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A study on *in-vitro* antimicrobial sensitivity pattern of the predominant bacterial isolates obtained from respiratory infection in sheep

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Abstract

The study was conducted to assess the prevalence of bacterial pathogens associated with respiratory infections in sheep and their *in-vitro* antimicrobial susceptibility in and around Hassan, Karnataka, India. A total of 150 samples, including 110 nasal swabs, 21 lung and 19 tracheal swabs, were collected for bacteriological isolation and antibiotic sensitivity testing. All samples yielded bacterial isolates, with *E. coli* (22.66%) being the most prevalent, followed by *Mannheimia haemolytica* (16.66%), *Klebsiella* spp. (15.33%), *Streptococcus* spp. (14.66%), *Staphylococcus* spp. (12.66%), *Pasteurella multocida* (7.33%), *Bacillus* spp. (6.66%), *Pseudomonas* spp. (3.33%) and *Serratia marcescens* (0.66%). Antibigram studies revealed that *P. multocida* showed highest sensitivity to co-trimoxazole, *M. haemolytica* was completely sensitive to β -lactam and macrolide antibiotics, while *E. coli* was most responsive to amoxycillin-sulbactam but resistant to erythromycin. The findings indicate rising antimicrobial resistance among bacterial agents of ovine respiratory infections.

Keywords: Sheep, respiratory infection, bacterial pathogens, *Escherichia coli*, *Mannheimia haemolytica*, *Pasteurella multocida*, anti-microbial susceptibility

Introduction

Sheep and goats were among the first animals domesticated by humans and continue to hold a vital place in the global livestock sector. In India and other tropical countries, these small ruminants are indispensable for sustaining rural livelihoods and strengthening agricultural economies. Their contribution to meat, milk and wool production, coupled with high reproductive efficiency, adaptability to varied agro-climatic conditions and low maintenance requirements, makes them an integral part of mixed farming systems (Abera and Mossie, 2023) [2].

Despite their economic and social importance, the productivity and survival of sheep and goats are often hindered by various diseases, particularly respiratory infections, which are among the leading causes of mortality and economic loss in small ruminant farming systems worldwide (Mohammed *et al.*, 2022) [16]. Respiratory diseases account for nearly 50% of total deaths in small ruminants, with pneumonia recognized as the predominant cause due to their low resistance to respiratory pathogens. Respiratory infections in sheep are multifactorial in origin, involving complex interactions among bacterial, viral and environmental factors (Kumar *et al.*, 2014) [11].

Bacterial pathogens such as *Pasteurella multocida* (*P. multocida*), *Mannheimia haemolytica* (*M. haemolytica*) and various *Mycoplasma* species are commonly implicated in the etiology of ovine respiratory diseases (Quinn *et al.*, 2002) [20]. These organisms may act as primary pathogens or as secondary invaders following viral infections caused by agents such as parainfluenza-3 virus (PI-3), bovine herpesvirus type 1, respiratory syncytial virus, adenoviruses and reoviruses (Bosch *et al.*, 2013) [6]. Environmental factors such as overcrowding, poor ventilation, nutritional deficiencies and abrupt changes in weather can compromise the animal's immune defences, predisposing them to infection and exacerbating disease progression. The clinical signs associated with respiratory infections include fever, nasal discharge, dyspnoea, anorexia, coughing and increased respiratory effort (Scott, 2011) [26].

Several studies have documented the presence of multiple bacterial pathogens in the respiratory tract and lungs of affected animals, including *P. multocida*, *M. haemolytica*, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Mycoplasma ovipneumoniae* (Franco *et al.*, 2019) ^[9]. In addition, opportunistic organisms such as *Bacillus* and *Micrococcus* species, which are normally part of the commensal flora, may act as secondary invaders under stress conditions (Garedew *et al.*, 2010) ^[10]. Accurate identification of the causative bacterial pathogens is essential for the effective management of respiratory diseases and for implementing appropriate therapeutic and preventive strategies, particularly in light of the growing concern over antimicrobial resistance in livestock pathogens.

Given the multifactorial nature of respiratory diseases and the emergence of antimicrobial resistance among bacterial pathogens, understanding the prevalence, isolation, and antimicrobial susceptibility of bacterial agents associated with respiratory infections in sheep is critical. Such knowledge can help design effective therapeutic protocols and preventive measures to reduce disease burden, improve animal health and enhance productivity in small ruminant farming systems.

Materials and Methods

A total of 150 samples were collected in Amies transport media (ATM) from in and around Hassan between February 2025 and September 2025. These included 110 nasal swabs from animals showing signs of respiratory infection, along with 21 lung and 19 tracheal swabs obtained from pneumonic lungs during necropsy, which were used for the present study.

Culture and biochemical tests

Swab samples collected in Amies transport media (ATM) were directly streaked onto Brain Heart Infusion (BHI) agar and incubated at 37 °C for 12-18 hours under anaerobic conditions. Following preliminary identification based on staining morphology on BHI agar, the isolates were subcultured onto Mannitol Salt Agar (MSA), MacConkey agar, Eosin Methylene Blue (EMB) agar and blood agar. Bacterial identification on primary culture was carried out based on hemolytic characteristics, colony morphology, Gram-staining reaction, and cellular shape and arrangement. Gram-negative bacteria grown on MacConkey agar were differentiated according to their growth features, catalase and oxidase reactions, triple sugar iron (TSI) agar results, urease activity and IMViC (indole, methyl red, Voges-Proskauer, citrate) tests. *P. multocida* and *M. haemolytica* were identified through their hemolytic pattern, oxidase and indole tests. *Staphylococcus* spp. was confirmed by their growth characteristics and catalase activity, while *Streptococcus* spp. isolates were identified based on colony and staining morphology along with catalase testing. *Bacillus* species were identified based on their staining characteristics and medusa head growth on BHI agar.

Antibiogram studies

The predominant bacterial isolates, viz, *E. coli*, *M. haemolytica* along with the isolates of *P. multocida* were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion technique. Seventeen commonly

used antibiotics for treating respiratory infections were tested, including amikacin (AK), ampicillin (AMP), amoxycillin with sulbactam (AMS), cefixime (CFM), cefotaxime (CTX), cefoxitin (CX), ceftriaxone (CTR), cefoperazone with sulbactam (CFS), chloramphenicol (C), ciprofloxacin (CIP), co-trimoxazole (sulfamethoxazole with trimethoprim) (COT), doxycycline (DO), enrofloxacin (EX), erythromycin (E), gentamicin (GEN), levofloxacin (LE) and neomycin (N). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2025.

Results

Of the 150 samples cultured for bacterial isolation, 17 showed no growth, while another 17 exhibited mixed infections with two bacterial species. The remaining 116 samples yielded single bacterial isolates. The most frequently isolated bacterium was *E. coli* (22.66%), followed by *M. haemolytica* (16.66%), *Klebsiella* spp. (15.33%), *Streptococcus* spp. (14.66%), *Staphylococcus* spp. (12.66%), *P. multocida* (7.33%), *Bacillus* spp. (6.66%), *Pseudomonas* spp. (3.33%) and *S. marcescens* (0.66%) (Table 1).

The predominance of *E. coli* in the present investigation corroborates the observations of Parthiban *et al.* (2018) ^[18], who reported *E. coli* as the most frequent isolate (22.85%) from pneumonic sheep lungs in Tamil Nadu and Ugochukwu *et al.* (2017) ^[30], who found *E. coli* in 32.47% of pneumonic lungs in Nigeria. Similarly, studies by Babu *et al.* (2019) ^[4] and Khalifa *et al.* (2021) ^[13] also identified *E. coli* as a major etiological agent, indicating its ubiquitous role as an opportunistic pathogen in ovine respiratory diseases. The frequent isolation of *E. coli* may be attributed to stress-induced immunosuppression, poor ventilation and unhygienic housing conditions, which facilitate colonization of the upper respiratory tract and subsequent pulmonary invasion.

The second most prevalent organism in this study was *M. haemolytica* (16.66%), a well-known primary pathogen of ovine pneumonic pasteurellosis. Similar findings were reported by Mahmoud *et al.* (2005) ^[15] in Egypt, Tabatabaei and Abdollahi (2018) ^[29] in Iran and Pavan *et al.* (2021) ^[19] in Andhra Pradesh, India, who identified *M. haemolytica* as a dominant bacterial species associated with ovine respiratory infections. Its frequent isolation reflects its pathogenic significance and the complex interplay between stress, viral co-infections and environmental factors that predispose animals to *M. haemolytica*-induced pneumonia. *Klebsiella* spp. were isolated from 15.33% of samples, aligning with findings from Fouad *et al.* (2022) ^[8] in Egypt, who reported *Klebsiella pneumoniae* in 27.15% of cases and Parthiban *et al.* (2018) ^[18], who found 18.57% isolation. The opportunistic nature of *Klebsiella* spp., coupled with their increasing resistance to antimicrobial agents, highlights their emerging importance as respiratory pathogens in small ruminants.

Streptococcus spp. (14.66%) and *Staphylococcus* spp. (12.66%) were also frequently detected in the current study, comparable with the results of Garedew *et al.* (2010) ^[10], Ugochukwu *et al.* (2017) ^[30] and Reddy *et al.* (2023) ^[22], who reported similar isolation frequencies. These organisms are often opportunistic commensals of the upper respiratory tract that may act as secondary invaders following viral or mycoplasmal infections, contributing to the polymicrobial etiology of respiratory disease.

The isolation of *P. multocida* (7.33%) in this study is well supported by the previous reports by Azizi *et al.* (2011) [3], Babu *et al.* (2019) [4] and Samadipoor *et al.* (2024) [24], emphasizing its role as a common pathogen in ovine pneumonia. However, its comparatively lower occurrence in the present study could be due to temporal and geographical variations or to competition with other bacterial flora in mixed infections. The detection of *Bacillus* spp. (6.66%) and *Pseudomonas* spp. (3.33%) is also noteworthy, as similar findings were described by Franco *et al.* (2019) [9] and Parthiban *et al.* (2018) [18], who observed these species as opportunistic contaminants or secondary pathogens in pneumonic lungs.

A low incidence of *S. marcescens* (0.66%) was observed in this study, which is in line with the report of Khalifa *et al.* (2021) [13] from Egypt, where *S. marcescens* was isolated at a rate of 1.3%. Although rarely reported in small ruminants, the presence of *Serratia* species suggests possible environmental contamination or emerging pathogenic potential under immunocompromised conditions.

Overall, the findings of this study reaffirm that *E. coli* and *M. haemolytica* remain the major bacterial pathogens implicated in respiratory infections of sheep, consistent with earlier global and Indian reports.

The antimicrobial susceptibility testing of bacterial isolates obtained in the present study provides valuable insights into the current resistance trends among respiratory pathogens of sheep in and around Hassan, Karnataka. Marked variability in susceptibility patterns was observed across the different bacterial species, reflecting both pathogen-specific differences and potential impacts of antimicrobial usage practices in the region.

In the present study, *P. multocida* isolates exhibited the highest sensitivity to co-trimoxazole (90.9%), followed by amoxycillin-sulbactam, cefoperazone-sulbactam, chloramphenicol, ciprofloxacin, enrofloxacin and levofloxacin (81.81% each). Resistance was mainly noted against cefoxitin (45.45%) and neomycin (36.36%). These findings are in accordance with earlier reports from India and Ethiopia (Maru *et al.*, 2013; Sahay *et al.*, 2020; Abate and Kassa, 2023) [1, 14, 23], where *P. multocida* isolates showed high sensitivity to chloramphenicol, sulfonamides, and β -lactam combinations. Similarly, Sarangi *et al.* (2015) [25] reported over 90% susceptibility of *P. multocida* isolates to enrofloxacin and chloramphenicol, while Singh *et al.* (2019) [27] found complete sensitivity to amoxycillin-clavulanic acid and tetracycline. In contrast, studies by Bahr *et al.* (2021) [5] and Faid *et al.* (2022) [7] described high resistance rates to ampicillin, tetracycline and streptomycin among *P. multocida* isolates, indicating geographical differences in antibiotic response likely due to variation in drug exposure and selective pressure. The relatively high sensitivity of *P. multocida* in the current study to co-trimoxazole and β -lactam/ β -lactamase inhibitor combinations suggests that these antimicrobials remain as effective therapeutic choices for ovine pasteurellosis in the study area.

All *M. haemolytica* isolates in the present study demonstrated complete sensitivity to ampicillin, amoxycillin-sulbactam, cefoxitin, cefoperazone-sulbactam, co-trimoxazole and erythromycin, while only 20% showed resistance to neomycin. These results are in strong

agreement with those of Sahay *et al.* (2020) [23] and Singh *et al.* (2019) [27], who reported 100% susceptibility of *M. haemolytica* to ampicillin and amoxycillin-clavulanic acid in pneumonic sheep. Similarly, Jackson *et al.* (2024) [12] and Muazzez and Ozgul (2025) [17] found that all *M. haemolytica* isolates were susceptible to ceftiofur, enrofloxacin and florfenicol, underscoring the continued efficacy of β -lactam and fluoroquinolone antibiotics. However, some regional studies have indicated increasing resistance trends, as noted by Bahr *et al.* (2021) [5] and Van *et al.* (2023) [31], who reported high resistance to erythromycin, oxytetracycline and penicillin. The consistent sensitivity of *M. haemolytica* to most antimicrobials in this study may reflect prudent antibiotic usage or lower exposure to selective antimicrobial pressure in local production systems.

In contrast, *E. coli* isolates exhibited a markedly higher degree of resistance. Although the highest sensitivity was observed to amoxycillin-sulbactam (85.29%) and cefoxitin (79.41%), substantial resistance was recorded against several classes of antibiotics, notably erythromycin (100%), neomycin (94.11%), ampicillin (88.23%) and cefotaxime (85.29%). High resistance rates were also noted against aminoglycosides (amikacin and gentamicin, 82.35% each) and fluoroquinolones (enrofloxacin and ciprofloxacin, 70.58% each). These results are consistent with previous findings by Rania *et al.* (2021) [21] and Fouad *et al.* (2022) [8], who reported variable susceptibility and emerging multidrug resistance among *E. coli* isolates from sheep respiratory infections. Similarly, Sonawane *et al.* (2012) [28] observed *E. coli* isolates susceptible to fluoroquinolones and co-trimoxazole but resistant to several other antibiotics such as chloramphenicol, doxycycline and nitrofurantoin. The resistance patterns observed in this study could be attributed to the indiscriminate or prolonged use of common antimicrobials in livestock, leading to selection and persistence of resistant *E. coli* strains in the respiratory tract. Overall, the results of this study highlight a clear divergence in antimicrobial susceptibility profiles between primary respiratory pathogens such as *M. haemolytica* and *P. multocida*, which remain largely sensitive and opportunistic bacterial pathogens like *E. coli*, which demonstrate significant resistance. These findings align with reports by Zagahawa *et al.* (2010) [32], Babu *et al.* (2019) [4] and Abate and Kassa (2023) [1], all of whom noted higher resistance in *E. coli* and *Klebsiella* spp. compared to *Pasteurella* and *Mannheimia* species.

The persistence of antimicrobial susceptibility among *P. multocida* and *M. haemolytica* isolates suggests that β -lactam/ β -lactamase inhibitor combinations, sulfonamides and macrolides continue to be effective for treating bacterial pneumonia in sheep. However, the emergence of multidrug resistance in *E. coli* emphasizes the need for rational and evidence-based antimicrobial use in field conditions. Periodic surveillance of antibiotic resistance trends, coupled with molecular characterization of resistance genes, will be essential to formulate effective treatment guidelines and mitigate the spread of resistant respiratory pathogens in small ruminants.

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Table 1: Bacterial isolates obtained from sample

S. No.	Organism	Number of isolates	Percentage
1	<i>E. coli</i>	34	22.66%
2	<i>M. haemolytica</i>	25	16.6%
3	<i>Klebsiella</i> spp.	23	15.33%
4	<i>Streptococcus</i> spp.	22	14.66%
5	<i>Staphylococcus</i> spp.	19	12.66%
6	<i>P. multocida</i>	11	7.33%
7	<i>Bacillus</i> spp.	10	6.66%
8	<i>Pseudomonas</i> spp.	5	3.33%
9	<i>S. marcescens</i>	1	0.66%
	Total	150	

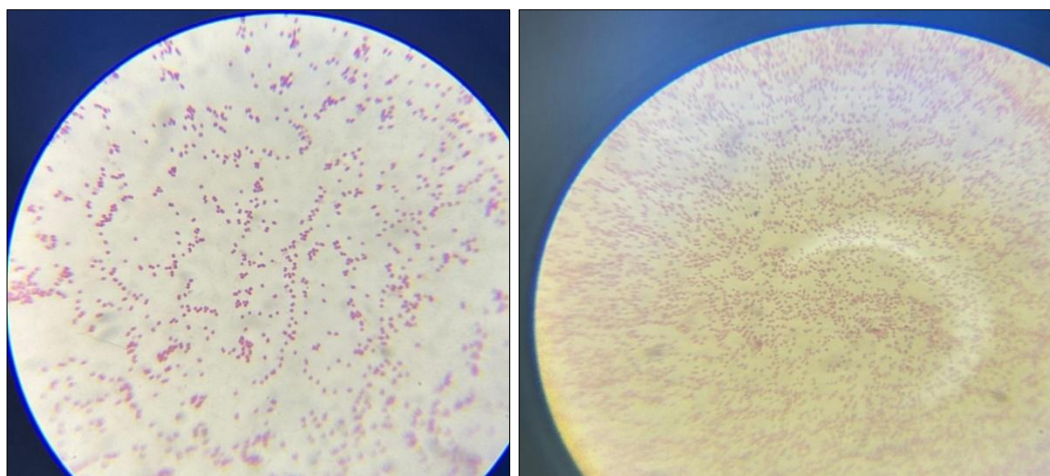
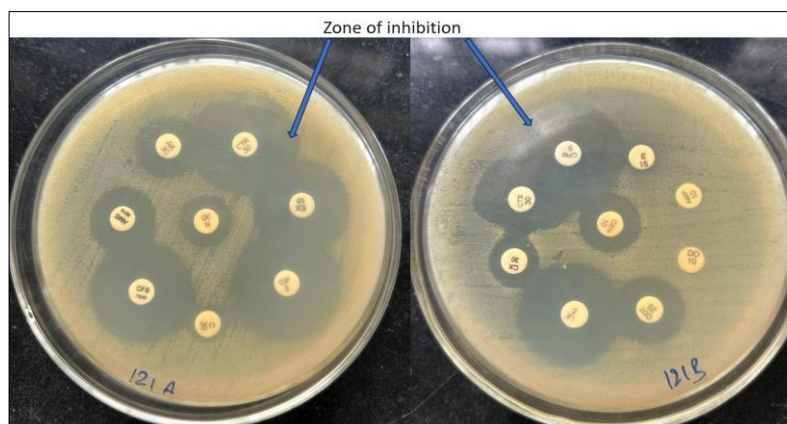
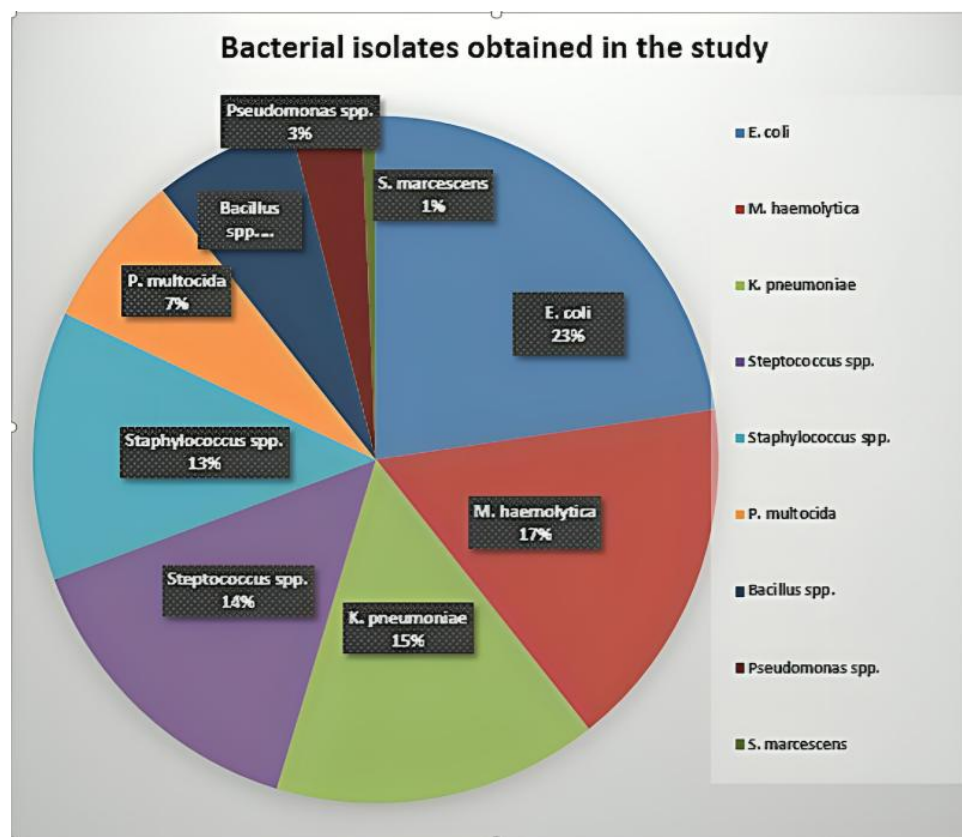
**Fig 1:** *M. haemolytica* colony morphology - honey type colonies on BHI agar (left); β -haemolysis on blood agar (right)**Fig 2:** *M. haemolytica* staining morphology- Gram's stain, **Fig 3:** *P. multocida* on staining morphology- Gram's stain**Fig 4:** Antimicrobial susceptibility pattern of *P. multocida*

Table 2: Phenotypic pattern of sensitivity of the bacterial isolates to the antimicrobial drugs under study

Species/family assigned (No of isolates)	Amikacin	Ampicillin	Amoxycillin with sulbactam	Cefixime	Cefotaxime	Cefoxitin	Cefoperazone with sulbactam	Ceftriaxone	Chloramphenicol	Ciprofloxacin	Co-trimoxazole	Doxycycline	Enrofloxacin	Erythromycin	Gentamicin	Levofloxacin	Neomycin
<i>P. multocida</i> (11)	6	7	9	8	7	6	9	8	9	9	10	9	9	6	8	9	6
<i>M. haemolytica</i> (25)	21	25	25	22	24	25	25	24	23	23	25	21	24	25	19	22	13
<i>E. coli</i> (34)	3	3	29	8	0	27	17	10	20	0	25	7	1	0	3	4	2
Total (70)	30	35	63	38	31	58	51	42	52	32	60	37	34	31	30	35	21

**Fig 5:** Graphical representation of prevalence of bacterial isolates

Conclusion

The present study provides valuable insights into the bacterial etiology and antimicrobial resistance patterns associated with respiratory infections in sheep from the study area. Among the isolates, *E. coli* emerged as the most prevalent pathogen, followed by *M. haemolytica* and *P. multocida*. The antibiogram profiling revealed notable variations in drug sensitivity among the bacterial species. *P. multocida* isolates exhibited high susceptibility to co-trimoxazole and β -lactam/ β -lactamase inhibitor combinations such as amoxycillin-sulbactam and cefoperazone-sulbactam, while showing resistance to cefoxitin and neomycin. All *M. haemolytica* isolates were completely sensitive to a broad range of antibiotics, indicating limited resistance development in this pathogen. Conversely, *E. coli* isolates demonstrated marked resistance to several commonly used antibiotics, including erythromycin, neomycin, ampicillin and cefotaxime, highlighting a growing concern over multidrug resistance. These findings emphasize the importance of routine bacteriological and antimicrobial susceptibility testing to guide rational antibiotic use and reduce the emergence of

resistant strains. Effective management of respiratory infections in sheep requires an integrated approach combining accurate diagnosis, judicious antibiotic use and improved herd health practices to minimize disease impact and promote sustainable livestock production.

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