



Original Scientific Article

OTITIS EXTERNA IN DOGS: DISTRIBUTION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *STAPHYLOCOCCUS* SPP. ISOLATES

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ABSTRACT

The study aimed to investigate the *Staphylococcus* species from dogs with chronic otitis externa in Istanbul and to determine their antibiotic susceptibility patterns. Ear swab samples were collected from 100 dogs suspected of otitis externa admitted at the clinics of Istanbul University - Cerrahpaşa, Faculty of Veterinary Medicine. The dogs were of different age, gender, and breed. The bacterial isolation was performed by conventional methods. BD Phoenix Automated Microbiology System was used to confirm bacterial identification by conventional methods and to test antimicrobial susceptibility. *Staphylococcus* spp. were isolated from 36% of the samples collected from the dogs. *S. pseudintermedius*, *S. aureus*, *S. epidermidis*, *S. hyicus* and *S. chromogenes* were identified in 41.6%, 22.2%, 11.1%, 5.5%, and 5.5%. In *Staphylococcus* spp. isolates, enrofloxacin, penicillin, and ampicillin-sulbactam resistance was 8.3%, marbofloxacin resistance was 11.1%, doxycycline resistance was 16.6%, amoxicillin-clavulanic acid, erythromycin, and gentamicin resistance was 19.4%, tetracycline, clindamycin, and sulphonamide resistance was 25%. Methicillin resistance was not observed in any of the isolates. However, multiple drug resistance (MDR) was detected in 11 (30.5%) of 36 isolates. In conclusion, the early detection and antimicrobial sensitivity testing of *Staphylococcus* spp in dog otitis externa cases that do not respond to empiric therapy could be beneficial for appropriate antibiotic selection and treatment thus preventing MDR.

Key words: antimicrobial resistance, *Staphylococcus*, canine, otitis externa

INTRODUCTION

The most common ear disease in dogs is otitis externa (OE), defined as acute or chronic inflammation of the ear canal and surrounding tissues. Its prevalence was reported between 5 and 20% (1, 2, 3).

The factors that cause OE are classified as primary, predisposing and permanent. The primary factors are parasitic infestations, foreign bodies, keratinization

disorders, autoimmune, hypersensitivity and allergic diseases. The predisposing factors (anatomic conformation of the ear canal, excessive moisture, iatrogenic factors and obstructive ear disease) make the ear more susceptible to the development of OE, but they do not cause infection alone. The persistent factors (bacteria, yeasts, otitis media and progressive pathological changes) are responsible for the exacerbation of the infection (2, 3, 4).

Regardless of the primary ear lesion, acute and suppurative canine otitis is mainly caused by microbial contamination. *Staphylococcus* species have been reported among the most frequently isolated bacteria from OE cases commonly observed in dogs (5, 6, 7).

Staphylococcus species are opportunistic pathogens that can be found on human and animal skin, on the mucous membranes of the upper respiratory tract and lower urogenital tract, and in the digestive tract. They might cause mastitis,

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enzootic pyemia, arthritis, and food poisoning in animals. In human medicine, they are commonly related to hospital infections, skin-soft tissue and respiratory-circulatory system infections, and food poisoning (8, 9).

Antimicrobial resistance in bacteria is defined as the ability of the agent to resist the bactericidal and bacteriostatic effects of an antimicrobial agent. It is frequently reported in both veterinary and human medicine in recent years (10) mostly due to the unethical and unrestricted use of antibiotics which leads to lower antibiotic effectiveness in the treatment of deadly infections and inconsistencies in obtained data.

Ensuring the rational use of antimicrobials is the most important step in solving the problem of antimicrobial resistance. However, the research is limited to companion animals such as dogs and cats. The frequent and regular monitoring is essential for planning and undertaking appropriate measures (3, 11).

The current study aimed to investigate the *Staphylococcus* species isolated from dogs with chronic otitis externa in Istanbul and to determine their antibiotic susceptibility by phenotypic methods.

MATERIAL AND METHODS

Swab samples were collected from 100 dogs of different age, sex and breed diagnosed with otitis externa, admitted to the Istanbul University-

Cerrahpaşa, Faculty of Veterinary Medicine clinics between October 2019 and March 2020. The samples were placed in Stuart’s transport medium and brought to the laboratory in a cold chain. (Istanbul University-Cerrahpaşa Rectorate Veterinary Faculty Unit Ethics Committee Presidency, Report no: 2019/10).

The swabs were inoculated in Mannitol Salt Agar and incubated at 37 °C for 24 hours. Suspected *Staphylococcus* spp. isolates were initially isolated and identified using standard biochemical tests (12, 13). BD Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) was used for bacterial identification and antimicrobial susceptibility testing according to the manufacturer’s instructions. Isolates with resistance to three or more than three of the fluoroquinolone, aminoglycoside, lincosamide, sulphonamide, tetracycline, macrolide, and β-lactam antibiotic groups were classified as having multiple drug resistance (MDR).

All statistical analyses were conducted using SPSS Statistics version 25 (IBM, USA). Statistical significance was set at p<0.05.

RESULTS

Staphylococcus spp. was isolated as pure culture in 36% of the samples. *S. pseudintermedius*, *S. aureus*, *S. epidermidis*, *S. hyicus* and *S. chromogenes* were identified in 41.6%, 22.2%, 11.1%, 5.5% and 5.5%, respectively.

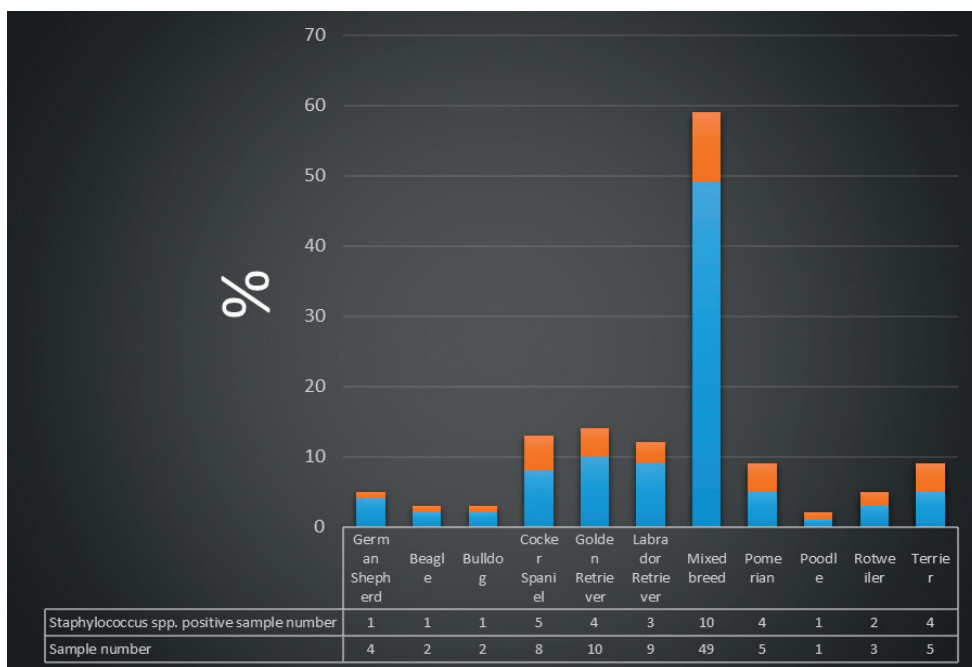


Figure 1. *Staphylococcus* spp. isolation rates in different breeds

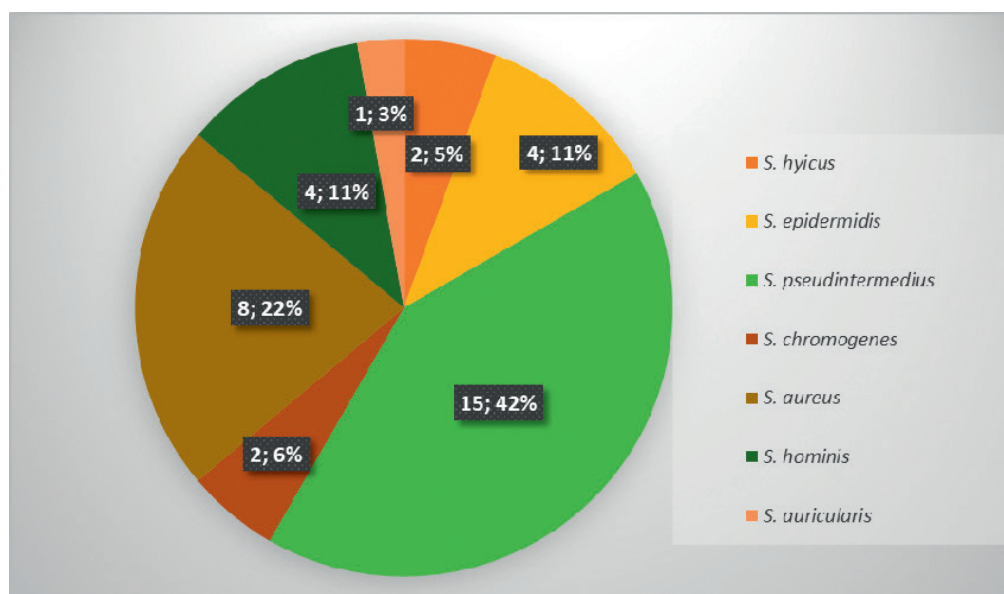


Figure 2. Distribution of *Staphylococcus* species isolates

Table 1. Antibiotic resistance of *Staphylococcus* spp. isolates

	<i>S. pseudintermedius</i> (n=15)	<i>S. aureus</i> (n=8)	<i>S. hominis</i> (n=4)	<i>S. epidermidis</i> (n=4)	<i>S. hyicus</i> (n=2)	<i>S. chromogenes</i> (n=2)	<i>S. auricularis</i> (n=1)	Total (n=36)
P	2	1	0	0	0	0	0	3 (8.3%)
AMC	3	3	0	0	1	0	0	7 (19.4%)
SAM	2	0	0	0	0	1	0	3 (8.3%)
ENR	1	1	0	1	0	0	0	3 (8.3%)
MA	2	1	0	1	0	0	0	4 (11.1)
CN	1	4	1	1	0	0	0	7 (19.4)
DO	0	3	1	0	2	0	0	6 (16.6)
TE	2	3	1	1	2	0	0	9 (25.0%)
SXT	4	3	0	1	1	0	0	9 (25.0%)
E	2	3	1	1	0	0	0	7 (19.4%)
DA	4	3	0	1	1	0	0	9 (25.0%)

Enrofloxacin (ENR; 5 µg), Marbofloxacin (MAR; 5 µg), Gentamicin (CN; 10 µg), Clindamycin (DA; 2 µg), Trimethoprim-sulfamethoxazole (SXT; 1.25/23.75 µg), Tetracycline (TE; 30 µg), Doxycycline (DO; 30 µg), Erythromycin (E; 15 µg), Penicillin G (P; 10 units), Amoxicillin/Clavulanic acid (AMC; 20/10 µg), Ampicillin/Sulbactam (SAM; 10/10 µg)

Staphylococcus spp. was detected in 15 (15/52, 28.8%) male and 21 (21/48, 43.75%) female dogs. *Staphylococcus* spp. positive samples were distributed in the following age groups: <1 year (9/26, 34.6%), 1-8 years (22/63, 34.9%), >8 years old (5/11, 45.4%). The presence of OE was found to be 80.0% in Terriers, 80.0% in Pomeranians, 66.6% in Rottweilers, 62.5% in Cocker Spaniels, 33.3% in Labradors, 25.0% in Golden Retrievers and 20.4% in mixed breeds. The distribution of *Staphylococcus* spp. positive samples according

to the breed is shown in Fig. 1. The isolates' distribution according to the *Staphylococcus* spp. is presented in Fig. 2. No significant differences were observed ($p>0.05$).

The antibiotic susceptibility of the isolates is presented in Table 1. *S. auricularis* was found to be sensitive to all antibiotics. Aztreonam resistance was not detected in any of the isolates. No methicillin resistance was observed in any of the isolates. MDR was found in 30.5% of the strains.

DISCUSSION

Otitis is a common disease in dogs due to the anatomical features of the ear (14, 15). The most commonly isolated bacterial agents from OE are Coagulase Positive *Staphylococcus* spp. (CPS) and Coagulase Negative *Staphylococcus* spp. (CNS) which are considered opportunistic agents (16, 17). Numerous authors have investigated the presence of *Staphylococcus* spp. in OE cases and have reported isolation rates between 36% and 60.3% (7, 15). The current study had 36 (36%) dog OE isolates positive for *Staphylococcus* spp., similar to other reports (7, 9, 18, 19, 20).

S. aureus in dog OE cases was reported between 9% and 55.76% (7, 9, 18, 19, 21). In this study, 8 (22.2%) of 36 *Staphylococcus* spp. isolates were identified as *S. aureus*.

S. pseudintermedius belongs to a community known as the *Staphylococcus intermedius* group (SIG), which includes three different species, *S. pseudintermedius*, *S. intermedius* and *S. delphini*. All members within the SIG group are known to colonize a large number of animal species. *S. pseudintermedius* is a commensal in animals, especially in dogs. Canine isolates previously identified as *S. intermedius* are currently referred to as *S. pseudintermedius* unless proven otherwise by genomic studies. *S. intermedius* isolation rates were reported between 13.6% and 73.9% in various studies (19, 20, 22, 23). In the current study, *S. pseudintermedius* was detected in 41.6% of the isolates. The genotypic methods for bacterial identification were considered more reliable rather than the phenotypic methods.

Members of the CNS group also play an important role in cases of otitis externa in dogs. Lilenbaum et al. (19) reported that 60.3% of the isolates were CNS and 25% were *S. epidermidis*. De Martino et al. (20) identified 28% *S. chromogenes*, 0.7% of *S. epidermidis*, and 2.1% *S. hyicus* in 143 isolates. Sarierler and Kırkan (21), Penna et al. (7) and Borum et al. (18) obtained CNS from their samples in 5.12%, 38%, and 17.3%, respectively.

Among the CNS isolates, *S. epidermidis*, *S. chromogenes*, and *S. hyicus* stand out in several studies. *S. epidermidis* has been reported in 25% (19) and 11% (7). In this study, *S. epidermidis* was detected in 11.1%, *S. hyicus* and *S. chromogenes* in 5.5% of the samples, similar to other reports. However, De Martino et al. (20) reported *S. epidermidis* 0.7%, *S. hyicus* 2.1%, and *S. chromogenes* 28% in 143 samples. In another study conducted in our country, it was reported

that CNS was detected in higher numbers than CPS (9). In this study, CPS were found to have a higher prevalence than CNS. The differences in the reports might be due to geographical specificities, or dog age and breed.

Numerous studies have been examining the relationship between OE and breed characteristics. It has been reported that Cocker Spaniels, Poodle, Labrador Retrievers, German shepherd, and Fox Terriers are predisposed to OE due to structural differences in the ear canal and auricle (excess hair follicles, long and drooping structures), excess apocrine gland tissue, or excess amounts of cerumen (15, 24, 25). The researchers found a higher rate of OE in dogs with floppy ears (73.2%) than in dogs with straight ears. The highest rate was obtained in Cocker Spaniels (26%) and Labradors (18%) which are lop-eared dogs, and in German Shepherds (14%) as straight-eared dogs (26, 27, 28). In this study, when the number of samples and the isolation of *Staphylococcus* species were compared, the presence of OE was found to be 80% in Terriers, 80% in Pomeranians, 66.6% in Rottweiler's, 62.5% in Cocker Spaniels, 33.3% in Labradors, 25% in Golden Retrievers and 20.4% in mixed breeds. The highest isolation rate was from the mixed breeds (10/36, 27.7%) which were the most numerous sample group (49%).

The relationship between the presence of OE and age has also been demonstrated in many studies. Topala et al. (24) reported the highest OE rate in dogs aged 5-8 years which was 33.4%. Kumar et al. (27) reported that they detected the presence of OE mostly in dogs over the age of 3 (66.6%). Fernández et al. (1) stated that OE was highest (43.4%) in dogs aged 2-5 years.

In the current study, *Staphylococcus* spp. was detected in 9 (9/26, 34.6%) dogs aged ≤ 1 year, in 22 (22/63, 34.9%) aged 2-8 years, and in 5 (5/11, 45.4%) aged ≥ 9 years. These results were found to be statistically non-significant ($p > 0.05$).

Comparing age and gender between different studies is difficult as they are defined differently (e.g., two age groups, young and old). Further studies are needed to establish an accurate correlation between these factors. The age can be additionally affected by other co-factors such as handling, hygienic conditions, geographical region, etc., which may define variable experimental or study conditions.

The researchers have demonstrated a relationship between gender and OE (24, 26, 27). Kumar et al. (27) stated that OE is more common in male animals than in females. On the contrary,

other studies reported a higher rate in female dogs than in males (1, 26). Topala et al. (24) reported 50.8% OE in female dogs, and they did not find a relationship between gender and OE. In the present study, *Staphylococcus* spp. was detected in 15 (15/52, 28.8%) male and 21 (21/48, 43.75%) female dogs, but no significant relationship between gender and OE was observed ($p>0.05$).

Numerous studies aimed to investigate the relationship between OE and antimicrobial resistance (21,31). Infections caused by *Staphylococcus* species are generally treated with antimicrobial agents that inhibit bacterial cell wall synthesis, especially β -lactams (29). The researchers reported penicillin resistance between 30.7% and 72.5% (21, 31). It was emphasized that the high level of resistance against penicillin was achieved due to β -lactamases produced by *S. aureus* and *S. intermedius*. Lyskova et al. (2) found that 66% of *S. intermedius* isolates were resistant to penicillin, but all of them were susceptible to amoxicillin/clavulanic acid (AMC). Kang et al. (30) reported penicillin resistance in 92% of the *S. pseudintermedius* isolates and AMC resistance in 6% of the samples. In other studies conducted in Türkiye, researchers reported that they did not detect AMC resistance in *S. aureus* and CNS isolates (22). Sığircı et al. (28) found penicillin resistance in 73.9%, AMC resistance in 22.2%, and ampicillin/sulbactam (SAM) resistance in 30% in *S. intermedius* isolates. In addition, recent studies reported that 77.3% of *S. pseudintermedius* isolates were resistant to penicillin and 6.8% to AMC resistance (23). Bourely et al. (10) reported penicillin resistance in 68.5% of *S. pseudintermedius* isolates and 70.9% of *S. aureus* isolates. In the present study, resistance to penicillin was found in 8.3%, AMC resistance in 19.4%, and SAM resistance in 8.3% of the isolates. The relatively low detection level of antimicrobial resistance compared to other studies was considered promising.

Fluoroquinolones obtained by adding fluorine at position six to the quinolone molecule, have a broad spectrum and are active against *Staphylococcus* species. Lyskova et al. (2) reported that they did not detect enrofloxacin resistance in all *Staphylococcus* spp. isolates. Penna et al. (7) found 48.6% of enrofloxacin resistance in *S. pseudintermedius* isolates. Petrov et al. (16) reported 32% resistance to enrofloxacin in CPS isolates. Öztürk et al. (22) noted enrofloxacin resistance in 40% of the *S. aureus* isolates and 11.1% in the CNS isolates. Sığircı et al. (28) found enrofloxacin resistance in 30.9% of the *S. intermedius* isolates. In this study,

enrofloxacin resistance was found in 8.3%, and marbofloxacin resistance at 11.1% of the isolates.

Amikacin and gentamicin aminoglycosides have been suggested for topical application in OE caused by Gram-positive bacteria (20). The authors demonstrated that the gentamicin resistance ranged from 3% to 84.1% (16, 19, 23, 28). Lyskova et al. (2) reported that gentamicin resistance was not found in all *S. intermedius* isolates. Penna et al. (7) emphasised that gentamicin is more resistant than amikacin and stated that gentamicin resistance was 54.3% in *S. pseudintermedius* isolates. In this study, gentamicin resistance was found in 19.4% of the isolates.

Tetracyclines, the first large group of antimicrobial agents, are among the most commonly used therapeutics in veterinary medicine (32). It is known that the use of tetracycline with standard and inappropriate methods may lead to the transfer of resistance genes to recipient bacteria. The researchers revealed tetracycline resistance ranged from 7.69% to 94% (23, 28, 30, 31). Petrov et al. (16) found doxycycline resistance in 49% of KPS isolates. In this study, tetracycline resistance was found in 25% and doxycycline resistance in 16.6% of the isolates. It was considered promising that the determined resistance values were in the lower limits.

Sulfonamides are synthetic antibiotics ranked as “High priority” among veterinary drugs. The authors demonstrated resistance to trimethoprim-sulfamethoxazole (SXT) between 15.38% and 88.8% (22, 23, 30, 31). In this study, sulfonamide resistance was determined in 25% of the isolates.

Macrolide resistance is also a growing problem, probably proportional to the number of drugs used. The researchers reported erythromycin resistance from 23% to 80%. In another study conducted in Türkiye, this rate was 75% (28). In the current study, erythromycin resistance was found in 19.4% and it was considered relatively lower compared to other studies.

The number of studies related to the lincosamide group, including lincomycin and clindamycin, is more limited. Oliviera et al. (31) reported clindamycin resistance in 23.07% of the *S. intermedius* isolates and 44.4% in the *S. aureus* isolates. Lyskova et al. (2) found 39% clindamycin resistance in *S. intermedius* dog isolates with otitis media. In this study, clindamycin resistance was detected in 25% of the isolated, similar to other studies.

Methicillin resistance was not observed in this study, but further studies must be continuously made for regular updates.

MDR is defined as a resistance of a microorganism to multiple antimicrobial drugs which are structurally unrelated and have different molecular targets. Lilenbaum et al. (20) reported that 90.9% of the isolates were resistant to at least one antibiotic, and MDR was detected in 36.4%. In the same study, it was noted that a single *S. haemolyticus* isolate was resistant to all tested antimicrobials. Penna et al. (7) emphasized that 89% of the 151 samples had multiple resistant isolates.

In this study, MDR was detected in 11 of 36 (30.5%) *Staphylococcus* spp. isolates. If antimicrobial resistance can be transmitted from pets to humans, there would be significantly lower antimicrobial effectiveness in both animals and humans. The prevalence of antimicrobial resistance in pets (primarily dogs and cats) is of paramount importance in veterinary and human medicine, and therefore, continuous studies are necessary for up-to-date information.

Some limitations have to be considered in the present study. First, the number of dogs enrolled in this study was small. Second, data on clinical history and antimicrobial usage were not available. Third, the molecular diagnostic identification of *S. pseudintermedius* was lacking. Finally, antimicrobial resistance genes were not included in the current study.

CONCLUSION

Consistent with previous studies, this study further confirms that *S. pseudintermedius* and *S. aureus* of staphylococcal strains are the most frequent causative agents of OE in dogs. In 25% of the staphylococci isolates SXT, tetracycline, and clindamycin resistance was found. MDR was detected in 30.5% of the isolates. There was no methicillin resistance found in any of the isolates.

CONFLICT OF INTEREST

The authors declare that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

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AUTHORS' CONTRIBUTIONS

MH collected the samples, made the isolation and writing the article. AIK and BH performed isolation and phenotypic assessments. BBK designed the project, wrote the article, and executed the final approval of the version to be published

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