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PREVALENCE AND CHARACTERIZATION OF *IN VITRO* SUSCEPTIBILITY PROFILE OF BACTERIA HARVESTED FROM OTITIS EXTERNA IN DOGS

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Abstract

Canine otitis externa is a complex disease with multiple causative factors, prominently involving bacteria. Antibiotics serve as the primary therapeutic approach for prevailing bacterial influence. However, indiscriminate antibiotic use results in the emergence of resistant strains. The escalating global threat of antimicrobial resistance (AMR) underscores the critical need for comprehensive investigations. The study involved 118 dogs with otitis externa from an animal hospital in Karachi, encompassing diverse breeds, ages, and genders. Ear swabs underwent bacterial culture, followed by incubation, colony examination, Gram staining, and biochemical tests for identification. Antibiotic susceptibility was assessed using the disc diffusion method following CLSI guidelines. Out of 118 dogs, 91(77.11%) exhibiting clinical symptoms were included, with 29.66% recorded positive in crossbreed, with 42.37% being females, according to age, the prevalence was higher in above four-year-old dogs (47.45%). Notably, Pseudomonas spp. (22.03%), followed by Staphylococcus spp. (17.79%), Escherichia coli (13.55%), Proteus spp. (9.32%), Streptococcus spp. (8.47%). Concerning the seasons, most cases of infection were recorded in spring (38 cases). The assessment of antibiotic sensitivity through the disc diffusion method revealed Enrofloxacin, Norfloxacin and Ciprofloxacin as generally effective. In contrast, notable resistance was observed for Amoxicillin, Gentamycin, Ampicillin and Penicillin, especially among both Gram-positive (G+) and Gram-negative (G-) bacteria. Comprehensive investigations into canine otitis externa are imperative due to the rising threat of antimicrobial resistance. The study highlights prevalent bacterial strains, seasonal trends, and antibiotic susceptibility patterns, emphasizing the need for judicious antibiotic use to mitigate resistance emergence.

Keywords: *Bacteria, Dog, Harvested, In vitro, Otitis Externa, Prevalence, Susceptibility*

INTRODUCTION

Otitis externa is considered a common disease of pets in veterinary practice. It causes great discomfort to animals and owners, as it can cause pain, itching, agitation, discharge, and unpleasant odor. In Pakistan, dogs and cats, are frequently acquired as pets, and these domestic animals within households seem to pose a significant risk for zoonotic diseases. Ear infections are most frequent reasons to be presented to veterinarians and may affect up to 20% of dogs, however, cases of canine otitis are often neglected by

professionals due to a lack of accurate diagnosis and essential examinations such as bacterial culture and antibiogram of ontological secretions (1). Otitis externa is the inflammation of the external ear canal, outside of the tympanic membrane, and sometimes the pinna. This dermatological disorder may be acute or chronic (2).

It can be triggered by various factors, including allergies, foreign bodies, excessive moisture, anatomical conformation, and microbial infections (3). Bacterial infections, along with fungal infections, are among the primary microbial culprits responsible for this condition in dogs. Common bacterial pathogens associated with otitis externa in dogs include *Staphylococcus pseudintermedius*, *Pseudomonas aeruginosa, Proteus spp., Lebsiella spp*., *Escherichia coli,* etc. (4).

The common clinical findings of otitis externa include headshaking, aural pruritus, pain, discomfort upon ear palpation, swelling, fibrosis, and calcification of the ear canal or pinna (5).

The prevalence of bacterial pathogens associated with this condition in different regions. For instance, a study by (5) examined 100 dogs with otitis externa in Brazil and found that *Staphylococcus pseudintermedius* was the most prevalent bacterial isolate, followed by *Pseudomonas aeruginosa*. In Poland (6) reported a high prevalence of Proteus spp. in dogs with otitis externa. Otitis externa is present in 15–20% of dogs examined clinically; the incidence of otitis externa varies depending on the age of the dog. The studies conducted by (7) show that 10% to 16% of otitis externa cases occur in 1-year-old dogs, 60% in dogs 1–5 years of age, and 24% to 44% – in dogs older than 5 years. Otitis externa is more common among the breeds (8).

The veterinarian practitioner may get frustrated as relapses are very common due to treatment failures and if left untreated the consequences may be very serious (9). Otitis externa is a syndrome, not a diagnosis. The successful treatment of the disease requires appropriate medications including anti-inflammatory drugs, antifungal agents, and chemotherapeutics about the sensitivity of the etiological agents (10).

As a serious global concern nowadays in humans, as well as in veterinary medicine, antimicrobial resistance (AMR) problems could be provoked by excessive antimicrobial use (AMU) and prolonged treatments (11). Furthermore, AMR bacteria can be transmitted from animals to humans through direct or indirect contact with contaminated food, and their shared environment (12). Interestingly, the possibility of AMR transmission from companion animals to humans has been the subject of recent research, where dogs and cats could be both, reservoirs, and transmission vectors (13).

Antimicrobial-resistant strains of Staphylococcus and Pseudomonas otitis have emerged as frustrating and difficult causes of otitis because of the development of resistance to most common antibiotics (14). Antimicrobial-resistant strains can cause a large nosocomial outbreak that is difficult to control in veterinary healthcare facilities (10). Due to an increase in extended-spectrum β-lactamase-producing Enterobacteriaceae, including *Proteus spp., Klebsiella spp., E. coli,* and Pseudomonas species (15).

In this regard, the present study aimed to investigate the prevalence of clinical and epidemiological aspects of different etiological agents responsible for canine otitis, particularly bacterial organisms, and to assess the antimicrobial susceptibility and resistance of bacterial isolates from such cases used in veterinary hospitals.

MATERIALS AND METHODS

STUDY DESIGN

The research was conducted following ethical guidelines for animal research, and all procedures were approved by the Institutional Animal Care and Use Committee (IACUC) or the relevant regulatory authority, Approval No DAS-125-2020.

SAMPLE COLLECTION

A total of 118 dogs diagnosed with clinical signs of otitis externa were included in the study. Dogs of different breeds, ages, and genders were recruited from an animal hospital in Karachi, within a specific geographic region. Informed consent was obtained from the dog owners before sample collection. For each

dog, a physical examination of the ear canal was performed using an otoscope to assess the severity and nature of the ear condition. Ear swabs were collected from both ears of each dog using sterile cotton-tipped swabs. Care was taken to avoid contamination and ensure proper labeling of each sample.

BACTERIAL CULTURE AND IDENTIFICATION

The collected ear swabs were immediately transported to the laboratory under appropriate storage conditions. Bacterial culture and identification were performed following standard microbiological techniques. Each swab was streaked onto blood agar and MacConkey agar plates and incubated at 37°C for 24-48 hours.

After incubation, bacterial colonies were examined for morphological characteristics, such as shape, size, and color. Gram staining was performed to differentiate between Gram-positive and Gram-negative bacteria. Biochemical tests, including catalase, coagulase, and oxidase tests, were carried out to further identify the bacterial isolates at the species level. The prevalence of bacterial isolates and their susceptibility profiles were analyzed.

ANTIBIOTIC SUSCEPTIBILITY TESTING (AST)

The in vitro susceptibility profiles of the bacterial isolates were determined using the disc diffusion method (Kirby-Bauer method) following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Briefly, pure cultures of bacterial isolates were prepared by inoculating a loopful of bacterial cells in sterile saline. The turbidity of the bacterial suspension was adjusted to match 0.5 McFarland standards.

The standardized bacterial suspensions were then spread onto Mueller-Hinton agar plates using a sterile cotton swab. Different antibiotic discs with predetermined concentrations were placed on the agar surface. The plates were incubated at 37°C for 16-18 hours.

The diameter of the inhibition zone around each antibiotic disc was measured in millimeters using a caliper. The results were interpreted based on the CLSI guidelines for susceptibility, intermediate, or resistance to each antibiotic.

STATISTICAL ANALYSIS

Descriptive statistics were used to summarize the prevalence of bacterial isolates and the distribution of susceptibility profiles. The results were presented as frequencies and percentages for categorical data. Chi-square tests were employed to assess associations between the prevalence of bacterial isolates and various demographic or clinical factors. Statistical 8.1 versions were used for analysis.

RESULTS

BREED-WISE PREVALENCE OF OTITIS EXTERNA IN DOGS

In Table I, from the prevalence of total cases with bacterial otitis externa, the breed of dogs infected with otitis externa percentage was recorded highest in cross-breed animals 35 (29.66%), followed by German Shepherd 35 (22.3%), Labrador Retriever 8 (6.77%), Rottweiler 7 (5.93%), Shihtzu and American bulldog (5.08%), and Pitbull 3 (2.53%). Regarding ear shape, the infection rate was 64 (71.11%) for dogs with erect ears, while 27 (29.67%) were recorded for dogs with pendulous ears. Statistically, the results are not significant (0.1270) differences among breeds.

Table I. Breed- wise prevalence of otitis externa in dogs

S. no	Breed	Type of ear	Number of animals examined	Number of infected animals	Percentage
1	German Shepherd	Erect	28	26	22.03
2	Shihtzu	Pendulous	8	6	5.08
3	Pitbull	Erect	6	3	2.54
4	Rottweiler	Pendulous	15	7	5.93
5	Labrador Retriever	Pendulous	10	8	6.77
6	American Bulldog	Pendulous	13	6	5.08
7	Crossbreed	Erect	38	35	29.66
	Total		118	91	77.11

*Overall Chi-Square=17.64, p-value=0.1270, Degrees of freedom=12

GENDER-WISE PREVALENCE OF OTITIS EXTERNA IN DOGS

The gender-wise prevalence shown in Table II included 91 infected animals, with 50 females (42.37%) and 41 males (34.74%). Statistically, no significant difference (p=0.8509) was found between genders.

Table II. Gender-wise prevalence of otitis externa in dogs

S .no	Gender	Examined dog	Infested dogs	Percentage
1	Male	56	41	34.74
2	Female	62	50	42.37
	Total	118	91	77.11

***Overall Chi-Square=0.32, p-value=0.8509, Degrees of freedom=2**

AGE-WISE PREVALENCE OF OTITIS EXTERNA IN DOG BREEDS

Table III shows the age-wise prevalence in infected animals. The highest infection rate was recorded in animals up to four years of age, with 56 (47.45%) positive samples. Animals aged one to four years had 26 (22.03%), while those less than one year old had 9 (7.62%).

Table III. Age-wise prevalence of otitis externa in dog breeds

S. no	Age	Number of animals examined	Number of infected animals	Percentage
1	Less than 1 year	21	9	7.62/42.85/8.19
2	1-4 year	32	26	22.03/81.25/28
3	Above 4 years	65	56	47.45/61
	Total	118	91	77.11

*Overall Chi-Square=2,79, p-value=0.5940, Degrees of freedom=4

PREVALENCE OF ISOLATED BACTERIA FROM OTITIS EXTERNA IN INFECTED DOGS

In Fig. 1, concerning bacterial identification, positive samples from the 91 infected animals were classified as follows: pseudomonas spp. (26 isolates, 22.03%), staphylococcus spp. (21 isolates, 17.79%), *Escherichia coli* (16 isolates, 13.55%), proteus spp. (11 isolates, 9.32%), streptococcus spp. (10 isolates, 8.47%), and less frequently, bacillus spp. (7 isolates, 5.93%).

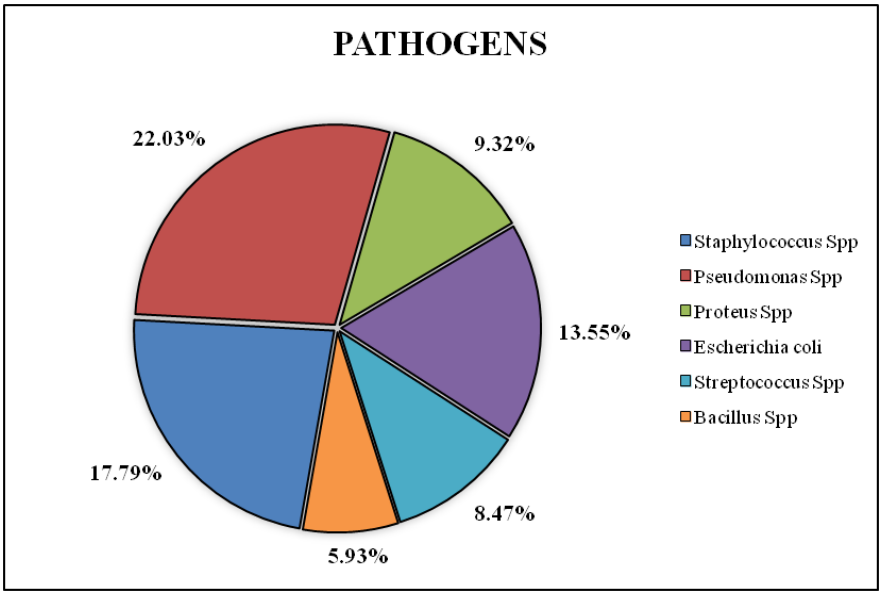

Fig. 1. Prevalence percentage of isolated bacteria from otitis externa from infected dogs

SEASONS WISE PREVALENCE OF OTITIS EXTERNA FROM INFECTED DOGS

Regarding the season of the year, most cases of infection were recorded in spring (38 cases, 32.2%) and autumn (28 cases, 23.72%), followed by summer (15 cases, 12.71%) and winter with only 10 (8.47%) cases (Fig. 2).

ANTIBIOTIC SUSCEPTIBILITY TESTING OF OTITIS EXTERNA ASSOCIATED BACTERIA

According to result of antimicrobial susceptibility testing of the isolated bacteria strains (Table IV). *Staphylococcus spp, Pseudomonas spp, Proteus spp, Escherichia coli, Streptococcus spp* and *Bacillus spp* were the most resistant to amoxicillin, gentamycin and ampicillin. On contrary, these bacteria have shown low resistance against oxytetracycline and cephotaxime. Meanwhile, enrofloxacin and norfloxacin show a highly sensitivity against the isolated bacteria.

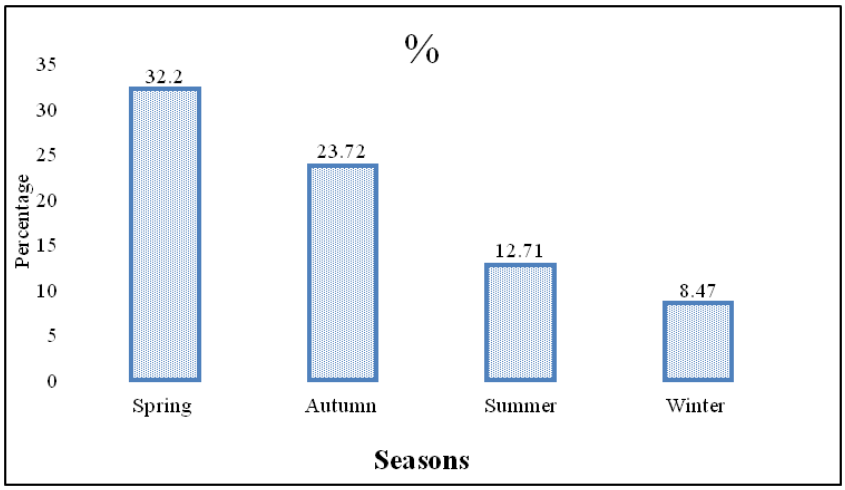

Fig. 2. Seasons-wise prevalence of otitis externa from infected dogs

Table IV. Antibiotic susceptibility testing of otitis externa associated bacteria

S. n	Antibiotics	Interpretive categories	Staphylococcus Spp.	Pseudomonas Spp.	Proteus Spp-	*E. coli*	Streptococcus Spp.	Bacillus Spp.
1	Amoxicillin 10 µg	Resistant	6mm	4mm	8mm	4mm	5mm	6mm
2	Enrofloxacin 5 µg	Sensitive	20mm	22mm	16mm	16mm	18mm	17mm
3	Gentamycin 10 µg	Resistant	6mm	5mm	0mm	0mm	8mm	6mm
4	Oxytetracycline 30 µg	Intermediate	10mm	12mm	10mm	15mm	15mm	10mm
5	Ampicillin 10 µg	Resistant	6mm	4mm	8mm	4mm	5mm	6mm
6	Norfloxacin 10 µg	Sensitive	18mm	20mm	19mm	20mm	19mm	16mm
7	Cephotaxime 10 µg	Intermediate	10mm	12mm	10mm	15mm	15mm	10mm
8	Ciprofloxacin 15 µg	Sensitive	17mm	18mm	20mm	22mm	17mm	16mm

DISCUSSION

It reveals that crossbreeds have the highest prevalence at 29.66%, followed by German Shepherds at 22.3%, with other breeds showing varying percentages. Additionally, it notes that 71.11% of dogs with erect ears had otitis externa, while only 29.67% of dogs with pendulous ears were affected. Importantly, the statistical analysis concludes that there are no significant differences among the breeds ($p = 0.1270$). A study found that mixed-breed dogs had a higher risk of ear infections compared to purebreds, which is in line with the observation that crossbreeds have the highest prevalence (16).

A report has shown that ear shape significantly influences the risk of otitis externa in dogs (17). The findings of the paragraph align with this research, emphasizing the importance of considering ear conformation when assessing the likelihood of infection. The higher infection rate in dogs with erect ears aligns with recent research indicating that ear shape can influence the risk of ear infections (18). This is attributed to the differences in airflow and moisture retention between erect and pendulous ears.

The finding that there is no significant difference in otitis externa prevalence between male and female dogs is in line with a study by (14). This study also found no statistically significant gender differences in ear infection rates. Such consistency suggests that gender may not be a major contributing factor to otitis externa. Although the possible causes of gender-based differences, the hormonal factors, such as estrogen and testosterone, may play a role in ear health. Gender-related behaviors and environmental factors could also contribute to otitis externa (19). A study explored that how certain behavioral traits, like swimming frequency, may affect ear health differently in males and females (18).

The observation that the highest infection rate occurs in dogs up to four years of age aligns with research conducted by Brame and Cain, 2021 (20). This study also found that younger dogs were more susceptible to otitis externa, likely due to their developing immune systems and increased exposure to ear irritants. Understanding the relationship between age and otitis externa often involves examining the immune system. Another study explored that the immune response of young dogs differs from that of older dogs, shedding light on younger animals who might be more prone to ear infections (4). Given the higher prevalence in younger dogs, a research focused on developing preventive strategies, including ear care routines and early screening, which could be particularly beneficial for animals in the one to four-year age group (21).

The various bacterial species identified and their respective prevalence are as follows: Pseudomonas spp. (22.03%), Staphylococcus spp. (17.79%), *Escherichia coli* (13.55%), Proteus spp. (9.32%), Streptococcus spp. (8.47%), and less frequently, Bacillus spp. (5.93%). The bacterial species commonly associated with otitis externa. Studies by (2) have reported similar bacterial profiles, emphasizing the prevalence of *Pseudomonas, Staphylococcus*, and *Escherichia coli* in ear infections. The interactions between different bacterial species within the ear canal are a subject of growing interest. The mentioned microorganisms interact and contribute to the pathogenesis of otitis externa (22). Such studies can provide insights into potential treatment strategies targeting multiple bacterial species simultaneously.

The bacterial otitis externa in dogs provides data on the distribution of cases across the seasons. It reveals that the majority of cases occurred in spring (32.2%) and autumn (23.72%), followed by summer (12.71%) and winter (8.47%). The higher prevalence of otitis externa in spring and autumn. This is consistent with research by Avberšek *et al.,* 2021 reported similar seasonal patterns, emphasizing increased cases during the warmer months and the transition to colder seasons. Seasonal variations in temperature and humidity can influence the proliferation of microorganisms in the ear canal (23). The link between climate and otitis externa, shedding light on how environmental factors contribute to infection rates (24). It's essential to address potential underlying factors and implement preventive measures. Regular ear cleaning with veterinarian-approved solutions can help remove excess wax and debris, reducing the risk of bacterial growth. Avoiding exposure to moisture, such as swimming or excessive bathing, and ensuring proper drying of ears after exposure to water can also prevent infections. Additionally, identifying and managing underlying conditions like allergies or hormonal imbalances can contribute to preventing recurrent otitis externa. Regular veterinary check-ups and prompt treatment of ear infections can further aid in prevention by addressing early signs and preventing progression to chronic conditions (22). In the present study, the highest resistance was observed in Staphylococcus spp, PseudomonaIs spp, Proteus spp, *Escherichia coli*, Streptococcus spp, and Bacillus spp against amoxicillin, gentamycin, and ampicillin. Conversely, these bacteria exhibited low resistance to oxytetracycline and cephotaxime, while enrofloxacin and norfloxacin displayed significant sensitivity. Similar findings regarding Pseudomonas spp, Proteus spp, Escherichia coli, and Streptococcus spp resistance to penicillin in dogs were reported by Bourély *et al.,* 2019 (2). Cefoxitin, ceftriaxone, and enrofloxacin were identified as the most effective antibiotics against Pseudomonas spp, Proteus spp, *Escherichia coli*, and Streptococcus spp. These results align with previous research conducted by Bourély *et al.,* 2019 indicating good sensitivity for the tested antibiotics (2). Additionally, Arain *et al.,* 2021 and Avberšek *et al.,* 2021 reported higher resistance percentages to amoxicillin + clavulanic acid in their study (23, 25). Understanding the prevalent bacterial isolates and their antibiotic susceptibility in a specific geographical area can guide the selection of initial therapy for uncomplicated canine otitis externa. The

study emphasizes the necessity for countries to implement robust measures, including antimicrobial resistance (AMR) monitoring and consumption record-keeping guided by antimicrobial stewardship (AMS). Notably, G- bacteria strains exhibited a higher resistance level compared to G+ strains. The urgent need for guidelines promoting prudent antibiotic use in veterinary medicine is underscored by the study's findings, with a focus on risk management to mitigate antibiotic consumption and subsequent AMR development. Additionally, preventive strategies such as vaccination, deworming, appropriate nutrition, and probiotics are highlighted as integral components of antimicrobial stewardship. The study emphasizes the potential for cross-resistance between antibiotics used in veterinary and human medicine, especially concerning critically important antimicrobials (CIAs) in small animal practice.

CONCLUSION

It is concluded from the present study that the highest prevalence of otitis externa was recorded crossbreed, among female dogs aged one to four years. The identified bacterial species from positive samples include Pseudomonas spp., Staphylococcus spp., Escherichia coli, Proteus spp., Streptococcus spp., and Bacillus spp. Most infections were recorded during the spring season. Enrofloxacin and norfloxacin exhibited high sensitivity against isolated bacteria. Notably, critical antimicrobials for human medicine (amoxicillin, gentamycin, and ampicillin) showed susceptibility, emphasizing the need for continuous education of veterinarians to address antimicrobial resistance (AMR). The paragraph stresses the importance of regular antibiotic susceptibility testing and advocates for the implementation of antimicrobial stewardship principles, including surveillance and monitoring programs, as crucial tools in the ongoing effort to reduce antimicrobial resistance.

Conflict of Interest:

The authors have no conflict of interest.

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