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Aerobic Bacteria and Fungi Isolated from External Ear Canal of Healthy Dogs and the Antibiotic Susceptibility of Staphylococci

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Abstract: The study examined 200 swab samples, which were taken from the external auditory meatus of 100 clinically healthy dogs. The dogs were aged between 2-10 years and had erect ears. Bacteria, yeast and mould were isolated in 20 of the samples. In 74 samples, bacteria and mould were isolated. Yeast and mould were isolated in 22 samples. A single microorganism was present in 44 of the samples. In 40 samples, one or more agents were isolated. *Staphylococcus* sp. (59%), *Bacillus* sp. (15%) and *M. pachydermatis* (14%) were the most frequently isolated microorganisms. About 113 *staphylococcus* strains, which were isolated in the study were susceptible to all antibiotics. However, one *Staphylococcus chromogenes/hyicus* strain was resistant to clindamycin, erythromycin, oxacillin, rifampin, one *Staphylococcus* equorum strain and one *Staphylococcus* haemolyticus strain were resistant to oxacillin, one *Staphylococcus* equorum strain was resistant to clindamycin and one *Staphylococcus* hominis strain was resistant to erythromycin. These strains were susceptible to all other antibiotics. Consequently, bacterial factors, predominantly *Staphylococcus* sp., *Bacillus* sp. and *M. pachydermatis* can be pathogens via predisposed factors in the ear canal of healthy dogs. The bacteria isolated in the study were generally susceptible to antibiotics. Therefore factors, which are present in the normal flora should not be ignored and an antibiogram test should be used in the treatment of otitis externa.

Key words: Healthy dog, ear, bacteria, fungi, microorganism, erythromycin, Turkey

INTRODUCTION

Otitis externa-acute or chronic inflammation of external ear canal epithelium-(OE) is common in small animal veterinary practice with a prevalence from 5-20% and is a disease of multifactorial etiology (August, 1988; Carlotti, 1991; Angus, 2004). OE is seen more often in dogs than cats due to the anatomical structure of external ear canal (August, 1988; Rosychuk, 1994). Several studies of the etiology of OE have that temperature, humidity, dermatitis, trauma, age, sex, breed, presence of sebum, stress, pH, parasites, foreign bodies, hypersensitivities, autoimmune diseases, keratinization disorders and microorganisms such as bacteria and yeasts play a role in the pathogenesis of OE (Carlotti, 1991; Keskin *et al*., 1999).

The bacteria most frequently isolated from OE in dogs are coagulase-positive staphylococci (Cole *et al*., 1998; May *et al*., 2005; Lyskova *et al*., 2007). Other commonly isolated pathogens include *Proteus* sp., Coagulase-Negative Staphylococci (CNS), haemolytic streptococci, enterococci, *Escherichia coli*, *Citrobacter* sp., *Pasteurella* sp., Corynebacterium sp. and *Pseudomonas* sp. (Keskin *et al*., 1999; Junco and Barrasa, 2002). The most common yeasts in OE is Malassezia pachydermatis (Lorenzini *et al*., 1985; Bernardo *et al*., 1998; Cafarchia *et al*., 2005; Nardoni *et al*., 2006). As an opportunistic yeast *M. pachydermatis* has been isolated from the ear canals of between 15-50% of healthy dogs however, it has been reported that this rate increases in OE (Crespo *et al*., 2000). *Candida* sp., *Aspergillus* sp., *Microsporum* sp., *Trichophyton* sp., Sporothrix schenckii and Paecilomyces may also be isolated from the external otitis of dogs (Kumar *et al*., 2002; Fernandez *et al*., 2006).

In addition to clinical signs and examination of the ear the bacteria and yeasts, which are play a major role in the etiology of OE must be isolated and identified (Scott *et al*., 2001). Moreover, knowledge of the composition of the normal flora in the external ear canal is an important factor in determining the etiology and epidemiology of infectious OE. However, detailed studies about staphylococci that are most frequently isolated from OE and some other opportunistic bacteria and fungi

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in the external ear canals of healthy dogs are not adequate. The aims of this study are the isolation and determination of the species distribution of micro-organisms in the external ear canals of healthy dogs and determination of the susceptibility of isolated staphylococci strains to antimicrobial agents.

MATERIALS AND METHODS

In this study, 200 samples were from the external ear canals of 100 healthy dogs. The dogs were crossbreeds and aged between 2-10 years, the group comprised 50 females and 50 males. All 100 dogs had erect ears. Samples were immediately transferred to the laboratory for culture.

Bacteriological cultures were grown on blood agar supplemented with 5% sheep blood, Edwards medium, MacConkey Agar and Mannitol Salt Agar (all Oxoid) and were incubated at 37°C for 24-48 h in aerobic conditions. The biochemical and physiological identification of all the strains was performed using the BD Phoenix™ 100 Automated Microbiology System (BD Diagnostic Systems, Sparks, USA)

Mycological culture swabs were cultured onto modified Dixon medium and Sabouraud dextrose agar with additional chloramphenicol (0.5%) and cycloheximide (0.5%). The Dixon's agar was incubated at 32°C and the Sabouraud's dextrose agar at room temperature (25°C) for up to10 days with daily monitoring. Fungal identification was based both on macroscopic appearance of colonies and microscopic cell morphology. In particular, *M. pachydermatis* was identified microscopically by its morphology and ability to grow on the medium without lipid supplementation (Sabouraud Dextrose Agar, Biolife) (Larone, 2002). The antibiotics susceptibility tests of *Staphylococcus* sp. isolated and identified from ear swabs were carried out using BD Phoenix™ 100 Automated Microbiology System. The antimicrobial cefazoline, Clindamycin, levofloxacin, linezolid, Moxifloxacin, ofloxacin, oxacillin, rifampin, tetracycline, sulfamethoxazol/trimethoprim (all Oxoid).

RESULTS AND DISCUSSION

In the study, samples obtained from the external ear canal of 100 healthy dogs were processed. Bacterial and/or fungal growth was observed in all samples. *Staphylococcus* sp. (46.7%), *Bacillus* sp. (23.0%) and *Malassezia pachydermatis* (10.7%) were the microorganisms most frequently isolated inform the external ear canal.

A single microorganism was present in 44 of the samples. In 40 samples, one or more agents were isolated. Bacteria, yeast and mould were isolated in 20 of the samples. Yeast and mould were isolated 22 of the samples. Bacteria and mould were isolated in 74 samples (Table 1).

Antimicrobial susceptibility tests: The susceptibility to antimicrobial agents was determined for *Staphylococcus* sp., (28 *S. felis*, 16 *S. capitis* sp. capitis, 12 *S. haemolyticus*, 8 *S. cohnii* sp. cohnii, 8 *S. simulans*, 6 *S. chromogenes/hyicus*, 6 *S. hominis*, 5 *S. intermedius*, 4 *S. schleiferi*, 4 *S. schleiferi* sp. coagulans, 4 *S. schleiferi* sp. schleiferi, 4 *S. warneri*, 4 *S. lugdunensis*, 3 *S. equorum*, 3 *S. aureus*, 2 *S. carnosus*, 1 *S. capitis*).

Table 1: Occurrence of microbial species from external ear canal samples of healthy dogs

Isolated bacteria	No. of isolates (n) and ratio (%)	Isolated bacteria	No. of isolates (n) and ratio (%)
Staphylococcus felis	28 (14)	*Arcanobacterium pyogenes*	6 (3)
Staphylococcus capitis sp. capitis	16 (8)	*Cellumonas turbata*	4 (2)
Staphylococcus haemolyticus	12 (6)	*Streptococcus pneumoniae*	4 (2)
Staphylococcus cohnii sp. cohnii	8 (4)	*Streptococcus agalactiae*	1 (0.5)
Staphylococcus simulans	8 (4)	*Corynebacterium matruchotii*	4 (2)
Staphylococcus chromogenes/hyicus	6 (3)	*Alloiococcus otitidis*	2 (1)
Staphylococcus hominis	6 (3)	*Gemella haemolysans*	2 (1)
Staphylococcus intermedius	5 (2.5)	*Kytococcus sedentarius*	2 (1)
Staphylococcus schleiferi	4 (2)	*Dermacoccus nishinomycensis*	2 (1)
Staphylococcus schleiferi sp. coagulans	4 (2)	*Pediococcus parvulus*	2 (1)
Staphylococcus schleiferi sp. schleiferi	4 (2)	*Aerococcus urinae*	1 (0.5)
Staphylococcus warneri	4 (2)	*Kocuria rosea*	1 (0.5)
Staphylococcus lugdunensis	4 (2)	*Globicatella sanguinus*	1 (0.5)
Staphylococcus equorum	3 (1.5)	**Isolated fungus**	
Staphylococcus aureus	3 (1.5)	*Malasseia pachydermatis*	28 (14)
Staphylococcus carnosus	2 (1)	*Candida* sp.	15 (7.5)
Staphylococcus capitis	1 (0.5)	*Mucor* sp.	14 (7)
Bacillus circulans	26 (13)	*Aspergillus* sp.	34 (17)
Bacillus coagulans	4 (2)	*Penicillium* sp.	42 (21)
Leifsonia aquatica	12 (6)	*Alterneria* sp.	10 (5)
Gardneralla vaginalis	8 (4)		
Micrococcus lylae	8 (4)		

All *Staphylococcus* sp. were susceptible to the antimicrobial agent tested, except that 1 *S. chromogenes/hyicus* was resistant to clindamycin, erythromycin, oxacillin and rifampin; 1 *S. equorum* and 1 *S. haemolyticus* starins were resistant to oxacillin 1 *S. equorum* was resistant to clindamycin, 1 *S. hominis* starin was resistant to erythromycin.

Otitis externa is prevalent in dogs and bacteria, yeast and fungi are the causes of the disease due to predisposed factors (Bornand, 1992). Damage resulting from a primary cause in the ear canal can cause microorganisms in microflora to form the disease secondarily. Therefore, it would be useful to know in detail the typical microflora of the external ear in healthy dogs. However, studies on this issue are limited. Lyskova *et al.* (2007) isolated mostly *Staphylococcus* sp. (46.7%), *Bacillus* sp. (23.0%) and *M. pachydermatis* (10.7%) from the external ear of healthy dogs. In another study, 154 swab samples were taken from the external ear of healthy dogs and of these samples, 15.5% *Staphylococcus* sp., 12.3% *M. pachydermatis* were isolated as the most frequent agents (Yoshida *et al*., 2002). According to the results of the study, *Staphylococcus* sp. (59%), *Bacillus* sp. (15%) and *M. pachydermatis* (14%) were the most frequently isolated microorganisms.

Yamashita *et al.* (2005) isolated *S. intermedius* (39.3%), *S. aureus* (3.6%), *S. schleiferi* sp. coagulans (3.6%), *S. capitis* (7.1%), *S. caprae* (7.1%), *S. chromogenes* (3.6%), *S. epidermidis* (25%), *S. hominis* (3.6%), *S. lentus* (3.6%), *S. saprophyticus* (7.1%), *S. sciuri* (3.6%), *S. warneri* (10.7%) and *S. xylosus* (10.7%) from ear canal samples taken from healthy dogs. May *et al.* (2005) isolated two schleiferi sp. schleiferi and a *S. schleiferi* sp. coagulans from the ears of 13 healthy dogs. Igimi *et al.* (1990) isolated *S. schleiferi* sp. coagulans in otitis externa cases. In the study, isolated staphylococci species were *S. felis* (14%), *S. capitis* sp. capitis (8%), *S. haemolyticus* (6%), *S. cohnii* sp. cohnii (4%), *S. simulans* (4%), *S. chromogenes/hyicus* (3%), *S. hominis* (3%), *S. intermedius* (2.5%), *S. schleiferi* (2%), *S. schleiferi* sp. coagulans (2%), *S. schleiferi* sp. schleiferi (2%), *S. warneri* (2%), *S. lugdunensis* (2%), *S. equorum* (1.5%), *S. aureus* (1.5%), *S. carnosus* (1%), *S. capitis* (0.5%). Staphylococci species isolated in the study are comparable with the findings of previous researchers. Lyskova *et al.* (2007) isolated *Micrococcus* sp. (6.2%), Streptococcus canis (2.8%), viridans streptococci (7.3%), non-hemolytic streptococci (0.6%), *Enterococcus* sp. (4%), *Bacillus* sp. (23%), *Corynebacterum* sp. (9.6%), *E. coli* (0.6%), *Klebsiella* sp.(0.6%), *Proteus* sp.(3.9%) and staphylococci in healthy dogs. In the study, *Bacillus circulans* (13%), *Bacillus coagulans* (2%), *Leifsonia aquatica* (6%), *Gardnerella vaginalis* (4%), *Micrococcus lylae* (4%), *Arcanobacterium pyogenes* (3%), *Cellumonas turbata* (2%), *Streptococcus pneumoniae* (2%), *Streptococcus agalactiae* (0.5%), *Corynebacterium matruchotii* (2%), *Alloiococcus otitidis* (1%), *Gemella haemolysans* (1%), *Kytococcus sedentarius* (1%), *Dermacoccus nishinomycensis* (1%), *Pediococcus parvulus* (1%), *Aerococcus urinae* (0.5%), *Kocuria rosea* (0.5%) *Globicatella sanguinus* (0.5%) were isolated in addition to staphylococci. The findings of the study are similar with the findings of other researchers, since the study was performed according to current nomenclature and identification on the basis of species.

Previous studies indicate that the most commonly isolated yeast in dog otitis cases is M. pachydermatis. (Cafarchia *et al*., 2005; Nardoni *et al*., 2005; Girao *et al*., 2006). The study of 200 swabs included 28 (14%) examples of *M. pachydermatis*. Lyskova *et al.* (2007) isolated *M. pachydermatis* in 10.7% of cases. In another study, of 154 swab samples taken from 77 healthy dogs, *M. pachydermatis* was isolated in 12.3% of cases (Yoshida *et al*., 2002). According to culture analysis of Gustafson (1955) and Gedek *et al.* (1979) the isolation rates of *M. pachydermatis* were 5.0 and 17.0% respectively. The isolation rates of the study are comparable with the findings of other researchers (Lyskova *et al*., 2007; Yoshida *et al*., 2002; Gustafson, 1955; Gedek *et al*., 1979). Kumar *et al.* (2002) Isolated *Candida* sp., *Aspergillus fumigatus* and *M. pachydermatis* in samples taken from healthy dogs. In the study, *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., *Mucr* sp. and *Candida* sp. were isolated in addition to *M. pachydermatis*.

According to studies, an increasing resistance to antimicrobials presents a problem in the treatment of otitis externa (Blue and Wooley, 1977 Keskin *et al*., 1999; Lilenbaum *et al*., 2000; Oliveira *et al*., 2000; Hariharan *et al*., 2006). In a study by Yamashita *et al.* (2005), 38 strains (59.4%) of 64 staphylococci strains of 15 species isolated from healthy dogs and dogs with otitis externa showed resistance to one or more of the 17 antibiotics tested. Researchers (Yamashita *et al*., 2005) detected resistance to penicillin G, ampicillin, kanamycin, erythromycin, norfloxacin, lincomycin, streptomycin, ciprofloxacin, ofloxacin, tetracycline and gentamicin, respectively with the percentages of 56.3, 54.7, 14.1, 14.1, 9.4, 7.8, 6.3, 3.1, 3.1, 3.1 and 1.6%. Junco and Barrasa (2002) indicated that 67 coagulase-positive staphylococci strains isolated from healthy dogs and dogs with otitis externa, had resistance to 16 tested antibiotics with the percentage of 35.8%.

Another study (Lyskova *et al*., 2007) detected that 100 *S. intermedius* strains isolated from the health dogs and dogs with otitis externa has resistance to Penicillin G, ampicillin, erythromycin, chloramphenicol, clindamycin, tetracycline, teicoplanin respectively with the percentages of 66, 7, 44, 34, 39, 35 and 8%, respectively. However, they were 100% susceptible to amoxicillin/clavulanic acid methicillin, oxacillin, piperacillin, ciprofloxacin, enrofloxacin, ofloxacin, gentamicin and vancomycin.

Lyskova *et al*. (2007) reported that isolated 48 KNS strains had resistance to Penicillin G, methicillin, oxacillin, ciprofloxacin erythromycin, chloramphenicol, clindamycin, tetracyclin, teicoplanin respectively with the percentages of 43.8, 6.3, 8.3, 2.1, 37.5, 12.5, 14.6, 25 and 2.1%. However, they were 100% susceptible to amoxicillin/clavulanic acid enrofloxacin, gentamicin and vancomycin. There was no significant difference in the susceptibility of strains to antibiotics in the three previous studies used for comparison. The study isolated *S. felis*, *S. capitis* sp. capitis, *S. haemolyticus*, *S. cohnii* sp. cohnii, *S. simulans*, *S. chromogenes/hyicus*, *S. hominis*, *S. schleiferi*, *S. schleiferi* sp. coagulans, *S. schleiferi* sp. schleiferi, *S. warneri*, *S. lugdunensis*, *S. equorum*, *S. aureus*, *S. intermedius*, *S. carnosus*, *S. capitis*. In total, 113 strains were susceptible to all antibiotics tested in the study. However, one *S. chromogenes/hyicus* strain was resistant to clindamycin, erythromycin, oxacillin and rifampin, one *S. equorum* strain and one *S. haemolyticus* strain were resistant to oxaacillin, one *S. equorum* strain was resistant to clindamycin and one *S. hominis* strain was resistant to erythromycin but they were susceptible to all other antibiotics.

In the study, 100% susceptibility to ciprofloxacin, ofloxacin and gentamicin was detected, which is compatible with of the findings reported by Lyskova *et al*., 2007. Susceptibility percentages of the study are higher than the findings of the other studies used for comparison. This may result from two factors: the dogs used in the study did not receive antibiotic treatment beforehand and there are regional differences in drug-resistance.

CONCLUSION

The bacterial factors, predominantly *Staphylococcus* sp., *Bacillus* sp. and *M. pachydermatis* may be pathogenic via predisposed factors in the ear canal of healthy dogs. These bacteria, when isolated were generally susceptible to antibiotics. Therefore, factors which are present in the normal flora should not be ignored. Furthermore, an antibiogram test can be useful in the treatment of otitis externa, since antibiotic resistance can be transferred due to the similarity of antibiotics used for people and small animals and people and animals are often in close contact.

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