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/haemolyticus* 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 pathogenic 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 the external ear canal epithelium (OE) is common in small animals. It is seen in 5-20% of small animals 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 the 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; Caffarelli et al., 2005; Nardon 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., *Mucor* 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 played 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 microorganisms 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 ear drops. 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 to 10 days with daily monitoring. Fungal identification was based 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 cefazolin, Clindamycin, levofloxacin, linezolid, Moxifloxacin, ofloxacin, oxacillin, rifampin, tetracycline, sulfamethoxazole/trimethoprim (all Oxid).

**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 from 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).

<table>
<thead>
<tr>
<th>Isolated bacteria</th>
<th>No. of isolates (n) and ratio (%)</th>
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<th>No. of isolates (n) and ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus felis</em></td>
<td>28 (14)</td>
<td>Arcanobacterium pyogenes</td>
<td>6 (3)</td>
</tr>
<tr>
<td><em>Staphylococcus capitis</em> sp. capitis</td>
<td>16 (8)</td>
<td>Cellumonos turbata</td>
<td>4 (2)</td>
</tr>
<tr>
<td><em>Staphylococcus haemolyticus</em></td>
<td>12 (6)</td>
<td>Streptococcus pneumoniae</td>
<td>4 (2)</td>
</tr>
<tr>
<td><em>Staphylococcus cohnii</em> sp. cohnii</td>
<td>8 (4)</td>
<td>Streptococcus agalactiae</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td><em>Staphylococcus simulans</em></td>
<td>8 (4)</td>
<td>Corynebacterium matruchotii</td>
<td>4 (2)</td>
</tr>
<tr>
<td><em>Staphylococcus chromogenes</em> hyicus</td>
<td>6 (3)</td>
<td>Aliiococcus otitidis</td>
<td>2 (1)</td>
</tr>
<tr>
<td><em>Staphylococcus hominis</em></td>
<td>6 (3)</td>
<td>Gemella haemolytica</td>
<td>2 (1)</td>
</tr>
<tr>
<td><em>Staphylococcus intermedius</em></td>
<td>5 (2.5)</td>
<td>Kytococcus sedentarius</td>
<td>2 (1)</td>
</tr>
<tr>
<td><em>Staphylococcus schleiferi</em></td>
<td>4 (2)</td>
<td>Dermacoccus nathanencomycetes</td>
<td>2 (1)</td>
</tr>
<tr>
<td><em>Staphylococcus schleiferi</em> sp. coagulans</td>
<td>4 (2)</td>
<td>Pedococcus parvulus</td>
<td>2 (1)</td>
</tr>
<tr>
<td><em>Staphylococcus schleiferi</em> sp. schleiferi</td>
<td>4 (2)</td>
<td>Aerococcus urine</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td><em>Staphylococcus warneri</em></td>
<td>4 (2)</td>
<td>Kocuria rosea</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td><em>Staphylococcus lugdunensis</em></td>
<td>4 (2)</td>
<td>Glohicellula sanguinis</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td><em>Staphylococcus equorum</em></td>
<td>3 (1.5)</td>
<td><em>Isolated fungus</em></td>
<td></td>
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<tr>
<td><em>Staphylococcus aurus</em></td>
<td>3 (1.5)</td>
<td>Malassezia pachydermatis</td>
<td>29 (14)</td>
</tr>
<tr>
<td><em>Staphylococcus carnosus</em></td>
<td>2 (1)</td>
<td><em>Candida</em> sp.</td>
<td>15 (7.5)</td>
</tr>
<tr>
<td><em>Staphylococcus capitis</em></td>
<td>1 (0.5)</td>
<td><em>Mucor</em> sp.</td>
<td>14 (7)</td>
</tr>
<tr>
<td><em>Bacillus circulans</em></td>
<td>26 (13)</td>
<td>Aspergillus sp.</td>
<td>34 (17)</td>
</tr>
<tr>
<td><em>Bacillus coagulans</em></td>
<td>4 (2)</td>
<td><em>Penicillium</em> sp.</td>
<td>42 (21)</td>
</tr>
<tr>
<td><em>Leuconostoc ammoea</em></td>
<td>12 (6)</td>
<td><em>Alz annoy</em> sp.</td>
<td>10 (5)</td>
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<tr>
<td><em>Gardnerella vaginalis</em></td>
<td>8 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Micrococcus lycae</em></td>
<td>8 (4)</td>
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</tbody>
</table>
All *Staphylococcus* sp. were susceptible to the antimicrobial agent tested, except that 1 *Staphylococcus hominis* was resistant to clindamycin, erythromycin, oxacillin and rifampin; 1 *S. equorum* and 1 *S. haemolyticus* strains were resistant to oxacillin 1 *S. equorum* was resistant to clindamycin, 1 *S. hominis* strain 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. sanguinis* (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. Imai 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*/*lycicus* (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 carni* (2.8%), viridans streptococci (7.3%), non-hemolytic streptococci (0.6%), *Enterococcus* sp. (4%), *Bacillus* sp. (23%), *Corynebacterium* sp. (9.6%), *E. coli* (0.6%), *Klebsiella* sp. (0.6%), *Proteus* sp. (3.5%) and staphylococci in healthy dogs. In the study, *Bacillus circulans* (13%), *Bacillus coagulans* (2%), *Listeria monocyctogenes* (6%), *Gardnerella vaginalis* (4%), *Micrococcus luteus* (4%), *Arachosia pyogenes* (3%), *Clostridium tetani* (2%), *Streptococcus pneumoniae* (2%), *Streptococcus agalactiae* (0.5%), *Corynebacterium matruchoti* (2%), *Alloccocus otitidis* (1%), *Gemella haemolytica* (1%), *Kytococcus sedentarius* (1%), *Dermacoccus nishimiyae* (1%), *Pedococcus parvulus* (1%), *Aerococcus urinae* (0.5%), *Kocuria rosea* (0.5%) *Globicatella sanguinis* (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., *Mucor* 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 Woolery, 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 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, tetracycline, 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 oxacillin, 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.

REFERENCES


