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Study on bacterial isolation, antibiogram and therapeutic management of canine otitis externa

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Abstract

Canine otitis externa (COE) is a multifactorial condition characterized by acute or chronic inflammation of the external ear, significantly impacting affected dogs and posing diagnostic and therapeutic challenges. The present study analyzed 92 clinical cases from the Junagadh region, focusing on bacterial etiology, incidence patterns and antimicrobial susceptibility profiles. *Staphylococcus* spp. was identified as the predominant pathogen (48.18%, 53/110), followed by *Pseudomonas* spp. (11.81%, 13/110) and *Proteus mirabilis* (10.91%, 12/110) isolates, respectively. Higher incidence was observed in non-descript breeds (33.70%, 31/92), male dogs (57.61%, 53/92) and dogs aged 6-10 years (55.43%, 51/92). Antimicrobial sensitivity testing (AST) demonstrated that *Staphylococcus* spp. was highly susceptible to amoxicillin-clavulanic acid (92.45%), gentamicin (86.79%) and chloramphenicol (83.01%) but exhibited resistance to penicillin-G (81.13%). *Pseudomonas* spp. showed the greatest sensitivity to gentamicin (84.61%) and amoxicillin-clavulanic acid (76.92%), while *Proteus* spp. was most sensitive to chloramphenicol and doxycycline (91.67%). These results emphasize the need for AST-guided therapy to mitigate antimicrobial resistance and improve treatment efficacy. These findings underscore the importance of targeted diagnostic approaches and precise antimicrobial therapies to enhance otitis externa management and improve canine health outcomes.

Keywords: COE, *Staphylococcus* spp., etiology, incidence, antimicrobial susceptibility

Introduction

Canine otitis externa (COE) is a non-life-threatening yet distressing condition characterized by acute or chronic inflammation of the external ear in dogs. This condition poses significant challenges, not only for affected dogs and their owners but also from diagnostic and therapeutic perspectives for practicing veterinarians [1, 2]. It is a multifactorial disease often associated with various microbial agents, affecting approximately 20% of the canine population [3]. COE is frequently complicated by mixed infections involving both bacteria and yeast. The normal microflora of the ear canal typically includes coagulase-positive and coagulase-negative *Staphylococcus* species, along with β -hemolytic *Streptococcus* species, *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and others [4]. Despite its incidence, there is a lack of published data on the incidence of otitis externa, the antimicrobial sensitivity patterns of associated bacterial isolates and its management in Gujarat. The present study aimed to address this gap by identifying the bacterial agents responsible for otitis externa, evaluating their antimicrobial susceptibility testing (AST) and providing insights into effective therapeutic strategies to alleviate the suffering of affected dogs.

Materials and Methods

A total of 92 ear swabs from clinical cases of COE were collected. All the samples were collected aseptically by gently rolling the sterile HiCulture collection device (PW 003, HiMedia Laboratories and Mumbai) moistened with sterile 0.9% saline solution from ear canal of affected dogs. They were having complaints of shaking of ears, tilting of the head, drooping of affected ears, ceruminous or purulent ear discharge, pawing of ears and foul smell from ears.

All the dogs underwent a thorough assessment that included, obtaining a detailed history, conducting clinical examinations, performing microbiological analyses and carrying out AST to guide therapeutic management. Primarily Brain Heart Infusion (BHI) agar (M211, HiMedia) was used to isolate bacteria and colonies were subjected to Gram's staining. While, Mueller Hinton (MH) agar (M173, HiMedia) was used for the AST. All the media and antibiotic discs used were procured from Hi-Media.

Results and Discussions

Breed-wise Incidence of COE

The breed-wise incidence of otitis externa in the present study revealed the highest incidence in Non-descript (ND) dogs (33.70%, N=31), followed by Labrador Retrievers (23.91%, N=22) and German Shepherds (16.30%, N=22) and German Shepherds (16.30%, N=15). The incidence of other species was as per (Table 1 and Figure 1).

Table 1: Breed-wise incidence of COE in dogs

Sr. No.	Dog breed	No of samples	Percentage (%)
1	Non-descript (ND)	31	33.70
2	Labrador Retriever	22	23.91
3	German Shepherd	15	16.30
4	St. Bernard	8	8.70
5	Pomeranian	7	7.60
6	Pug	3	3.26
7	Golden Retriever	3	3.26
8	Rottweiler	2	2.17
9	Doberman	1	1.09
	Total	92	100

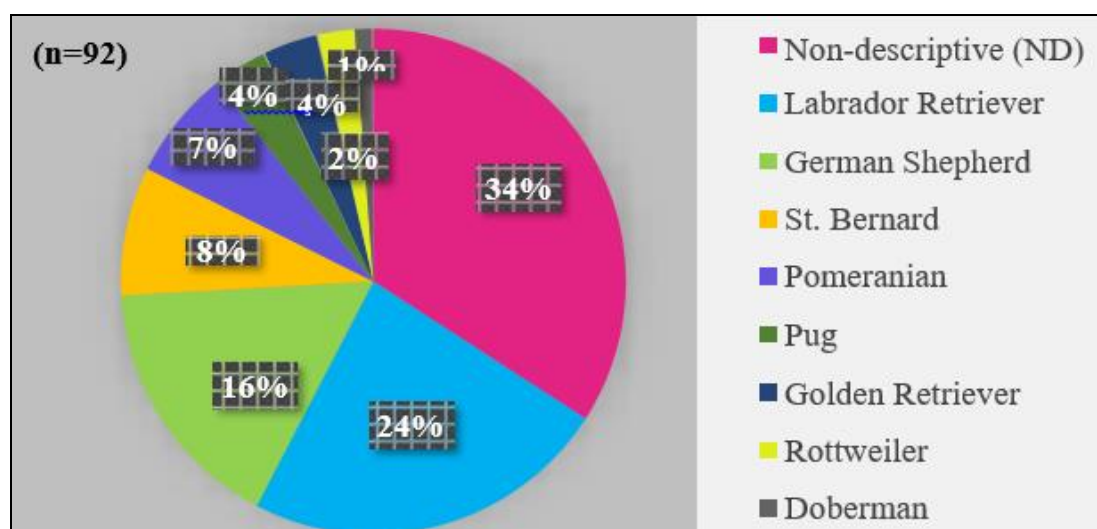


Fig 1: Breed-wise incidence in cases of COE

Bacterial Incidence in COE

The bacterial incidence in 92 COE samples revealed 110 pathogenic bacterial isolates, with *Staphylococcus* spp. being the most prevalent, accounting for 53 isolates (48.18%). It was followed by *Pseudomonas* spp. (13 isolates, 11.81%) and *Proteus mirabilis* (12 isolates, 10.91%). Other notable pathogens included *Micrococcus* spp., *Corynebacterium* spp. and *Malassezia pachydermatis* (7 isolates each, 6.36%). *E. coli* and *Klebsiella* spp. each contributed 4 isolates (3.64%), while *Streptococcus* spp. was identified in 3 isolates (2.73%) (Table 2 and Figure 2). Some samples showed single and mixed infection of two or multiple organisms. These findings highlighted the diverse bacterial population involved in otitis externa, with *Staphylococcus* species being the dominant pathogens.

Kiss *et al.* [5] also found similar findings for *Staphylococcus* spp. (39.22%) and *Pseudomonas* spp. (12.62%). Murugan *et al.* [2] also observed similar findings for *Staphylococcus* spp. (48.6%) followed by *Pseudomonas* spp. (21.5%) and *Proteus* spp. (14.95%). Luciani *et al.* [6] also reported similar results for *Staphylococcus* spp. (48.8%) and *Pseudomonas* spp. (30.2%). A comparable bacterial distribution was reported by Kumar *et al.* [7] and Suneja *et al.* [8], emphasizing the

consistent role of *Staphylococcus* spp. in COE. Lakshmi & Tirumala [1] reported the presence of similar bacteria in COE in their study.

Moreover, Hariharan *et al.* [9] identified *Staphylococcus intermedius* as the predominant species in otitic dogs, followed by *Pseudomonas* spp., which aligns with present findings. Similar observations were made by Mateus *et al.* [10], who also highlighted *Staphylococcus pseudintermedius* as a major pathogen, often found in single or mixed infections. Kania *et al.* [11] further supported the dominant role of *Staphylococcus* species, attributing their incidence to their ability to form biofilms and evade host immunity.

The *Staphylococcus* spp. isolates were identified based on their morphological and cultural characteristics (white or golden-yellow colonies). It was confirmed as Gram-positive, catalase-positive, and oxidase-negative. The *Pseudomonas* spp. isolates in the present study were identified microscopically as Gram-negative, rod-shaped bacteria. It exhibited a characteristic greenish discoloration on BHI agar due to the production of the pigment pyocyanin, a hallmark of the genus [12]. *Proteus mirabilis* was identified by its swarming growth on agar, Gram-negative microscopic appearance, and biochemical characteristics as catalase-

positive and oxidase-negative. *Micrococcus* spp. was confirmed based on its Gram-positive staining properties and biochemical tests, including catalase-positive and oxidase-positive reactions. Gram-positive bacteria with a distinctive Chinese letter arrangement were identified as *Corynebacterium* spp. The Gram-positive, typically oval to round and larger yeast cells compared to *Staphylococcus* species' cocci were identified as *Malassezia pachydermatis* [13]. Gram-negative bacteria were further cultured on Eosin

Methylene Blue (EMB) agar. *Escherichia coli* (*E. coli*) produced distinctive metallic green sheen colonies, while *Klebsiella* spp. formed circular, dome-shaped colonies ranging from pink to purple on EMB agar. Biochemical testing confirmed both *E. coli* and *Klebsiella* spp. as catalase-positive and oxidase-negative. Gram-positive cocci arranged in chains were identified as *Streptococcus* spp. based on their cultural characteristics and biochemical properties, such as catalase-negative and oxidase-negative reactions [13].

Table 2: Overall incidence of bacterial isolates from otitic ears

Sr. no.	Bacteria species	No of isolates	Incidence (%)
1	<i>Staphylococcus</i> spp.	53	48.18
2	<i>Pseudomonas</i> spp.	13	11.81
3	<i>Proteus mirabilis</i>	12	10.91
4	<i>Micrococcus</i> spp.	7	6.36
5	<i>Corynebacterium</i> spp.	7	6.36
6	<i>Malassezia pachydermatis</i>	7	6.36
7	<i>E. coli</i>	4	3.64
8	<i>Klebsiella</i> spp.	4	3.64
9	<i>Streptococcus</i> spp.	3	2.73
Total isolates		110	100

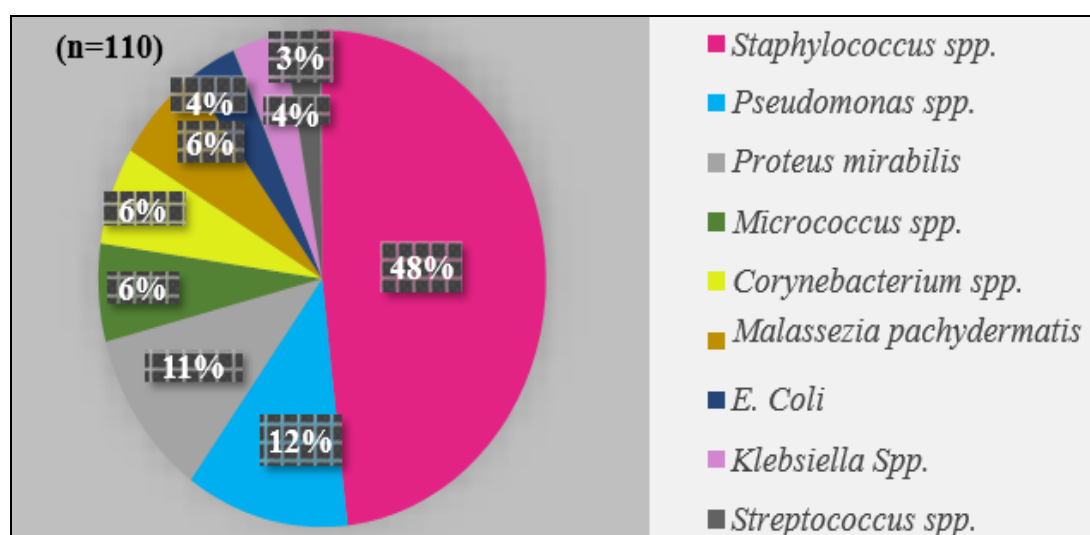


Fig 2: Overall incidence of different bacteria associated with COE

Sex-wise Incidence of COE

The sex-wise incidence of clinical cases of COE was comparatively higher in male dogs (53, 57.61%) than in female dogs (39, 42.39%) (Table-3 and Fig.-3). That might be because people prefer to keep male dogs as pets [14]. Male dogs are often more prone to otitis externa, and various biological and behavioral factors may contribute to this predisposition. Hormonal influences, particularly testosterone, may play a role in immune modulation, affecting males' susceptibility to infections like otitis externa [15]. Additionally, certain behaviors, such as increased outdoor activity or engagement in muddy areas, can lead to greater exposure to environmental pathogens, and moisture is a common risk factor for ear infections [16]. Anatomically, male dogs may have slightly thicker ear canals, potentially altering ear ventilation and trapping moisture, which fosters bacterial and yeast growth, particularly in breeds already predisposed due to ear structure [17]. Agnihotri *et al.* (2014) [18] observed similar results, with otitis externa being more common in male dogs, with an incidence of 68.71%, compared to 31.29% in female dogs. Subramanian *et al.* (2015) [19] also reported the same incidence in male dogs (61.03%). Manju *et al.*

(2018) [20] reported that otitis externa was higher in old age and male dogs.

Table 3: Sex-wise incidence of COE

Sr. No.	Sex	No of isolates (%)
1	Male	53 (57.61)
2	Female	39 (42.39)

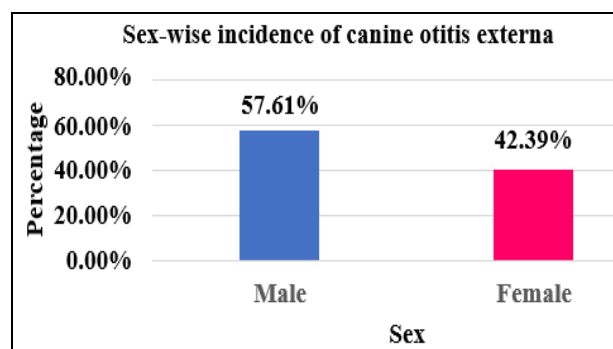


Fig 3: Sex-wise incidence of COE

Age-wise Incidence of COE

For the age-wise incidence, most cases were observed in dogs aged 6-10 years, representing 55.43% (51/92) of the sample population. It was followed by younger dogs aged 0-5 years, accounting for 27.17% (25/92) of cases. The lowest incidence was observed in dogs older than 11 years, comprising 17.39% (16/92) of cases (Table 4 and Figure 4). Suneja *et al.* [8] reported a higher incidence of canine otitis in the 2-7 years age group, and male dogs were more affected than females. Reddy [21] observed a significant incidence of otitis externa in dogs aged between 5-10 years. Singh *et al.* [22] reported the highest incidence of otitis externa in dogs aged 3-6 years.

Subramanian *et al.* [19] also reported a peak incidence of otitis externa in the age group of 5-10 years (31.46%). However, Fraser *et al.* [23] and Nuttall [24] reported no apparent relationship between the incidence of canine otitis and the age of the affected animals.

Table 4: Age-wise incidence of COE

Sr. No.	Age groups (in yrs.)	No. of dogs	Incidence (%)
1	0-5	25	27.17
2	6-10	51	55.43
3	>11	16	17.39
Total		92	100

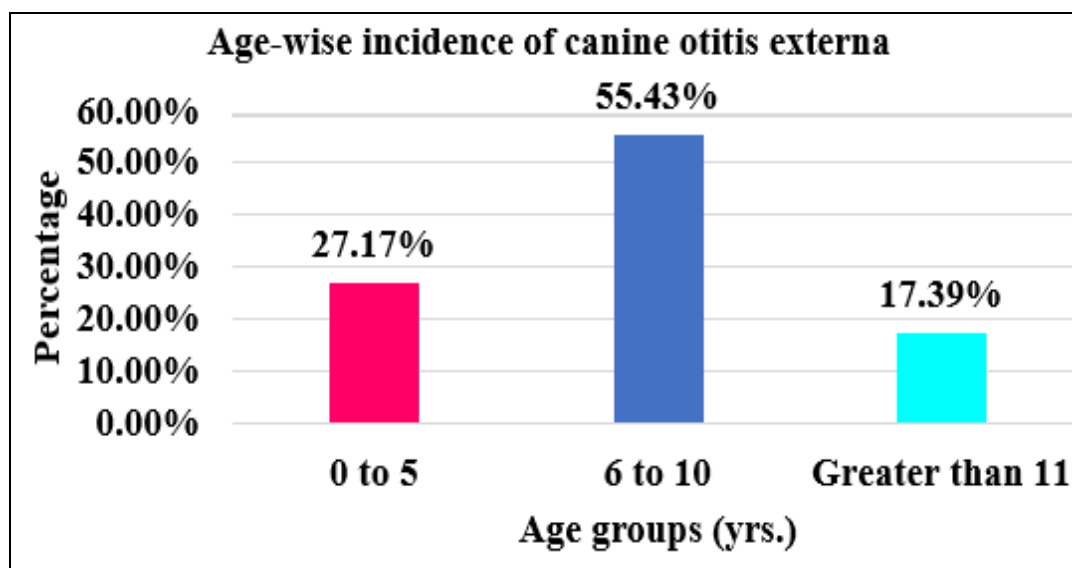


Fig 4: Age-wise incidence of COE

Antibiogram of Major Bacteria

Antibiotic susceptibility testing (AST) was performed on Muller-Hinton agar (MHA) as suggested by the Kirby-Bauer disk diffusion method [25]. Three to four distinct pure bacterial colonies (cultured for 24 hours) were suspended in a sterile 0.85% NaCl solution to achieve a concentration corresponding to 0.5 McFarland turbidity. The turbidity was verified using a McFarland densitometer. A cotton swab mounted on a wooden stick was dipped into the suspension and then used to evenly streak the surface of a Muller-Hinton agar plate until the inoculum fully covered the surface. Afterward, antibiotic disks were placed on the agar using a disk dispenser, and the plate was incubated at 37°C for 18 hours. After incubation, the zones of inhibition surrounding each antibiotic disk were measured in millimeters and compared to the reference guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) [26].

The AST results highlighted distinct susceptibility patterns among *Staphylococcus* spp., *Pseudomonas* spp., and *Proteus* spp. *Staphylococcus* spp. showed high sensitivity to amoxicillin-clavulanic acid (92.45%), gentamicin (86.79%), and chloramphenicol (83.01%), while resistance was notable for penicillin-G (18.87%). *Pseudomonas* spp. exhibited significant sensitivity to gentamicin (84.61%) and amoxicillin-clavulanic acid (76.92%) but low response to doxycycline (15.38%) and cefoxitin (0%). *Proteus* spp. displayed high susceptibility to chloramphenicol (91.67%) and doxycycline (91.67%), with complete resistance to penicillin-G and clindamycin (both 0%) (Table 5). These findings were corroborated by Murugan *et al.* [2] and Parmar *et al.* [27]. Bugden [28] also found similar findings of high

sensitivity for gentamicin against all three bacterial genera. These findings underscore the importance of AST-guided antibiotic selection to address resistance variability effectively. Shobha *et al.* [29] also reported the highest resistance against penicillin-G in their study.

Table 5: Antibiotic sensitivity of major isolated bacteria

Sr. No.	Antibiotic discs	Antibiotic sensitivity (%)		
		<i>Staphylococcus</i> spp. (n= 53)	<i>Pseudomonas</i> spp. (n= 13)	<i>Proteus</i> spp. (n= 12)
1	Chloramphenicol (30 mcg)	83.01	61.53	91.67
2	Doxycycline (30 mcg)	83.01	15.38	91.67
3	Cefpodoxime (10 mcg)	45.28	23.07	50
4	Cefoperazone (75 mcg)	58.49	61.53	58.33
5	Amikacin (30 mcg)	75.47	61.53	33.33
6	Clindamycin (10 mcg)	71.69	0	0
7	Ceftriaxone (10 mcg)	62.26	23.97	50
8	Gentamicin (10 mcg)	86.79	84.61	75
9	Penicillin-G (10 units)	18.87	15.38	0
10	Methicillin (5 mcg)	66.03	0	0
11	Amoxicillin-Clavulanic acid (30 mcg)	92.45	76.92	83.33
12	Enrofloxacin (10 mcg)	60.37	46.15	75
13	Levofloxacin (5 mcg)	56.6	69.23	58.33
14	Cefoxitin (30 mcg)	39.62	0	25

Treatment

Ear canals of all affected dogs were cleaned with sterile saline solution and it was advised to clean ears with ear cleanser Virbac EpiOtic (Salicylic acid-0.2%). Patients were treated based on AST results. Amoxicillin-clavulanic acid 12.5-25 mg/kg BW PO or Chloramphenicol 25-50 mg/kg BW BID PO

was prescribed for 7 days. In addition to antibiotic therapy, there are several dermatologic conditions where glucocorticoids are helpful, even when antimicrobial treatment or sepsis is present. Glucocorticoids help reduce inflammation and swelling in the ear canal, playing a crucial role in the successful management of these conditions. After one week of treatment, all the affected dogs demonstrated significant improvement, with a noticeable reduction in head shaking, ear scratching, and erythema of the pinna.

Conclusions

The present study's findings reveal COE's intricate nature, highlighting significant trends in its incidence and management. ND breeds, male dogs and those aged 6-10 years were identified as the most susceptible groups, emphasizing the potential influence of genetic and hormonal factors. Among the bacterial pathogens isolated, *Staphylococcus* spp. emerged as the most prevalent, followed by *Pseudomonas* spp. and *Proteus mirabilis*, underscoring their role in disease progression. Antimicrobial sensitivity testing provided critical insights, with notable effectiveness of amoxicillin-clavulanic acid, gentamicin and chloramphenicol against key pathogens, while resistance to penicillin-G and certain other antibiotics was evident. These findings stress the importance of tailoring antibiotic regimens based on sensitivity profiles to combat resistance and optimize therapeutic outcomes. Integrative management, including thorough ear cleaning, strategic antibiotic use and adjunctive glucocorticoids, demonstrated efficacy in alleviating clinical signs and improving recovery rates. The present study underscores the necessity of comprehensive diagnostic and treatment approaches to effectively address COE and enhance the quality of life in affected dogs.

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Conflict of Interest

All authors declare no conflict of interest

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