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Research Article

Antibiotic Susceptibility Patterns of *Pseudomonas aeruginosa* Strains Isolated from Dogs with Otitis Externa

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Abstract: The aims of this study were to isolate *Pseudomonas aeruginosa* from dogs with otitis externa (OE), to determine the susceptibility of isolated strains to antibiotics, and to evaluate the incidence of the infection in the Aydın region. For this purpose, ear swab samples were obtained from 92 dogs with symptoms of OE infection and the samples were examined bacteriologically. Bacteria were isolated from 88 (96%) of 92 samples but no isolation was performed on 4 (4%) dogs. In total 93 microorganisms were identified. Among these microorganisms, staphylococci species were the most isolated one in number (43%), followed by *P. aeruginosa* (17%), *Escherichia coli* (11%), *Proteus* spp. (9%), *Streptococcus* spp. (9%), *Pasteurella* spp. (3%), *Citrobacter* spp. (3%), *Corynebacterium* spp. (3%), and *Enterococcus* spp. (2%). Of the 16 *P. aeruginosa* strains examined, 13 (81%) were sensitive to gentamycin, 12 (75%) to penicillin G, 7 (44%) to danofloxacin, 6 (38%) to streptomycin, 3 (19%) to ampicillin, 2 (13%) to lincomycin, and 2 (13%) to tetracycline. In conclusion, gentamycin and penicillin could be more effective for the treatment of OE caused by *P. aeruginosa* in the Aydın region.

Key Words: *Pseudomonas aeruginosa*, otitis externa, dog

Otitis Eksternalı Köpeklerden İzole Edilen *Pseudomonas aeruginosa* Suşlarının Antibiyotik Duyarlılık Şekilleri

Özet: Bu çalışmanın amaçları otitis eksternalı (OE) köpeklerden *Pseudomonas aeruginosa*'nın izolasyonu, izole edilen suşların antibiyotiklere duyarlılıklarının ve Aydın yöresinde otitis eksternanın insidensinin belirlenmesidir. Bu amaçla, OE infeksiyonu semptomu bulunan 92 köpekten kulak sıvap örnekleri alındı ve bunlar bakteriyolojik olarak incelendi. Alınan 92 kulak sıvabının 88 (% 96) tanesinden izolasyon yapılırken; 4 (% 4)'ünden izolasyon gerçekleştirilemedi. Toplam 93 mikroorganizma identifikasyonu yapıldı. Bu mikroorganizmalar arasında en yüksek oranda *Staphylococcus* ssp. (% 43) izolasyonları gerçekleştirildi. Bu mikroorganizmayı sırası ile *Pseudomonas aeruginosa* (% 17), *Escherichia coli* (% 11), *Proteus* spp. (% 9), Streptecoccus spp. (% 9), *Pasteurella* spp. (% 3), *Citrobacter* spp. (% 3), *Corynebacterium* spp. (% 3) ve *Enterococcus* spp. (% 2) izledi. İncelenen 16 *P. aeruginosa* suşunun 13 (% 81)'ü gentamisine, 12 (% 75)'si penisillin G'ye, 7 (% 44)'si danofloksasine, 6 (% 38)'sı streptomisine, 3 (% 19)'ü ampisilline, 2 (% 13)'si linkomisine ve 2 (% 13)'si tetrasikline duyarlı bulundu. Sonuç olarak, Aydın yöresinde *P. aeruginosa*'nın neden olduğu OE'nın tedavisinde gentamisin ve penisillinin faydalı olabileceği düşünülmektedir.

Anahtar Sözcükler: *Pseudomonas aeruginosa*, otitis eksterna, köpek

Introduction

Otitis externa (OE) is defined as acute or chronic inflammation of the epithelium of the external auditory canal, usually considered the most common disease of the ear canal in the dog (1). Bacterial OE is a common disease of dogs. Although OE is not a life-threatening disease, it can be frustrating for both the patient and the owner. Antibiotic susceptibility tests are done for the treatment of chronic OE (1,2).

The etiology of canine OE is complicated and involves many aspects that can be classified as predisposing, primary, and perpetuating. Microorganisms are considered perpetuating factors as they are responsible for the aggravation of the process. Common organisms isolated from dogs with OE include *Staphylococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Streptococcus* spp., *Escherichia coli*, *Klebsiella* spp., and *Pasteurella* spp. (2,3).

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Pseudomonas aeruginosa is a Gram-negative, glucose-nonfermenting aerobic bacterium. *P. aeruginosa,* either alone or in combination with other microorganisms like *Proteus mirabilis,* is the dominant species in chronic supurative OE (4,5). It was reported that *P. aeruginosa* was isolated from 9.64% of the dogs suffering OE (6). Akay et al. (7) reported that *P. aeruginosa, Staphylococcus aureus*, *S. intermedius, P. mirabilis, Escherichia coli, Corynebacterium pyogenes, Streptococcus pyogenes, Citrobacter* spp., and anthracoid agents were isolated from dogs with OE at the percentages of 27%, 18%, 18%, 12%, 9%, 6%, 3%, 3%, and 3%, respectively. Uchida et al. (8) reported that various microorganisms (*S. intermedius*, *S. hycus, S. haemolyticus*, *Pseudomonas* spp., *Bacillus* spp., *S. aureus*, *Corynebacterium* spp., and *Candida* spp.) may be involved in the etiology of OE in dogs.

In most of the previous studies, the antibiotic susceptibility of the microorganisms isolated from dogs with OE is variable (9-15). However, the most important problem in the treatment of OE caused by *P. aeruginosa* strains is the development of fast and high antibacterial resistance (4,5,9). There is evidence indicating that the efficacy of enrofloxacin was relatively poor, when all bacterial isolates (38% susceptibility) as well as Gram-negative bacilli (25% susceptibility) were considered (10). Martín Barrasa et al. (11) reported that *Pseudomonas* strains from chronic canine OE were sensitive to tobramycin (100% susceptible), marbofloxacin (91%), ceftazidime (91%), ticarcillin (86%), and gentamicin (65%), whereas lower susceptibility was found when enrofloxacin (52%) was administered probably due to its indiscriminate use. However, many practitioners treat OE on the basis of their clinical impressions and an examination of a stained smear of the exudate. If the smear shows only Gram-positive cocci, they may use a drug that is likely to be effective against staphylococci, streptococci, and enterococci. If the smear shows Gram-negative rods in large numbers, they need to consider that the rods represent multi-drug resistant strains of *P. aeruginosa* (14).

Many factors influence antimicrobial susceptibilities of a specific bacterium. Prevalence and etiological reasons of OE vary depending on the geographical area. In the available literature, there is no report concerning the antibiotic susceptibility of *P. aeruginosa* causing OE in the Aydın region. Thus, the aim of this study was to isolate *P. aeruginosa* from the dogs with OE to determine their susceptibility to the antibiotics of isolated strains and to evaluate the incidence of this disease in the Aydın region.

Materials and Methods

Samples: A total of 92 dogs suffering OE from different veterinary clinics in Aydın region were included in this study between January and December in 2004. No antibiotic had been administered to the dogs before the ear swabs were obtained. The samples were collected from the horizontal portion of the external ear canal with swabs moistened with sterile saline solution. They were sent to the laboratory in transport medium (Aimes). The breeds, age, and sex of the dogs are shown in Table 1.

Table 1. The breeds, age, and sex of dogs.

Breeds of dogs	No. of examined samples	No. of isolated samples	Isolation rate (%)	Of *P. aeruginosa*					
				n (Isolation number)	Ages of the dogs			Sex	
					0-3	4-6	> 7	Female	Male
Poodle	24	23	26	4	2	2	-	3	1
Cocker spaniel	23	22	25	7	2	4	1	2	5
Terrier	23	22	25	4	1	2	1	3	1
Labrador retriever	11	11	13	-	-	-	-	-	-
Boxer	6	5	6	1	1	-	-	1	-
German shepherd	3	3	3	-	-	-	-	-	-
Pointer	2	2	2	-	-	-	-	-	-
Total	92	88	100	16	6	8	2	9	7

Bacteriological examinations: For isolation, sheep blood agar (Difco) and MacConkey Agar (Difco); for passage of microorganisms, Nutrient Broth (Difco) and Tripticase Soya Broth (Difco); and for antibiotic susceptibility test, Mueller Hinton Agar (Difco) was used.

Ear swab samples were streaked onto sheep blood agar (Columbia agar with 5% sheep blood) and MacConkey agar plates. Plates were incubated at 37 °C in aerobic conditions and examined daily for 5 days for the presence of bacterial growth. The isolated bacteria were identified on the basis of cultural, morphological, and biochemical characteristics (16). The isolates were regarded as significant in causing the condition if there was serious growth of a single bacterial species in a mixed culture or a moderate growth in a pure culture (14).

Antibiotic sensitivity test: Antibiotic sensitivity was tested using disk diffusion (17) with the following antibiotics: penicillin G (10 IU), gentamycin (10 µg), danofloxacin (5 µg), ampicillin (10 µg), streptomycin (10 µg), trimethoprim-sulphamethoxazole (1.25 µg/23.75 µg), tetracycline (30 µg), and lincomycin (15 µg). Isolates were categorized as susceptible (S), moderately susceptible (I), and resistant (R), based upon the interpretive criteria developed by the Clinical and Laboratory Standards Institute (CLSI) (18).

Results

Bacteriological examination: Bacteria were isolated from 88 (96%) of the 92 ear swab samples, whereas the isolation of 4 samples (4%) was not performed. A total of 93 microorganisms were identified from the samples. The cultures of 92 ear swabs with Gram-negative bacteria yielded 40 strains: 16 (17% of all isolates) *P. aeruginosa,* 10 (11%) *E. coli,* 8 (9%) *Proteus* spp., 3 (3%) *Pasteurella* spp., and 3 (3%) *Citrobacter* spp. The most common Gram-positive species recovered from the 93 dog isolates was 22 (24% of all isolates) coagulase-positive staphylococci (CoPS), followed by 18 (19%) coagulase-negative staphylococci (CoNS, not identified to species level), 8 (9%) *Streptococcus* spp., 3 (3%) *Corynebacterium* spp., and 2 (2%) *Enterococcus* spp. All isolated microorganisms were considered etiological agents in the disease. Monthly incidence and frequency of the organisms isolated from OE are given in Table 2.

P. aeruginosa was the most often isolated Gram-negative microorganism (17/40; 43%) and it was the most frequently isolated in August (5 dogs). *P. aeruginosa* was the most frequently isolated from cocker spaniel (7/23; 30%), terrier (4/23; 17%), boxer (1/6; 17%), and poodle (4/24; 16%) suffering OE (Table 1).

Table 2. Monthly incidence and frequency of organisms isolated from otitis externa.

Isolation microorganism	Isolated number (n) and rate (%)	Months											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
CoPS	22 (24)	1	2	2	2	1	3	4	2	2	1	1	1
CoNS	18 (19)	1	1	2	1	2	2	2	1	2	3	1	-
P. aeruginosa	16 (17)	-	-	-	-	2	3	2	5	2	1	1	-
E. coli	10 (11)	1	2	1	1	1	1	-	2	1	-	-	-
Proteus spp.	8 (9)	-	-	-	-	1	1	1	1	2	1	1	-
Streptococcus spp.	8 (9)	-	1	1	1	-	-	2	-	-	-	2	1
Pasteurella spp.	3 (3)	1	-	-	-	-	-	1	-	-	1	-	-
Citrobacter spp.	3 (3)	-	-	-	1	-	-	-	-	1	-	-	1
Corynebacterium spp.	3 (3)	-	1	-	-	1	-	-	1	-	-	-	-
Enterococcus spp.	2 (2)	-	-	-	-	-	-	-	-	-	1	1	-
TOTAL	93 (100)	4	7	6	6	8	10	12	12	10	8	7	3

It was determined that *P. aeruginosa* was the most frequently identified in 4- to 6-year-old dogs (8/16; 50%) and followed by 0- to 3-year-old (6/16; 38%), and 7-year-old and older dogs (2/16; 13%) with OE. Moreover, the pathogen was more frequently isolated in females (9/16; 56%) compared to males (7/16; 44%).

Antibiotic sensitivity test: Thirteen of the 16 *P. aeruginosa* strains examined (81%) were sensitive to gentamycin, 12 (75%) to penicillin G, 7 (44%) to danofloxacin, 6 (38%) to streptomycin, 3 (19%) to ampicillin, 2 (13%) to lincomycin, and 2 (13%) to tetracycline. Most of the *P. aeruginosa* isolates (14 strains) were found resistant to (88%) trimethoprim-sulphamethoxazole. Results of in vitro sensitivity testing of 16 *P. aeruginosa* isolates are given in the Figure.

The results of the antimicrobial susceptibility testing of the 6 most common bacterial isolates are summarized in Table 3.

All of the Gram-positive and Gram-negative tested microorganisms were highly susceptible to gentamycin and penicillin. Resistance was most frequently detected to trimethoprim-sulphamethoxazole and lincomycin in Gram-positive microorganisms, and streptomycin, lincomycin, tetracycline, and trimethoprim-sulphamethoxazole in Gram-negative microorganisms.

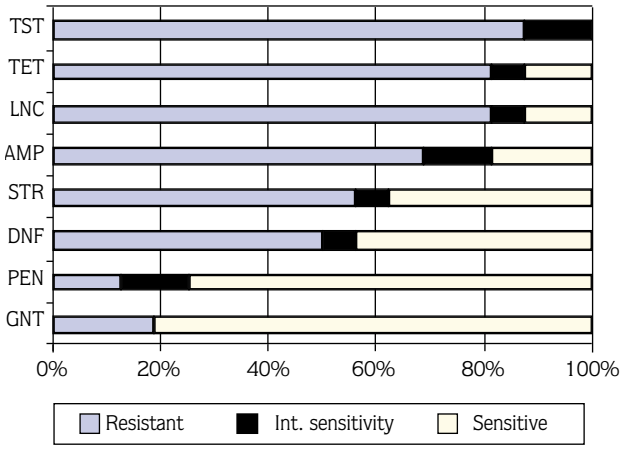

Figure. Results of in vitro sensitivity testing of 16 *P. aeruginosa* isolates.

Discussion

Development of antibiotic resistance is of great concern. The treatment of infections caused by *P. aeruginosa* is getting more difficult because of widespread resistance, existing multi-resistance patterns, and the development of quick resistance during treatment (1,2,5). It is crucial to determine the convenience of antimicrobial susceptibility tests in animal infections.

Various microorganisms may be involved in the etiology of OE in dogs. In the present study, 93 strains

Table 3. Results of antimicrobial susceptibility testing of the 6 most common bacterial isolates from dogs with otitis externa.

	CoPS n = 22, S(%)	CoNS n = 18, S(%)	*P. aeruginosa* n = 16, S(%)	*E. coli* n = 10, S(%)	*Proteus* spp. n = 8, S(%)	*Streptococcus* spp. n = 8, S(%)
GNT	14(70)	16(89)	13(81)	8(80)	8(100)	6(75)
PEN	13(59)	11(61)	12(75)	7(70)	7(88)	8(100)
DNF	16(73)	18(100)	7(44)	8(80)	8(100)	7(88)
STR	11(50)	7(39)	6(38)	1(10)	2(25)	4(50)
AMP	11(50)	10(56)	3(19)	6(60)	7(88)	7(88)
LNC	8(36)	5(28)	2(13)	0(0)	0(0)	3(38)
TET	13(59)	15(83)	2(13)	3(30)	5(63)	3(38)
TST	2(9)	4(22)	0(0)	5(50)	3(38)	3(38)

S = number of sensitive isolates

n = Isolate numbers

GNT: Gentamycin; PEN: Penicillin G; DNF: Danofloxacin; STR: Streptomycin; AMP: Ampicillin; LNC: Lincomycin; TET: Tetracycline; TST: Trimethoprim-sulphamethoxazole

were isolated. Fifty-three of these strains were identified as Gram-positive bacteria and 40 as Gram-negative bacteria. Keskin et al. (6) reported that the pathogens chiefly involved were *S. aureus* (47%), *Pseudomonas* spp. (10%), *S. epidermidis* (7%), *Proteus* spp. (5%), *E. coli* (2%), *Streptecoccus* spp. (2%), *Pasteurella* spp. (1%), and *Citrobacter* spp. (1%). In another study (8), of the strains isolated, 8 were identified as *S. intermedius*, 6 as *S. hyicus*, 5 as *S. haemolyticus*, 2 as *Pseudomonas* spp., 2 as *Bacillus* spp., 1 as *S. aureus*, and 1 as *Corynebacterium* spp. Kumar and Rao (12) isolated *Staphylococcus* spp. (75%), *Streptococcus* spp. (60%), *E. coli* (25%), and *Pseudomonas* spp. (18%). Hariharan et al. (14) reported that the most common species recovered from 1819 canine isolates were *S. intermedius*, followed by *P. aeruginosa, Streptecoccus* spp., *Proteus* spp., *E. coli*, and *Enterococcus* spp. The proportions of the microorganisms isolated in the present study are not different from those of previous studies.

P. aeruginosa was isolated from 16 of the 93 microorganisms (17%) in the present study. The findings here agreed with the previous studies (12,14,15). The incidence of Gram-negative bacteria isolated in our study corresponds with the previous studies: a high incidence of *Pseudomonas* spp. followed by *Proteus* spp. and *E. coli* (5,11,14).

The CoPS were the most prevalent species in our study, representing 24% of the isolates. This was an expected result, since previous studies reported that CoPS species are the most frequent isolates in OE of dogs (6,14,15). CoNS species as a group constitute a major component of the normal microflora of human beings, dogs, and cats, and are considered important opportunistic pathogens in those species. The presences of these staphylococcal species in animals have received little attention to date (19). Further studies are necessary in order to evaluate the real role of CoNS species in OE of the dogs.

In a previous study, it was observed that there was a high correlation between species and the hair length of dogs with OE (20). According to this study, OE was clinically more often seen in some breeds with longer hair such as spaniel cocker, terrier, and poodle. In accordance with this finding, the most infected dog breeds in the present study are also spaniel cocker, terrier, and poodle.

Another study reported that OE most frequently occurred in 4- to 6-year-old dogs and the findings in the present study agree with this (21).

Infectious diseases and their treatment are an important part of veterinary practice. Antibiotic sensitivity testing in veterinary practice is an essential tool for treatment. Aminoglycosides, such as gentamicin, have been suggested for topical application in OE caused by Gram-negative bacteria (22,23). In the present study, the in vitro antibiotic sensitivity test of isolated *P. aeruginosa* strains examined revealed gentamycin to be the most effective antibiotic, followed by penicillin G, danofloxacin, streptomycin, ampicillin, lincomycin, and tetracycline in decreasing order. It is found that most of the investigated *P. aeruginosa* strains have noticeable resistance to trimethoprim-sulphamethoxazole. Hariharan et al. (14) reported that most isolates were susceptible to gentamycin but resistant to ampicillin, penicillin, and trimethoprim-sulphamethoxazole. Schick et al. (24) reported that most isolates were susceptible to gentamycin (81%) and enrofloxacin (56%). Keskin et al. (6) reported that most bacterial strains from dogs with OE were sensitive to enrofloxacin, gentamycin, and lincomycin, but resistant to tetracycline and ampicillin. Martín Barrasa et al. (11) reported that most isolates were susceptible to tobramycin (100%), marbofloxacin (90%), ceftazidime (90%), gentamycin (68%), and enrofloxacin (42%), but resistant to trimethoprim-sulphamethoxazole. These findings indicate that the antimicrobial sensitivities of the bacteria isolated from dogs with OE are variable; therefore, antimicrobial agents should be selected on the basis of bacterial culture and sensitivity tests.

In conclusion, these findings suggested that staphylococci and *P. aeruginosa* play an important role in the pathogenicity of canine OE in Aydın. Isolation and identification of microbial agents responsible for the perpetuation of OE is a fundamental point for the diagnosis of the process and the initiation of a correct treatment. Therefore, antimicrobial susceptibility tests should be performed, especially in cases in which multiresistant bacteria are suspected. The present study indicates that gentamycin and penicillin could be the most effective agents for the treatment of OE caused by *P. aeruginosa*. To prevent the development of antibiotic resistance, rational antibiotic prescribing must be established.

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