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Identification and Antimicrobial Susceptibility of Bacteria Isolated from Dogs with Chronic Otitis Externa

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Abstract : Otitis externa (OE) is an inflammatory disease of the externa auditory meatus that occurs commonly in dogs. Antimicrobial susceptibility tests should be performed in case of chronic OE for successful treatment. In this study, identification and antimicrobial susceptibility test of bacteria isolated from dogs with chronic OE was performed. From 60 dogs with chronic OE, 60 bacterial species were identified. The most frequently identified species were *Staphylococcus* spp. (51%), followed by *Pseudomonas* spp. (15%) and *Enterococcus* spp. (14%). A single bacterial infection and multiple bacterial infections were observed in 67.5% and 32.5%, respectively. *Staphylococcus* spp. was susceptible to imipenum. *Pseudomonas* spp. was found to be susceptible to amikacin, cefepime, imipenum and piperacillin-tazobactam. *Enterococcus* spp. was susceptible to ampicillin-sulbactam, imipenum and piperacillin-tazobactam. Imipenum was highly susceptible antibiotic against both Gram-positive and negative bacteria whereas aztreonam and vancomycin were highly resistant. These results could suggest the optimal choice of antimicrobial agents for canine OE treatment.

Key words **:** Antimicrobial susceptibility test, dog, otitis externa.

Introduction

Otitis externa (OE) is an acute or chronic inflammatory disease of the external auditory meatus that is one of the frequently encountered diseases in the small animalclinic (20). There are various causes for OE such as stenosis of the external auditory meatus due to an abnormal anatomical structure, increased moisture from lack of ventilation within the canal, obstruction by tumor growth, atopy and allergies. That said, microbial infections are among the most predominant causative and perpetuating factors of the disease (2,3,17)

The treatments available for OE include ear cleaning, topical or systemic medication and surgery. Topical medication is the first line therapy for OE, while chronic OE may require systemic medications including antibiotics (10).

The frequent systemic administration of empirically chosen antibiotics can increase antibiotic resistance and lead to treatment failure, necessitating antibiotic susceptibility tests for the successful treatment of chronic OE. Several studies performed antimicrobial susceptibility tests on OE-causing bacteria in dogs (2-4,6,8,12,13). Although, these previous studies addressed the necessitation of antibiotic sensitivity tests early in the treatment process, susceptibility to empirically used antibiotics in Korea have not been examined recently.

In this study, bacteria from chronic OE in dogs were isolated and identified. Antimicrobial susceptibility tests were then performed for 16 antibiotics, not only those frequently used but also the recently potentiated antibiotics.

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Materials and Methods

Animals and samples

The subjects of this study were 60 dogs of different breeds that have been affected with chronic OE. They were diagnosed with chronic OE based on clinical examination. The secretion inside the external auditory meatus of one ear was collected with a sterilized cotton swab (COPAN, Italy). The collection was conducted at 6 different animal hospitals in Seoul between February 2015 and August 2015.

Microbiological tests

Bacterial colonies that grew in primary culture were used for antibiotic susceptibility tests. Samples were cultivated on 5% sheep blood agar plates (Asan Pharmaceutical, Korea) and MacConkey agar plates (Difco, USA), and aerobically incubated for 24 hours at 37°C. Single colonies were inoculated in Mueller-Hinton Broth (OXOID, UK) and cultivated for 4 hours at 37°C. The broth culture was streaked on Mueller Hinton Agar (OXOID, UK), on which antibiotic discs were dispensed, and incubated for 24 hours at 37°C. The antibiotics disks used are listed in Table 1. Sensitivity or resistance was determined by measuring the zone of inhibition. Isolated bacterial identification was performed using the VITEK 2 (bioMeriéux, France).

Results

Bacterial isolation and identification

A total of 60organisms were isolated from the clinical specimens through which 9 bacterial species were identified.

Table 1. The antibiotics disks used

Class/Antimicorbial agents	Disc content (μg)
Aminoglycoside	
Amikacin (AK)	30
Carbapenem	
Imipenem (IPM)	10
Cephalosporin	
Cefotaxim (CTX)	30
Cephalexin (CL)	30
Cefepime (FEP)	30
Macrolide	
Clindamycin (DA)	20
Monobactam	
Aztreonam (ATM)	30
Penicillin	
Ampicillin (AMP)	10
Vancomycin (VA)	30
Penicillin & beta-lactamase inhibitor	
Amoxicillin-clavulanic acid (AMC)	30
Ampicilli-sulbactam (SAM)	20
Piperacillin-tazobactam (TZP)	110
Quinolone	
Ciprofloxacin (CIP)	5
Enrofloxacin (ENR)	5
Sulfonamide	
Trimethoprim-sulfamethoxazole (SXT)	25
Tetracycline	
Tetracycline (TE)	30

Table 2. Bacterial isolation and identification results

Species	Number of isolates
Staphylococcus spp.	31
Pseudomonas spp.	9
Enterococcus faecalis	8
Escherichia coli	3
Klebsiella pneumoniae	3
Acinetobacter spp.	2
Proteus mirabillis	1
Other species	3
Total	60

The most frequently found species were *Staphylococcus* spp. (51%), *Pseudomonas* spp. (15%) and *Enterococcus faecalis* (14%). A list of all identified species is shown in Table 2.

In 67.5% of the cases (n = 27), there was only one dominant species found, while 25% (n = 10) had two species, and 7.5% (n = 3) had three. Table 3 presents the distribution of bacteria that are related to multiple infections in dogs with OE.

Antimicrobial susceptibility test

The susceptibilities of 9 bacterial species to 16 antibiotics were examined (Table 4). The greatest antimicrobial effects for *Staphylococcus* spp. were observed from imipenum (100%), followed by ampicillin-sulbactam (90%), amikacin and piperacillin-tazobactam (77%), respectively. *Pseudomonas* spp., a gram negative bacteria, exhibited sensitivity to amikacin, cefepime, imipenum and piperacillin-tazobactam (100%, respectively) and resistance to amoxicillin-clavulanic acid, ampicillin, ampicillin-sulbactam, clindamycin, trimethoprim-sulfamethoxazole and vancomycin (100%). *Enterococcus faecalis* was observed to maintain higher resistance to antibiotics than the two aforementioned species while *Proteus mirabilis* developed resistance to 11 of the16 drugs. Most pathogens were resistant to aztreonam and vancomycin.

Table 3. Distribution of bacteria related to multipleinfection of the dogs with bacterial otitis externa

Species	%
Staphylococcus spp.	42.5
Pseudomonas spp.	12.5
Enterococcus faecalis	7.5
Gardnerellavaginalis	2.5
Proteus mirabillis	2.5
Enterobacter faecalis + *Acinetobacter* spp.	2.5
Klebsiella spp. + *Esherichia coli*	2.5
Klebsiella spp. + *Pseudomonas* spp.	2.5
Staphylococcus spp. + *Acinetobacter* spp.	2.5
Staphylococcus spp. + *Enterococcus faecalis*	2.5
Staphylococcus spp. + *Esherichia coli*	7.5
Staphylococcus spp. + *Pseudomonas* spp.	5.0
Pseudomonas spp. + *Enterococcus faecalis* + *Granulicatellaelegans*	5.0
Staphylococcus spp. + *Enterococcus faecalis* + *Esherichia coli*	2.5

Discussion

Canine OE results from inflammation of epithelial lining of the external auditory canal (9,20). The inflammation may arise within the external ear canal or result from conditions affecting the pinna or middle ear. A number of factors have been shown to cause OE and it has proven useful to classify these as primary, predisposing or perpetuating. Bacterial infection is an important perpetuating factor which exacerbates the otitis and prevents resolution (2,3).

Canine OE occurs frequently in the population that seeks veterinary care, listed as the second most common condition for US canine health insurance claims. It is often a chronic and recurrent problem with limited options for treatment. Systemic antibacterial agents are prescribed when the tympanic membrane has been ruptured and infection is present in the middle ear, as well as when topical treatments yield poor response (10).

In this study, the most frequently identified species was

Table 4. Antibiotic resistance of bacterial species isolated from dogs with chronic otitis externa (n = 60, %)

	AK	AMC	AMP	SAM	ATM	FEP	CTX	CL	CIP	DA	ENR	IPM	TZP	TE	SXT	VA
Staphylococcus spp.(n = 31)	23	37	93	10	100	40	37	47	47	63	43	0	23	67	50	90
Pseudomonas spp. (n = 9)	0	100	100	100	33	0	56	100	22	100	33	0	0	89	100	100
Enterococcus faecalis (n = 8)	88	50	100	0	100	88	100	13	88	100	63	13	13	88	13	100
Escherichia coli (n = 3)	67	100	100	33	100	0	33	0	33	100	0	0	100	33	33	100
Klebsiella spp. (n = 3)	67	100	100	100	100	33	67	0	33	100	33	0	67	67	33	100
Acinetobacter spp. (n = 2)	0	100	100	0	100	0	0	0	50	100	50	0	0	0	0	100
Granulicatella elegans (n = 2)	0	0	100	0	100	50	0	0	0	0	0	0	0	0	0	0
Gardnerellavaginalis (n = 1)	0	100	100	0	100	100	0	0	0	100	0	0	0	100	0	100
Proteus mirabillis (n = 1)	0	100	100	100	100	100	100	100	0	100	0	100	100	100	0	100

AK: Amikacin, AMC: amoxicillin-clavulanic acid, AMP: ampicillin, SAM: ampicillin-sulbactam, ATM: aztreonam, FEP: cefepime, CTX: cefotaxim, CL: cephalexine, CIP: ciprofloxacin, DA: clindamycin, ENR: enrofloxacin, IPM: imipenum, TZP: piperacillin-tazobactam, TE: tetracycline, SXT: trimethoprimsulfamethoxazole, VA: vancomycine

Staphylococcus spp. (51%), followed by *Pseudomonas* spp. (15%), *Enterococcus faecalis* (14%), *Escherichia coli* (6%) and *Klebsiella* (6%). Although the prevalence ratio for each isolated bacterial species is different from other studies, their classification and distribution remain similar (4,8). Multiple infection was also commonly found, which is consistent with the findings of other authors (3,6,12,13,16).

The most effective antibiotics for *Staphylococcus* spp. were imipenum, ampicillin-sulbactam, amikacin and piperacillin-tazobactam, in decreasing order in the study. Yoon *et al*. reported that *Staphylococcus* spp. isolates from dogs with pyoderma and OE in Korea were resistant to penicillin (95%) or tetracycline (91.9%), but highly susceptible to amoxicillin/clavulanic acid (90.5%). While, 13-mecA-positive and methicilline resistant *S. pseudintermedius* strains were high level resistant (46.2%) to amoxicillin/clavulanic acid than other *S. pseudintermedius*strains (19). In contrast, we determined a 63% susceptibility of *Staphylococcus* spp. to amoxicillin/clavulanic acid, a significant drop from the previously study. A potential explanation for this discrepancy is that many veterinary clinics in Korea have been empirically using amoxicillin/clavulanic acid for most infectious cases such as OE and cystitis or the proportion of MRSP has been increased recently.

The most effective antibiotics of *Pseudomonas* spp. were amikacin, cefepime, imipenum and piperacillin-tazobactam. On the other hand, all 9 isolates of *Pseudomonas* spp. isolates showed absolute resistance to antibiotics belonging to the family of beta-lactamase, except for piperacillin-tazobactam. These results support findings from earlier studies.The resistance of *Pseudomonas* spp. to quinolone agents corresponds with results from many other studies (3,4,12,14). Fifty-eight of the 60 organisms were resistant towards aztreonam, while 57 were resistant to vancomycin. Thus, the use of these agents is not recommended to treat for OE.

Most isolates were highly sensitive to imipenum, a member of the carbapenum class of antibiotics, as it targets both gram positive and negative bacteria. Imipenum may be useful for the treatment of serious infections such as UTI, but are expensive and not active against methicillin-resistant *Staphylococci* and resistant strains of *Enterococcus faecium*. In addition, imipenem is used as a last-resort treatment for gram-negative infections in humans. As such, the use of this drug in companion animals cannot be justified unless stringent requirements are met (1,11).

Recently, there is an emerging problem on multi-resistant bacteria of both gram positive and negative natures in human and veterinary medicine (5,7,15,18). To prevent the rapid international spread of these bacteria, optimal choice of antimicrobial agents is critical. The selection should rely on stringent bacterial culturing and antimicrobial susceptibility tests. However, these procedures are difficult to implement in clinical practice due to time constraints. An additional hindrance is the variability of antimicrobial susceptibility with changes in location and time. Therefore, regular investigations regarding antimicrobial susceptibility are needed. In conclusion, our results, in combination with earlier data, can suggest the optimal choice for canine chronic OE treatment.

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