal for microorganism growth. On the other hand, the ambient temperature and humidity can result in slight but significant changes in the microenvironment of the ear **(Huang and Huang, 1999).**

There have been relatively few recent local surveys of the variety and sensitivity of bacterial species associated with otitis externa.While there is general reliability in the common bacteria present, there are differences in prevalence as well as in resistance to antibiotics.

Herein, this study aimed at investigating bacteria associated with dog OE.The obtained results indicated the prevalence of bacterial species causing OE with the highest prevalence was of *Staphylococcus spp* (58.4%) where *S. aureus* and *S. epidermidis* were the most common *Staphylococcus* species. Similar findings were recorded in previous studies **(Malayeri *et al.*, 2010; Sfaciotte *et al.*, 2015).** An Iranian study recorded a high prevalence of normal *Staphylococcus* species reported in the ears of 58% of healthy dogs **(Sarchahi *et al.*, 2007).** Previous studies described a higher incidence of coagulase-positive staphylococci, but also the coagulase-negative staphylococci, were recovered which have long been believed to be nonpathogenic, recently have presumed a significant role as pathogens because of their increasing incidence as cause of bacteremia. While **Lyskova *et al*. (2007) and Oliveira *et al*. (2008)** reported lower incidence rates; **Penna *et al*. (2011)** recorded coagulase-positive staphylococci in a high percentage, while coagulase-negative staphylococci were in lower incidence.Concerning Gram-negative species, *K. pneumoniae*, *E. coli* and *P. aeruginosa* were isolated in descending rates. These findings are similar to those of **De Martino *et al*. (2016),**

Malayeri *et al*. (2010) and Fernández *et al*. (2006). The differences in the incidences of different bacterial species may be related to the stage or severity of the disease and could be attributed to different geographical distribution of different districts and regions with various climatic conditions as well as the dog breeds and the community levels in which those dogs are reared.

Zur *et al*. (2011) reported that, Gram-negative rods have been more frequently isolated from Cocker spaniel and German shepherd dog breeds and they concluded that this could be correlated with the breed's predisposition to allergies.

The most common antimicrobial classes used in pet animals in veterinary medicine are penicillins,cephalosporin,macrolides,lincosamides,fusidicacid,tetracyclines, chloramphenicol, potentiated sulphonamides,aminoglycosidesandfluoroquinolones.

Aminoglycosides, such as gentamicin and neomycin, are usually used for topical application in dog otitis mainly caused by *P. aeruginosa* **(Penna *et al*., 2011; Bugden, 2013).**

Considering the susceptibility results, all Staphylococci isolates were susceptible for vancomycin, piperacillin and Imipenem/meropenem. Only 20% of *S. epidermidis* isolates were resistant to amikacin, chloramphenicol, erythromycin and 7% of the isolates were resistant to trimethoprim/sulfamethoxazole. **Malayeri *et al*. (2010)** reported that all isolated Staphylococcus spp. were sensitive to amikacin, enrofloxacin, and rifampin, and had low resistance to gentamicin, cephalothin and ceftriaxone.

On the other hand, all Gram-negative bacterial isolates recovered in this study, were sensitive to amikacin and enrofloxacin, and had low resistance to ceftriaxone and gentamicin. Meanwhile, they were highly resistant to penicillin, erythromycin, and cephalothin and susceptible for vancomycin, piperacillin and imipenem/meropenem.

P. aeuroginosa isolates were resistant to both chloramphenicol and trimethoprim/ sulfamethoxazole and (50%) and resistant to amikacin and cefoxitin which represents serious evidence of emergence of multidrug-resistant strains in dog infections. This study results combined with data from the literature support the premise that antimicrobials should be selected basically on bacterial culture and antimicrobial susceptibility test. Until recently, multidrug-resistance was overall seen in Gram-positive bacteria **(Giannakaki and Miyakis, 2012)** However, the recent rapid international spread of multidrug-resistant Gram-negative bacteria is much more ominous, and the optimal management of serious infections with these

bacteria remains to be determined **(Schmiedel *et al*., 2014).** Consistent with previous studies, this study is a further confirmation that coagulase-positive species of staphylococci are the most causative microorganisms of otitis externa in dogs. Knowledge of the microbial species present locally and their sensitivity to specific antibiotics should accelerate more successful treatment and decrease the possibility of the development of chronic cases or antibiotic-resistant bacteria. Likewise, large differences between the results of experimental studies and actual outcomes in veterinary practices can be informative and can reflect the need for changing medical interventions and procedures.

REFERENCES

Bajwa, J. (2019): Canine otitis externa-Treatment and complications. *The Canadian Veterinary Journal*, *60* (1), p.97**.**

Boda, C., Liège, P. and Rème, C.A. (2011): Evaluation of Owner Compliance with Topical Treatment of Acute Otitis Externa in Dogs: A Comparative Study of Two Auricular Formulations. *International Journal of Applied Research in Veterinary Medicine*, *9* (2), p.157.

Bugden, D.L. (2013): Identification and antibiotic susceptibility of bacterial isolates from dogs with otitis externa in an ustralia. *Australian veterinary journal*, *91*(1-2), pp.43-46. https: // doi. org/ 10.1111/avj.12007

De Martino, L., Nocera, F.P., Mallardo, K., Nizza, S., Masturzo, E., Fiorito, F., Iovane, G. and Catalanotti, P. (2016): An update on microbiological causes of canine otitis externa in Campania Region, Italy. *Asian Pacific Journal of Tropical Biomedicine*, *6* (5), pp.384-389. https://doi.org/10.1016/j.apjtb.2015.11.012

Doshi, D.M., Bhanderi, B.B., Mathakiya, R.A., Nimavat, V.R. and Jhala, M.K. (2021): Isolation, Characterization, Antibiogram and Molecular Detection of Antibiotic Resistance Genes from Bacteria Isolated from Otitis Externa in Dogs. DOI: 10.21887/ijvsbt.17.2.8.

Fernández, G., Barboza, G., Villalobos, A., Parra, O., Finol, G. and Ramirez, R.A. (2006): Isolation and identification of microorganisms present in 53 dogs suffering otitis externa. Revista cientifica-universidad Del Zulia facultad de ciencias veterinarias division de investigacion, 16 (1), p.23.

Giannakaki, V. and Miyakis, S.(2012): Novel antimicrobial agents against multi-drug-resistant gram-positive bacteria:an overview. *Recent patents on anti-infective drug discovery*,*7* (3), pp.182-188.

Hayes Jr, H.M., PICKLE, L.W. and Wilson, G.P. (1987): Effects of ear type and weather on the hospital prevalence of canine otitis externa. *Research in veterinary science*, *42* (3), pp.294 -298.

Huang, H.P. and Huang, H.M. (1999): Effects of ear type, sex, age, body weight, and climate on temperatures in the external acoustic meatus of dogs. American journal of veterinary research, 60 (9), pp.1173-1176.

Kasai T, Fukui Y, Aoki K, Ishii Y and Tateda K. (2021): Changes in the ear canal microbiota of dogs with otitis externa. Journal of Applied Microbiology 130:1084-91. https://doi.org/10.1111/jam.14868

Kumar, K.S., Selvaraj, P., Vairamuthu, S., Shammi, M. and Kathiresan, D. (2010): Antibiogram patterns of microbes isolated from otitis externa of dogs. *Indian J Vet Anim Sci*, *6*, pp.145-147.

Lyskova, P., Vydrzalova, M. and Mazurova, J. (2007): Identification and antimicrobial susceptibility of bacteria and yeasts isolated from healthy dogs and dogs with otitis externa. *Journal of Veterinary Medicine Series A*, *5 4* (10), pp.559-563. https://doi.org/10.1111/j.1439-0442.2007.00996.x

Malayeri, H.Z., Jamshidi, S. and Salehi, T.Z. (2010): Identification and antimicrobial susceptibility patterns of bacteria causing otitis externa in dogs. *Veterinary research communications*, *34* (5), pp.435-444. DOI 10.1007/s11259-010-9417-y

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. *The Canadian veterinary journal*, *49* (8), p.785.

Penna, B., Thomé, S., Martins, R., Martins, G. and Lilenbaum, W. (2011): In vitro antimicrobial resistance of Pseudomonas aeruginosa isolated from canine otitis externa in Rio de Janeiro, Brazil. *Brazilian journal of microbiology*, *42*, pp.1434-1436.

Petrov, V., Zhelev, G., Marutsov, P., Koev, K., Georgieva, S., Toneva, I. and Urumova, V. (2019): Microbiological and Antibacterial Resistance Profile in Canine Otitis Externa--A Comparative Analysis. *Bulgarian Journal of Veterinary Medicine*, *22* (4).

Rosser, E.J. (2004): Causes of otitis externa. *Veterinary Clinics: Small Animal Practice*, *34* (2), pp.459-468.

Sarchahi, A.A., Haghkhah, M., Firouzi, R. and Yarinasab, F. (2007): Bacterial and fungal normal flora of the external ear canal of dogs. *Online Journal of Veterinary Research*, *11*.

Schmiedel, J., Falgenhauer, L., Domann, E., Bauerfeind, R., Prenger-Berninghoff, E., Imirzalioglu, C. and Chakraborty,T.(2014): Multiresistant extended-spectrum β-lactamase-producing Enterobacteriaceae from humans, companion animals and horses in central Hesse, Germany. BMC microbiology, 14 (1), pp.1-13.

Sfaciotte, R.A.P., Coronel, L.G., Osaki, S.C. and Wosiacki, S.R. (2015): Gram-positive bacterial resistant strains of interest in animal and public health. *Semina: Ciências Agrárias*, *36* (4), pp.2693-2712. DOI: 10.5433/1679-0359.2015v36n4p2693

Terziev, G. and Urumova, V. (2018): Retrospective study on the etiology and clinical signs of canine otitis. *Comparative Clinical Pathology*, *27* (1), pp.7-12.

Zur, G., Lifshitz, B. and Bdolah-Abram, T. (2011): The association between the signalment, common causes of canine otitis externa and pathogens. *Journal of Small Animal Practice*, *52* (5), pp.254-258. https://doi.org/10.1111/j.1748-5827.2011.01058.x.