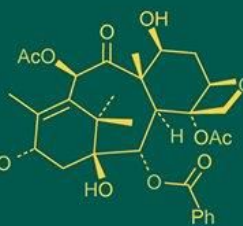


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Isolation, cultural and biochemical identification of bacteria causing canine otitis in the north-eastern Karnataka region

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Abstract

Canine otitis is a frequently encountered and often recurrent clinical condition in dogs, resulting from a complex interplay of infectious and non-infectious factors. The present study was undertaken to isolate, identify and characterize the predominant bacterial pathogens associated with canine otitis in the north-eastern Karnataka region (Bidar, Kalaburagi, Yadgir, Raichur and Koppal).

A total of 104 ear swabs were collected from clinically suspected otitis cases, of which 86 samples yielded bacterial growth. The *Staphylococcus* spp. were the most predominant isolates (55.81%), followed by *Pseudomonas* spp. (15.12%), *Escherichia* spp. (11.63%), *Klebsiella* spp. (9.30%), and *Proteus* spp. (2.33%), with mixed infections observed in 5.81% of cases. Biochemical characterization revealed that the predominant *Staphylococcus* isolates were coagulase positive. The findings highlight the predominant role of *Staphylococcus* spp. in canine otitis in this region and emphasize the importance of bacteriological diagnosis for effective clinical management.

Keywords: Canine, otitis, bacteria, *Staphylococcus* spp., *Escherichia* spp., north eastern Karnataka

Introduction

Otitis is defined as inflammation of the ear canal and may involve the external, middle or inner ear, with otitis externa accounting for the majority of clinical cases. Otitis is one of the commonly diagnosed disorders in companion animal practice, particularly in dogs, and often necessitates repeated veterinary intervention. The condition may be present in acute or chronic forms, depending on the duration and severity of inflammation. Otitis is categorized into otitis externa, media, and interna based on anatomical involvement, with otitis externa accounting for the majority of clinical cases [6, 14]. The pathogenesis of canine otitis externa is multifactorial and involves primary, predisposing and perpetuating factors that disrupt the normal microenvironment of the ear canal [10]. Secondary microbial invaders, including bacteria and yeasts that are normally commensal, become opportunistic pathogens in compromised ear canals, often leading to chronic or complicated infections [11]. Common isolates include *Staphylococcus pseudintermedius*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus* spp., *Streptococcus* spp., *Corynebacterium* spp., *Escherichia coli*, *Enterobacter* spp., and *Klebsiella pneumoniae* [6, 12]. Several of these organisms, especially *P. aeruginosa* and *S. pseudintermedius*, are also associated with zoonotic risks and increasing concerns of antimicrobial resistance (AMR) and multidrug resistance (MDR) (Jin *et al.*, 2023) posing challenges for effective treatment.

In many field situations, empirical therapy is initiated without prior laboratory confirmation, which may contribute to treatment failure and emergence of resistant strains [13, 15]. Therefore, accurate isolation and identification of the etiological agents through morphological, cultural and biochemical characterization are essential for rational therapeutic decision-making.

The north-eastern Karnataka region including Bidar, Kalaburagi, Yadgir, Raichur, and Koppal houses a diverse canine population but lacks systematic studies on the bacteriology of canine otitis. Understanding the bacterial flora responsible for otitis in this region is essential for formulating region-specific treatment protocols and for monitoring AMR trends.

Therefore, the present study aims to isolate the bacterial pathogens associated with canine otitis, characterize them based on morphological, cultural, biochemical properties and contribute baseline data for improving diagnostic and therapeutic strategies for canine ear infections in north-eastern Karnataka region.

Materials and Methods

The study was conducted at the Department of Veterinary Microbiology, Veterinary College, Bidar (KVAFSU), Karnataka. All laboratory procedures were performed under strict aseptic conditions. Standard bacteriological media and reagents were procured from HiMedia and prepared as per manufacturer's instructions. A total of 104 ear swabs were collected from dogs exhibiting clinical signs suggestive of otitis, including head shaking, pruritus, erythema and ear discharge, from Bidar, Kalaburagi, Yadgir, Raichur and Koppal districts. Samples were transported in cold chain to the laboratory. Swabs were first enriched in nutrient broth and incubated at 37 °C overnight. Enriched cultures were streaked onto nutrient agar, MacConkey agar, EMB agar, mannitol salt agar and blood agar, and incubated at 37 °C for 24-48 hours. Distinct colonies were purified and stored for further testing. Colony characteristics (colour, size, shape, haemolysis) were noted. Gram staining was performed to determine Gram reaction, morphology and arrangement. Standard biochemical tests including indole, methyl red, Voges-Proskauer, citrate utilization, oxidase and catalase were conducted. Identification of isolates was based on colony morphology, Gram staining, and biochemical profiles. Pure cultures were maintained on nutrient agar slants at 4 °C and preserved in nutrient broth at -20 °C for long-term storage.

Results

Out of 104 ear swabs examined, 86 samples (82.69%) were positive for bacterial growth. The highest occurrence of otitis was observed in dogs aged between 1-5 years (53.49%), followed by 5-10 years (29.07%) are tabulated in table-1 and depicted in fig-1. Sex-wise incidence was noted highest in males (52.33%) than their counter parts (47.67%) (Table-2; Fig-2). The highest incidence was observed in non-descript dogs (36.05%), followed by German Shepherds (27.91%) and Labrador Retrievers (24.42%). Unilateral otitis (86.05%) was more common than bilateral (13.95%) (Table-3; Fig-3). Unilateral affections were observed in 74

(86.05%) dogs. Of these 74 unilateral cases, 43 (51.16%) were noticed in right ear and 31 (34.88%) in left ear. Bilateral affections were noticed in 12 (13.95%) dogs (Table-4 and Fig-4).

Table 1: Age wise occurrence of canine otitis

Age group	Number of samples screened	Number of positive samples	Per cent positivity (%)
0-1 Year	14	08	9.30
1-5 Year	52	46	53.49
5-10 Year	31	25	29.07
10 year and above	07	07	8.14
Total	104	86	100

Table 2: Sex wise occurrence of canine otitis.

Sex	Number of samples screened	Number of positive samples	Percentage (%)
Male	63	45	52.33
Female	41	41	47.67
Toatal	104	86	100

Table 3: Breed wise occurrence of canine otitis.

Breed	Number of samples screened	Number of positive samples	Percentage (%)
German Shepherd	27	24	27.91
Non Descript	36	31	36.05
Labrador Retriever	25	21	24.42
Pomeranian	05	03	3.49
Rottweiler	04	02	2.33
Doberman Pinscher	05	03	3.49
Saint Bernard	01	01	1.16
Great Dane	01	01	1.16
Total	104	86	100

Table 4: Occurrence of canine otitis with respect to Side of ear involvement.

Sr. No.	Side of the ear involved	Number of positive samples	Percentage (%)
1	Right	43	51.16
2	Left	31	34.88
3	Bilateral	12	13.95
	Total	86	100.00

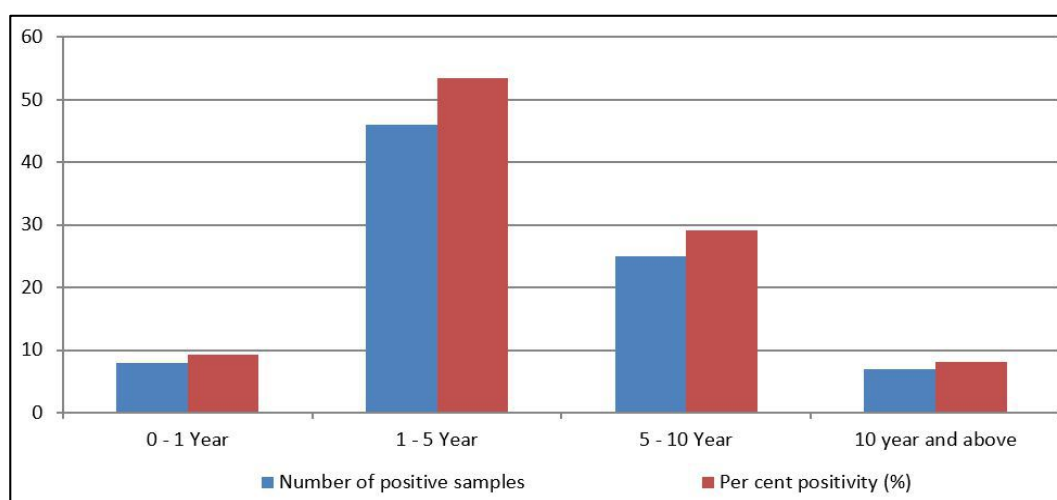


Fig 1: Age wise occurrence of canine otitis

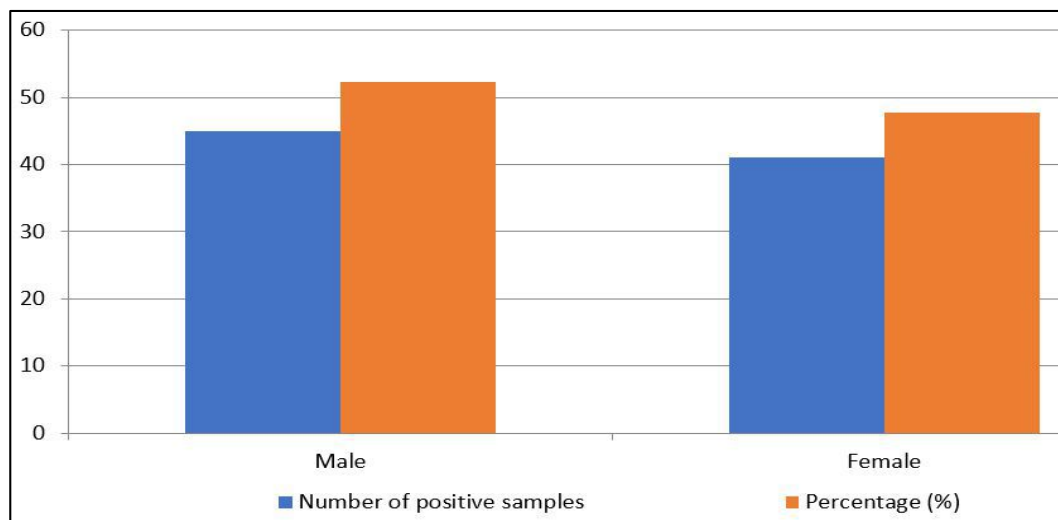


Fig 2: Sex wise occurrence of canine otitis.

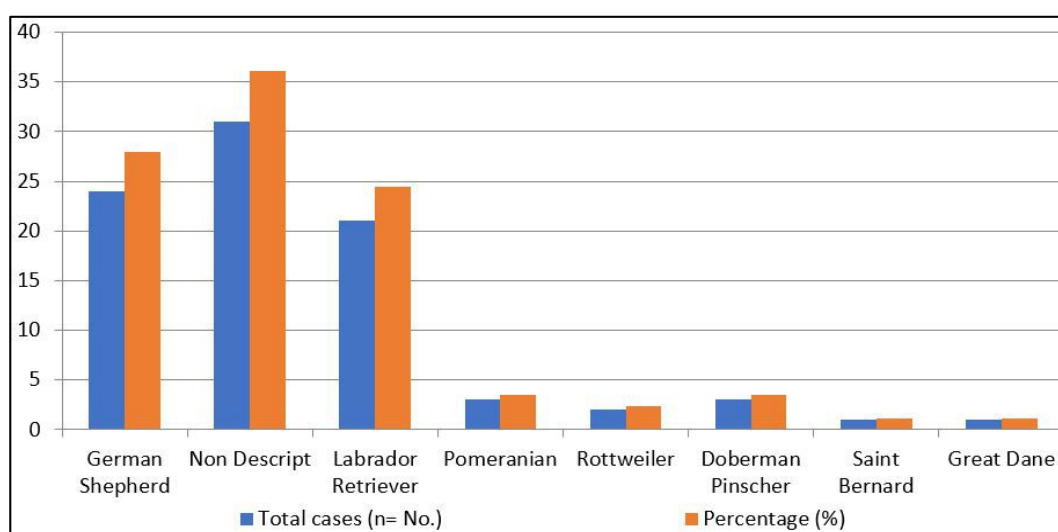


Fig 3: Breed wise occurrence of canine otitis.

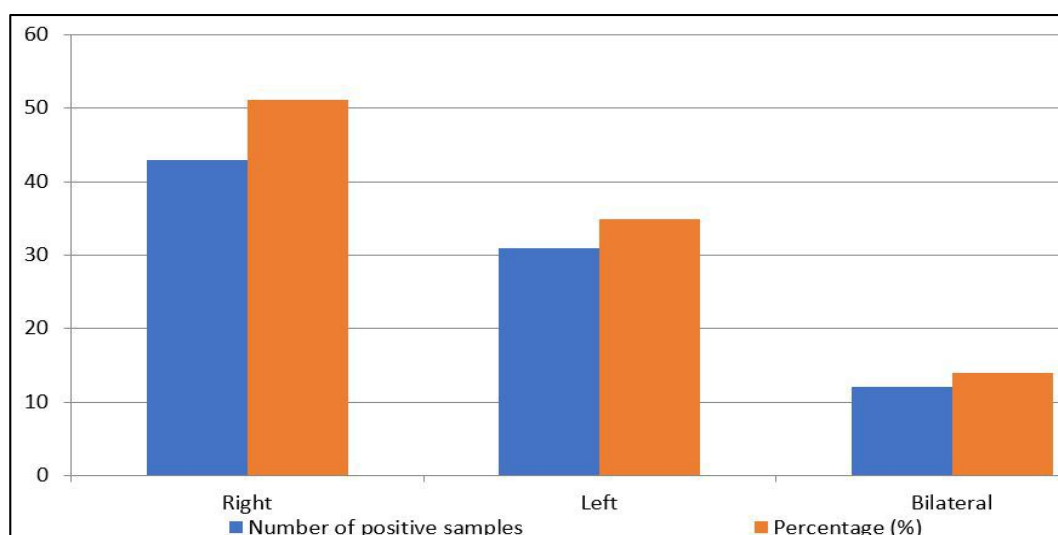


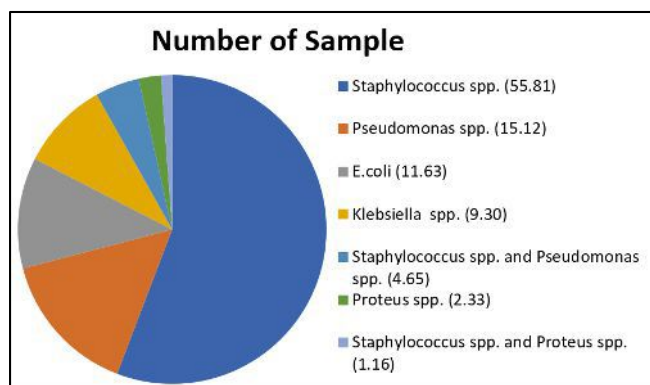
Fig 4: Occurrence of canine otitis with respect to Side of ear involvement.

Among the 86 culture-positive samples, the following isolates were recovered, and their incidence was tabulated in table-5 and depicted in fig-5. The predominant bacteria isolated were *Staphylococcus* spp. constituting a total of 55.81 per cent (48 dogs) followed by *Pseudomonas* spp. were 15.12 per cent (13 dogs), *Escherichia* spp. were 11.63

per cent (10 dogs), *Klebsiella* spp. were 9.30 per cent (8 dogs) and *Proteus* spp. 2.33 per cent (2 dogs) whereas, *Staphylococcus* spp. + *Pseudomonas* spp: 4.65 per cent (4 dogs) and *Staphylococcus* spp. + *Proteus* spp: 1.16 per cent (1 dog). 18 (17.31%) samples showed negative for bacterial growth.

Table 5: Occurrence of predominant bacteria

Organism found	Number of Sample	Percentage (%)
<i>Staphylococcus</i> spp.	48	55.81
<i>Pseudomonas</i> spp.	13	15.12
<i>Escherichia</i> spp.	10	11.63
<i>Klebsiella</i> spp.	08	9.30
<i>Staphylococcus</i> spp. and <i>Pseudomonas</i> spp.	04	4.65
<i>Proteus</i> spp.	02	2.33
<i>Staphylococcus</i> spp. and <i>Proteus</i> spp.	01	1.16
Total	86	100

**Fig 5:** Occurrence of predominant bacteria

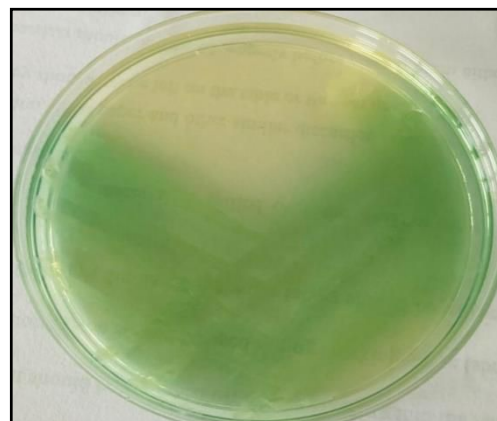
Morphological and Cultural Characteristics

A total of 53 bacterial isolates producing large, yellow colonies surrounded by a yellow zone due to mannitol fermentation on Mannitol salt agar were subjected to Gram staining. Microscopic examination revealed Gram-positive, purple-coloured cocci arranged in grape-like clusters, consistent with the typical characteristics of *Staphylococcus* spp (Plate-1 & 2). Additionally, 17 isolates displaying greenish colonies on Nutrient agar were Gram-stained. These showed Gram-negative, pink-colored, rod-shaped cells, which are consistent with *Pseudomonas* spp (Plate-3). Ten bacterial isolates that produced a characteristic metallic sheen on Eosin Methylene Blue agar were also examined via Gram staining (Plate-4). The results indicated Gram-negative, rod-shaped bacteria, consistent with the morphology of *Escherichia* spp. On MacConkey agar, eight isolates formed large, mucoid, pink to red colonies. Gram staining revealed Gram-negative, rod-shaped bacteria, consistent with the characteristics of *Klebsiella* spp (Plate-5). Finally, Three isolates exhibited swarming growth on Nutrient Agar, forming a thin, spreading film instead of discrete colonies. These were identified as Gram-negative, rod-shaped bacteria, consistent with *Proteus* spp (Plate-6).

Biochemical Characterization

All 53 pure cultures of *Staphylococcus* spp. isolates underwent biochemical testing. The isolates showed the following results: Positive for coagulase test, positive for Catalase and Oxidase negative (Plate-7a, 7b & 7c). All 17 pure cultures of *Pseudomonas* spp. (Plate-8) isolates underwent biochemical testing. The isolates showed the following results negative for the Indole, Methyl Red, and Voges-Proskauer tests, but were positive for Citrate utilization, Oxidase and Catalase. 10 pure cultures of suspected *Escherichia* spp. isolates underwent biochemical testing. The isolates showed the following results: positive for the Indole and Methyl Red tests, but were negative for the Voges-Proskauer and Citrate utilization tests (Plate-9).

They also showed a negative result for Oxidase and tested positive for Catalase. Eight pure cultures of *Klebsiella* spp. isolates underwent biochemical testing. The isolates showed the following results: negative for both Indole and Methyl Red tests, but were positive for the Voges-Proskauer and Citrate utilization tests (Plate-10). They also tested negative for Oxidase and positive for Catalase test. Three pure cultures of *Proteus* spp. isolates underwent biochemical testing. The isolates showed the following results: negative for the Indole and Voges-Proskauer tests, but showed positive results for the Methyl Red, Citrate utilization, and Catalase tests. They were also negative for the Oxidase test (Plate-11).

**Plate 1:** Growth of *Staphylococcus* spp. on mannitol salt agar showing pinpoint golden yellow pigmented colonies.**Plate 2:** *Staphylococcus* spp. culture on 5% sheep blood agar showing haemolytic colonies.**Plate 3:** Growth of *Pseudomonas* spp. on Nutrient agar showing greenish colonies.

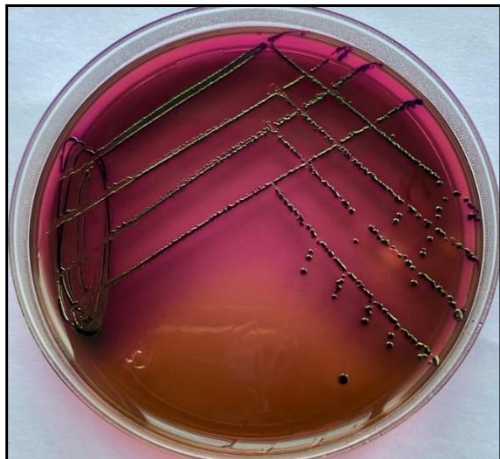


Plate 4: Growth of *Escherichia* spp. on EMB agar showing green metallic sheen

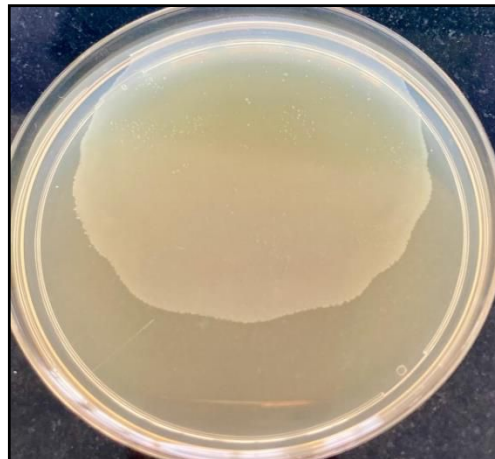


Plate 6: Growth of *Proteus* spp. on Nutrient agar showing swarming growth.



Plate 5: Growth of *Klebsiella* spp. on MacConkey agar showing mucoid colonies.

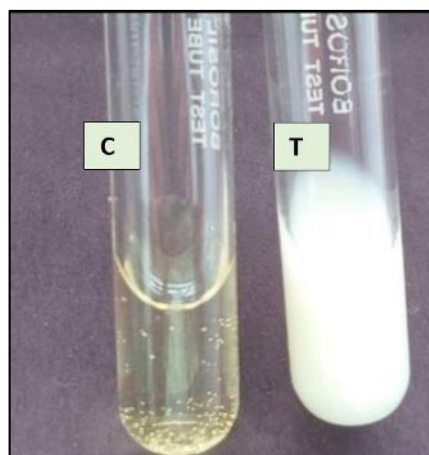


Plate 7a: Coagulase test for *Staphylococcus* spp. showing formation of fibrin clump. (T: Test sample; C: Control)

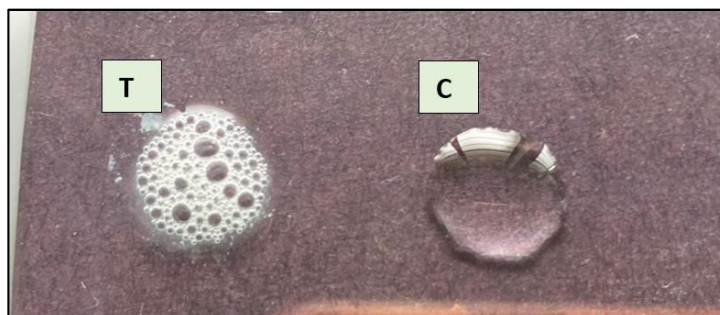


Plate 7b: Catalase test for *Staphylococcus* spp. showing bubble formation. (T: Test sample; C: Control)



Plate 7c: Oxidase test *Staphylococcus* spp. showing no change in colour (T: Test sample; PC: Positive Control)

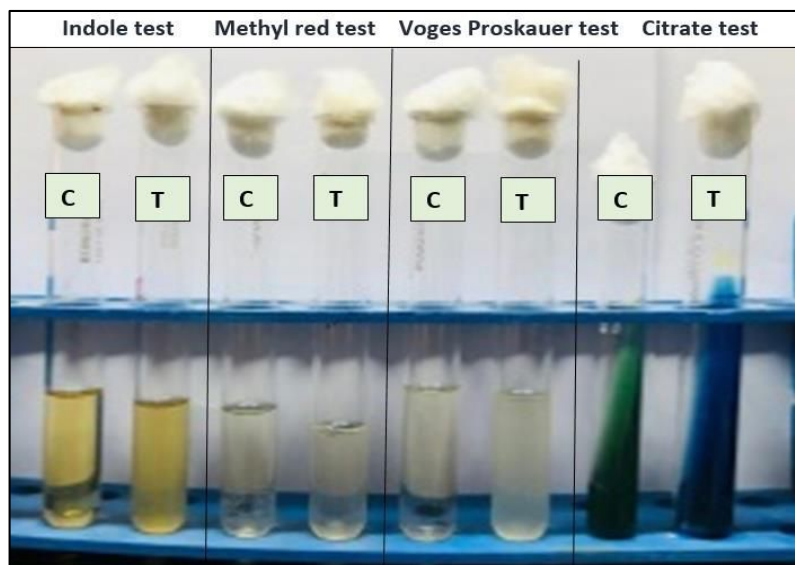


Plate 8: IMViC test for *Pseudomonas* Spp. Indole-ve, Methyl red-ve, Voges Proskauer-ve, Citrate +ve (showing change in green to blue colour) C: Control sample, T: Test sample

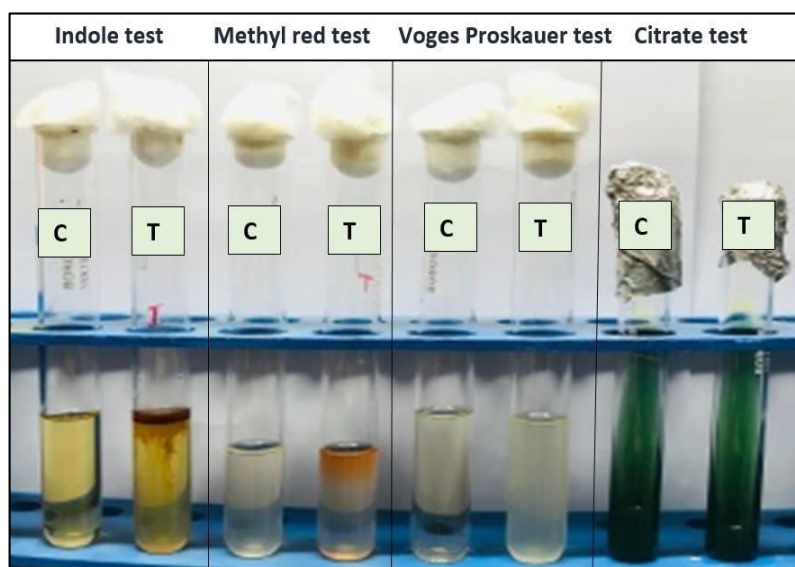


Plate 9: IMViC test for *Escherichia* spp. Indole +ve (formation of red color), Methyl red +ve (formation of red color), Voges Proskauer-ve, Citrate-ve, C: Control sample, T: Test sample

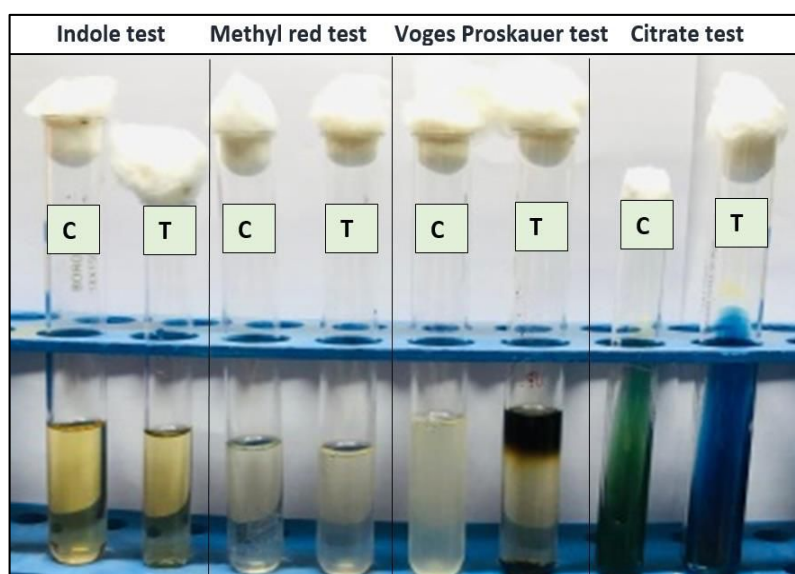


Plate 10: IMViC test for *Klebsiella* spp. Indole-ve, Methyl red-ve, Voges Proskauer +ve (formation of pinkish-red colour), Citrate +ve (showing change in green to blue colour) C: Control sample, T: Test sample

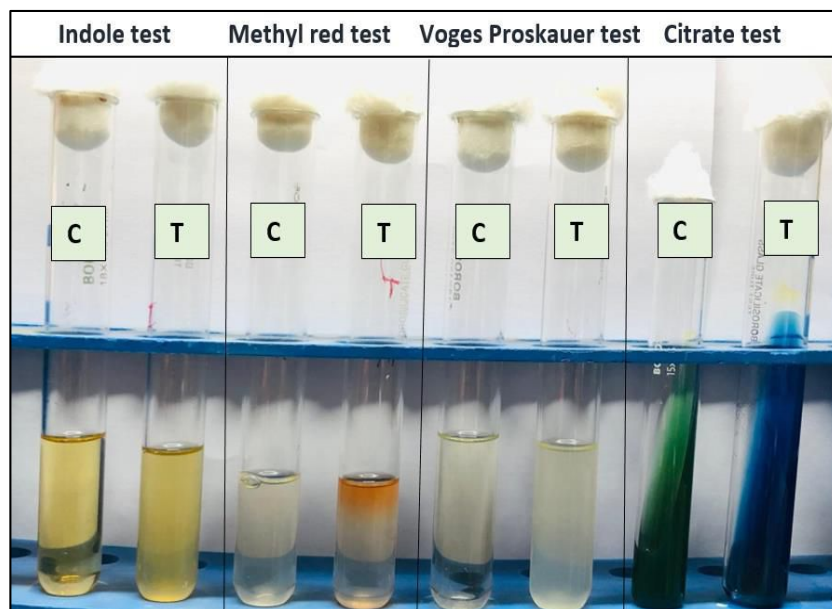


Plate 11: IMViC test for *Proteus* spp. Indole-ve, Methyl red +ve (formation of red colour), Voges Proskauer-ve, Citrate +ve (showing change in green to blue colour) C: Control sample, T: Test sample

Discussion

The present study evaluated the bacterial aetiology of canine otitis, along with their morphological, biochemical tests. Out of 104 suspected otitis cases, 86 (82.69%) yielded bacterial growth. The high rate of bacterial isolation observed in the present study underscores the significant role of bacterial pathogens in the aetiology of canine otitis. *Staphylococcus* spp. emerged as the predominant pathogen (55.81%), followed by *Pseudomonas* spp., *Escherichia* spp., *Klebsiella* spp., and *Proteus* spp. Similar dominance of Staphylococci in canine ear infections, corroborates earlier reports by [4, 5, 7] and reflects their opportunistic behaviour in compromised ear canals. Age-wise occurrence showed higher prevalence in dogs aged 1-5 years, agreeing with earlier reports [2, 4], likely due to increased outdoor activity and higher exposure risk during this age. Male dogs exhibited slightly higher incidence than females, consistent with previous studies, which attribute this to hormonal factors and owner preferences of owning a male dog over female dogs [1, 4]. Breed-wise, non-descript dogs showed the highest occurrence, followed by German Shepherds and Labrador Retrievers, aligning with earlier findings where breeds with pendulous ears or hairy canals were more predisposed. Unilateral otitis was more common than bilateral involvement, with the right ear more frequently affected a trend also documented by [3, 9]. Biochemical identification confirmed species characteristics such as catalase and coagulase positivity in *Staphylococcus* spp., IMViC (+ +--) for *Escherichia* spp., (--- +) for *Klebsiella* spp., and (---+) for *Pseudomonas* spp., which matched previously established biochemical profiles. The detection of mixed infections highlights the polymicrobial nature of canine otitis, particularly in chronic cases. Overall, the study reaffirms that *Staphylococcus* spp. remains the major contributors to canine otitis and accurate identification through morphological and biochemical approaches is essential for effective management.

Conclusion

The present study establishes that bacterial infections are a major cause of canine otitis in the north-eastern Karnataka

region, with *Staphylococcus* spp. being the most predominant pathogen, followed by *Pseudomonas* spp., *Escherichia* spp., *Klebsiella* spp., and *Proteus* spp. Morphological, cultural, and biochemical characterization proved effective in accurately identifying the etiological agents. The presence of mixed infections highlights the complexity of otitis cases and the need for thorough laboratory diagnosis. The findings provide valuable baseline data for the region and reinforce the need for laboratory-based diagnosis to guide appropriate therapeutic interventions.

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