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RESEARCH ARTICLE

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Bacterial and Fungal Species Isolated From Dogs With Otitis Externa#

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ABSTRACT

This study was conducted to detect the distribution of bacterial and mycotic agents and the antimicrobial susceptibility of bacterial isolates from dogs with infective otitis externa for an 11-year period. Samples, collected from the external ear canal of 475 dogs, were analysed by conventional bacteriological and mycological methods between the years of 2005 and 2016. Antimicrobial susceptibility of the isolates was determined by Kirby-Bauer disc diffusion method. Bacterial growth was observed in 328 of 475 swab samples collected from the dogs. Of 434 isolated bacteria, 281 isolates (64.7%) were Gram-positive cocci, 151 isolates (34.8%) were Gram-negative rods and 2 isolates (0.5%) were Gram-positive rods. The most frequently isolated microorganisms was *Staphylococcus intermedius* (18.7 %), followed by *Pseudomonas aeruginosa* (12.9%), *Escherichia coli* (7.1%) *Proteus mirabilis* (6.7 %) *Micrococcus* spp (4.1%) and *Streptococcus canis (*2.5 %). Mycological growth was also observed from 213 of 475 matching swabs. The results showed that the need for bacterial culture and antimicrobial susceptibility tests for appropriate antimicrobial therapy. Mycological culture should also be performed in infectious otitis externa cases of dogs.

Keywords: Infectious otitis externa, Dog, Culture, Microorganism, Antimicrobial susceptibility

Otitis Eksternalı Köpeklerden İzole Edilen Bakteri ve Maya Türleri

ÖZ

Bu çalışma, 11 yıllık bir süre boyunca infektif otit eksternaları olan köpeklerden bakteriyel ve mikotik ajanların dağılımını ve bakteriyel izolatların antimikrobiyal duyarlılıklarını saptamak amacıyla yapıldı. 2005-2016 yılları arasında, 475 köpeğin dış kulak kanalından toplanan numuneler, geleneksel bakteriyolojik ve mikolojik yöntemlerle incelendi. İzolatların antimikrobiyal duyarlılıkları Kirby-Bauer disk difüzyon yöntemi ile belirlendi. Köpeklerden toplanan 475 sürüntü örneğinin 328'inde bakteriyolojik üreme gözlendi. İzole edilen 434 bakteriden 281'i (% 64.7) Gram pozitif kok, 151'i (% 34.8) Gram negatif çomak ve 2 izolat (%0.5) Gram pozitif basil olarak belirlendi. En sık izole edilen mikroorganizma *Staphylococcus intermedius*'tu (% 18.7), bunu *Pseudomonas aeruginosa* (% 12.9), *Escherichia coli* (% 7.1), *Proteus mirabilis* (% 6.7), *Micrococcus* spp (% 4.1) ve *Streptococcus canis* (% 2.5) izledi. Aynı zamanda, 475 swabın 213'ünde mikolojik üreme de görüldü. Sonuçlar, uygun antimikrobiyal tedavi için bakteri kültürü ve antimikrobiyal duyarlılık testlerine ihtiyaç duyulduğunu göstermektedir. Bunun yanısıra, köpeklerin enfeksiyöz otitis eksterna olgularında mikolojik kültür de yapılmalıdır.

Anahtar Kelimeler: İnfeksiyöz otitis eksterna, Köpek, Kültür, Mikroorganizma, Antimikrobiyal Direnç

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INTRODUCTION

Otitis externa (OE) which is the inflammation of the external auditory meatus, is the most common ear disease of the canine and feline (Guedeja-Marron et al.1998, Rosser 2004). The prevalence of the OE is estimated between 5 and 20% (Rougier et al. 2005).

Otitis has many predisposing factors which can be classified as primary, predisposing and perpetuating. The primary causes such as parasites, foreign bodies, hypersensitivity and allergic diseases, keratinization disorders, autoimmune diseases initiate otitis externa in otherwise normal ears. The predisposing factors such as anatomic and conformational factors, excessive moisture, iatrogenic factors, and obstructive ear disease make the ear more susceptible to the development of OE but do not cause it alone. Bacteria, yeast, otitis media, progressive pathologic changes are considered as perpetuating factors and they are responsible for aggravation of the process and therefore avoid spontaneous resolution (Rosser 2004, Lyskova et al. 2007).

Regardless of the primary ear lesion, acute and suppurate otitis of canine are predominantly caused by the microbial contamination (Guedeja-Marron et al.1998, Bernardo et al. 1998). The microorganisms the most commonly isolated from canine otitis externa are *Staphylococcus intermedius* and *Malassezia pachydermatis* (Kiss et al. 1997).

This study was conducted to detect the distribution of bacterial and mycotic agents and the antimicrobial susceptibility of bacterial isolates from dogs with infective otitis externa for an 11-year period.

MATERIALS and METHODS

Collection of samples

Canine cases clinically suspected of otitis externa and presented at the Department of surgery were included in the study. Diagnosis of the disease was based on historical data, clinical signs or findings on physical examination. At eleven year period, between 2005 and 2016, the samples were obtained from 475 dogs. In each case, two sterile bacteriological swabs were used to collect cerumen from the external ear canal. Swabs were processed within 2 hours.

The animals belonged to both sexes, with ages ranging from 2 months to 19 years old. The dog breeds were Golden Retreiver, Cocker spaniels, Terrier, German shepherd dogs and the other breeds (mix, Rottweiler, Anatolian Shepherd, Pekingese, Bulldog, Siberian Husky, Setter, Chow Chow, Boxer, Pointer, Beagle, Collie, Labrador Retreiver, Akbash, Miniature Pincher, Chihuahua, King Charles, Yorkshire Terrier, Dalmatian, Dogo Argentina, Pug, Kopay, , Saint Bernard, Mastiff).

Microbiological analysis

In each case, one of the swabs was inoculated in Nurient Agar containing 7% sheep blood and Nutrient Broth containing horse serum and incubated microaerobically at 37°C for 24-48 hours (Quinn et al. 2002). Gram staining was performed from the cultures and identification conducted by biochemical identification kits API Staph, API 20 Strep API 20 E, API 20 NE (BioMérieux; Marcy-L'Etoile, France). The other swab set was inoculated onto Sabouraud Dextrose Agar (SDA) and the plates were incubated at 37°C for 1 week. After the incubation, Gram staining was performed from the cultures and standard methods were used for the identification of the yeast (Quinn et al. 2002).

Antibiotic susceptibility test

The *in vitro* susceptibility of isolated strains was investigated by using Kirby-Bauer agar disk diffusion method compliant with the Clinical and Laboratory Standards Institute (CLSI 2006). For this purpose, gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), enrofloxacin (5 μg), amoxicillin–clavulanic acid combination – AMC (20 μg), ampicillin (10 μg), penicillin G (10 unit), ampicillin/sulbactam (10 μg), cefoperazone (75 μg), erythromycin (15 μg) and tetracycline (30 μg) were tested.

RESULTS

Isolation and identification findings

In this study, the most commonly represented breeds were: Golden Retreivers (103/475), Cocker spaniels (89/475), Terriers (47/475), German shepherd dogs (32/475) and the other breeds (204/475) (mix (85), Rottweilers (17), Anatolian Shepherds (16), Pekingeses (13), Bulldogs (9), Siberian Huskies (8), Setters (8), Chow Chows (6), Boxers (6), Pointers (5), Beagles (5), Collies (4), Labrador Retreivers (4), Akbashs (3), Miniature Pinchers (3), Chihuahuas (2), King Charles (2), Yorkshire Terriers (2), Dalmatian (1), Dogo Argentina (1), Pug (1), Kopay (1) , Saint Bernard (1) and Mastiff (1)).

Bacterial growth was observed in 328 of 475 swab samples collected from the dogs. In 233 of the cases bacteriological culture revealed single species. In 84 cases, two species were cultured from the sample. Three or more species isolated from 11 samples. Of 434 isolated bacteria, 281 isolates (64.7%) were Gram-positive cocci, 151 isolates (34.8%) were Gram-negative rods and 2 isolates (0.5%) were Gram-positive rods. The most frequently isolated microorganisms were *Staphylococcus intermedius* (81, 18.7 %) and *Pseudomonas aeruginosa* (56, 12.9%), followed by *Escherichia coli* (31, 7.1%), *Proteus mirabilis* (29, 6.7%), *Micrococcus* spp (18, 4.1%) and *Streptococcus canis* (11, 2.5 %). The dispersions of the isolates are summarized in the table 1.

Mycological growth was also observed from 213 of 475 (45.05%) matching swabs. 149 isolates (70%) were *Malassezia* spp, and 64 isolates (30%) were *Candida* spp. When isolated microorganisms evaluated according to dog breeds, *S. intermedius* was the most frequently bacteria in all breeds (except cocker), whereas in Cocker spaniels, *P. aeruginosa* was the most frequently isolated bacteria. The distribution of the isolates according the dog breeds are summarized in the table 2.

Antibiotic susceptibility test findings

In all strains, the most active susceptibility occurred to ciprofloxacin (72%), enrofloxacin (66.3%), amikacin (66.2%) and cephoperazone (65.3%). All *Pseudomonas aeruginosa* strains were resistant to eritromisin (100%), and most all to penicillin (97.5%), and tetracycline (96.4%). The rates of resistance of the most frequently isolated bacteria are summarized in the table 3.

Table 1. Distribution of the isolates

Isolates		Number of isolates	Percentage of results (%)
Bacteria	***S. intermedius***	81	18.7
	P. aeruginosa	56	12.9
	E. coli	31	7.1
	P. mirabilis	29	6.7
	***Micrococcus* spp.**	18	4.1
	S. canis	11	2,5
	Other Gram negative rods *	35	8.1
	Other Gram positive bacteria**	173	39.9
Total		434	100
Yeasts	***Malassezia* spp.**	149	70
	***Candida* spp.**	64	30
Total		213	100

* Other Gram negative rods : Members of the *Enterobactericeae* family

** Other Gram positive bacteria: Members of the *Staphylococcaceae* family, *Streptococcaceae* family, *Micrococcaceae* family and *Enterococcaceae* family

Table 2. Distribution of the isolates according the dog breeds

	Isolates	Breeds					Total
		Golden Retreiver No (%)	Cocker spaniels No (%)	Terrier No (%)	German shepherd No (%)	Mix breeds No (%)	
Bacteria	***S. intermedius***	11 (13.6)	17 (21)	8 (9.9)	10 (12.3)	35 (43.2)	81
	P. aeruginosa	9 (16.1)	19 (33.9)	5 (8.9)	3 (5.4)	20 (35.7)	56
	E. coli	6 (19.3)	6 (19.3)	2 (6.5)	7 (22.6)	10 (32.3)	31
	P. mirabilis	2 (6.9)	12 (41.4)	3 (10.3)	-	12 (41.4)	29
	***Micrococcus* spp.**	2 (11.1)	6 (33.3)	-	1 (5.6)	9 (50)	18
	S. canis	1 (9)	5 (45.5)	-	-	5 (45.5)	11
	Other Gram negative rods *	6 (17.1)	7 (20)	3 (8.6)	2 (5.7)	17 (48.6)	35
	Other Gram positive bacteria**	31 (17.9)	28 (16.2)	24 (13.9)	12 (6.9)	78 (45.1)	173
Yeasts	***Malassezia* spp.**	41 (27.5)	25 (16.8)	15 (10.1)	8 (5.4)	60 (40.2)	149
	***Candida* spp.**	12 (18.8)	10 (15.6)	5 (7.8)	7 (10.9)	30 (46,9)	64

Table 3. Percentages of *in vitro* resistance to antimicrobial agents

Resistance rate (%)	Antibiotic										
	GN	AM	CIP	ENR	AMC	AMP	PEN	SAM	CPZ	E	TE
S. intermedius	38,9	25,7	18,1	30,9	22,2	62,5	73,9	30	24,7	75	71,8
P. aeruginosa	34,9	22,2	9,3	40	96,2	91,3	97,5	93,3	30,2	100	96,4
E. coli	43,3	36,4	58,6	25	85,7	0	53,8	86,9	64	25,9	79,2
P. mirabilis	25	33,3	48,1	33,3	78,6	28,6	92,9	88,2	30,4	20	95,7
***Micrococcus* spp**	69,2	37,5	33,3	31,3	73,3	50	69,2	92,3	41,2	38,9	43,8
S. canis	100	88,9	60	44,4	75	100	100	66,7	60	44,4	100
Total	38,6	33,8	28	33,7	59,7	64,2	78,4	63,6	34,7	53,7	81,3

GN: Gentamicin AM: Amikacin CIP: Ciprofloxacin ENR: Enrofloxacin AMC: Amoxicillin/Clavulanic acid AMP: Ampicillin PEN: Penicillin SAM: Ampicillin/Sulbactam CPZ: Cefoperazon E: Erythromycin TE: Tetracycline

DISCUSSION

Otitis externa may occur in any dog. Although a predisposition has been recognized in Cocker Spaniels, Poodles, Pyrenean shepherds and Labrador retrievers. Saridomichelakis et al. (2007) indicated that this breed predisposition is more important in cocker spaniels, in which a combination of conformational factors including the long, pendulous and hairy ear pinnae and the increased density of compound hair follicules and ceruminous glands in the ear canal may contribute to the higher frequency of OE. In this study, similar to the other studies the most commonly represented breeds were Golden Retreivers, Cocker spaniels, Terriers and German shepherd dogs (Kiss et al. 1997, Bernardo et al. 1998, Cafarchia et al. 2005, Saridomichelakis et al. 2007).

In this study, most frequently isolated microorganism was *S. intermedius* (18.7%). Oliveira et al. (2008) reported that many studies have described the presence of *S. intermedius* as components of the normal microbiota of the canine ear and pointed their association with canine OE. Other researchers have isolated most frequently *S. intermedius* in canine otitis externa (Kiss et al.1997, Morris et al.2006). The results of some researchers are disagreeing with these findings. Sarıerler et al. (2004) have reported that 11.53% *S. aureus* and 5.12% coagulase-negative Staphylococci were isolated and *S. aureus* was the most frequent bacteria for canine otitis externa. *P. aeruginosa* was the next most common, followed by *P. mirabilis* and *E. coli*. Kuyucuoğlu and Sarıtaş (2010) indicated that the most frequently isolated microorganism from dog ears was *S.aureus* (31.5%), followed by *Streptococcus spp* (16.4%) and *Bacillus spp.* (12.3%). Similar to the results, Martin Barrasa et al. (2000) reported the incidence of Gram-negative bacteria isolated in their study corresponds with that reported previously: a high incidence of *Pseudomonas*, followed by *P. mirabilis* and *E. coli*. *S. canis* isolation rate was 2.5 % in dogs. Similar to this results, by Hariharan et al. (2006), *S. canis* rate was reported as 9.9% of otitic ears of dogs. On the contrary, Lyskova et al. (2007) reported were isolated 29.9% *S. canis* in dogs. The geographical location and previous drug use might be cause this argumentative results.

There are many bacteria in healthy ears as well as a small number of *Staphylococcus* genus which are the most common pathogens in otitis externa. Gram negative microorganisms are not routinely identified from the healthy ear canal. for this reason *P. aeruginosa, P. mirabilis, K. pneumoniae* and *E. coli* are important Gram-negative bacteria causing otitis externa (Penna et al. 2010). Also, *P. aeruginosa* is commonly isolated in otitis externa and often shows resistance to multiple antimicrobial agents, including fluoroquinolones (Colombini et al. 2000). In this study, *P.aeruginosa* isolates were resistant to enrofloxacin (40%) as the report but contrary susceptible to ciprofloxacin (90.7%). However, it has been well known fact that previous misusage of fluoroquinolones (ciprofloxacin, enrofloxacin or marbofloxacin) lead to the development of resistant to others (Gebru et al. 2011). The history of the individuals was investigated; excessive or inaccurate use of fluoroquinolones was not verified (unpublished data).

Sarıerler et al. (2004) indicated that the yeasts may be isolated from normal ear canals but if enviromental conditions are suitable, the otitis externa can be created by yeasts. *Malassezia pachydermitis* is the most common yeast isolated from otitis externa case. *Candida sp.* may also be found in canine otitis externa . In this study, mycological growth was also observed from 213 of 475 (44.8%) matching swabs. 149 isolates (70%) were *Malassezia* spp, and 64 isolates (30%) were *Candida* spp. These results are consistent with the findings of the other studies (Bernardo et al. 1998, Sarıerler et al. 2004, Cafarchia et al. 2005, Lyskova et al. 2007, Saridomichelakis et al. 2007).

Sfaciotte et al. (2015) reported that the major bacterial pathogens were *Staphylococcus* spp. (65.85%), *Pseudomonas* spp. (12.19%) and *Enterobacteria* species (19.51%) in 36 dogs with clinical otitis and they emphasized that the antimicrobial agents against this pathogens considered most resistant were penicillin (75%) and tetracycline (50%). In the current study, in a similar vein, tetracycline and penicillin resistance rates were found relatively high as 81.3% and 78.4% respectively. The lowest resistance rates were found to ciprofloxacin (28 %), enrofloxacin (33.7 %), amikacin (33.8 %) and cephoperazone (34.7 %). All of *P. aeruginosa* strains were resistant to eritromycin and % 97.5 to penicillin. In addition, all of *S.canis* strains were resistant to gentamicin, tetracycline, penicillin and ampicillin.

Aminoglycosides, such as amikacin and gentamicin, have been suggested for topical application in otitis externa caused by Gram-negative bacteria (Hariharan et al. 2006). In this study, amikacin (70%) and gentamicin (65.3%) were sensitive against to Gram-negative bacteria.

The patient material comprised 26 dog breeds, of which the Golden Retreiver (103, 21,7%) and the Cocker spaniels (90, 18,9%) were the most frequently affected. The cocker spaniel is said to be predisposed to the disease by its long, pendulous ears, its liking for water and the frequent entry of grass awns into the ear canal(Kiss 97) while, the Golden Retreiver dog may be predisposed by the hyperactivity of its cerumen producing glands.

CONCLUSION

Treatment of OE is generally challenging for the small animal practitioner due to multi-factorial structure of the disease, probability of long-term antimicrobial therapy usage. Consequently, bacterial culture and susceptibility test are very important factors for treatment success. Mycological culture should also be performed in infectious otitis externa cases of dogs.

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