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## Isolation, characterisation and prevalence pattern of bacterial flora on pneumonic cases of goats slaughtered at Thanjavur abattoir, Cauvery Delta Zone, Tamilnadu

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**Abstract**

Respiratory infections of goats irrespective of the breed cause indirect economic loss to the farmer and the industry. The present study was aimed to isolate and identify the bacterial population associated in goats slaughtered at Thanjavur District Abattoir of Cauvery Delta Zone (CDZ), Tamil Nadu, India. The pneumonic cases were segregated from the list of slaughtered goats and trachea and lungs were collected and proceeded as per standard protocols from 230 goats during August 2019 to February 2020 (6 month period) brought to the Thanjavur District abattoir. A total of 178 were affected with various types of pneumonia based on goat's morphology of lungs with two types of pneumonia. Bacterial pneumonia contributed 88.7% and verminous pneumonia existed in 11.3% of slaughtered goats. Out of 178 pneumonic lungs collected from August 2019 to January 2020 (6 months) upon bacterial investigation revealed *Staphylococcus* spp, *Pasteurella multocida*, *Streptococcus pyogenes* and *E.coli* predominantly with a lesser contamination of *Candida* spp. The population of Gram positive bacteria was more than the Gram positive ones from the above study.

**Keywords:** Pneumonia, gram negative bacteria, goats, bacterial investigation

**1. Introduction**

Goats, called as poorman's cow contribute in large to the rural economy and are the backbone of the rural farming community in India. Respiratory diseases affecting goats are multifactorial [16]. Lamb losses are generally connected with bacterial infections leading to pneumonia, diarrhoea, and subsequent sepsis, which is a potential complication of pneumonia [1]. Lungs are the most exposed organs to different aggressions because of their anatomical and histological particularities [3, 6]. Among the inflammatory and non-inflammatory disease conditions, pneumonia either acute or chronic causes debility and death leading to great economic loss to the farmers [8]. Globally abattoir / slaughter house surveillance has got an important role in disease control especially goats reared in rural conditions and act as an indicator to measure disease incidence. Respiratory disease bacterial affection on slaughtered goats have not been studied do far in this Cauvery Delta Zone of Tamilnadu and the knowledge of pathogenesis, gross changes of lung and prevalence of microorganisms and the isolated bacteria will help the veterinarian for diagnosis and treatment of the diseases. Hence this study was carried out during August 2019 to February 2020 with the objective to determine the prevalence of pneumonia in goat's slaughtered in this CDZ of Tamilnadu. This paper describes the occurrence of lung lesions, gross changes and isolation, identification of bacteria from the affected lungs with effect of season, breed and identify the causative organism accordingly.

**2. Materials and Methods**

Three corporation and one rural slaughter house in Thanjavur District were considered for study during the period of Aug 2019 to February 2020. A total of 178 goats carcasses affected with pneumonic condition were examined and data was collected. The age of slaughtered goats ranged from 7 months to 4 years and among which 124 numbers were male and 54 numbers were female. The specimens of pneumonic cases (178) were collected aseptically in separate containers, labelled & kept in ice and transported to the Bacteriological laboratory of Department of Veterinary Microbiology, VCRI, Orathanadu.

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## 2.1 Sample collection and study design

Upon the cross-sectional survey for 6 months, the slaughtering of goats with low body weight, wounds and aged brought to abattoir of Thanjavur Dt. were examined and a total of 178 numbers were selected and a portion of sample was used for bacteriological studies.

## 2.2 Bacteriological Culturing and identification by biochemical tests

**Lung:** The lung parenchymal tissue was cut and cultured on blood agar (Hi media, Mumbai, India) which contain 5% sheep blood using sterile forceps. Each lung sample was inoculated into Blood agar medium for aerobic and anaerobic culture. The anaerobic Jar and Anerobicbox (Hi media, Mumbai) were used and then placed at 37°C for 24 hours in an incubator. The derivation of pure culture was made as per the procedures [2, 4].

**2.3 Tracheal swabs:** After 24 hours of incubation in the Trypti case Soya Broth for enrichment, the lung and tracheal swabs samples were vortexed and a loop full of culture was taken and streaked over identified Blood agar plates supplemented with 5% sheep blood and MacConkey (Hi media, Mumbai, India [11]).

## 2.4 Bio chemical characterisation of isolates

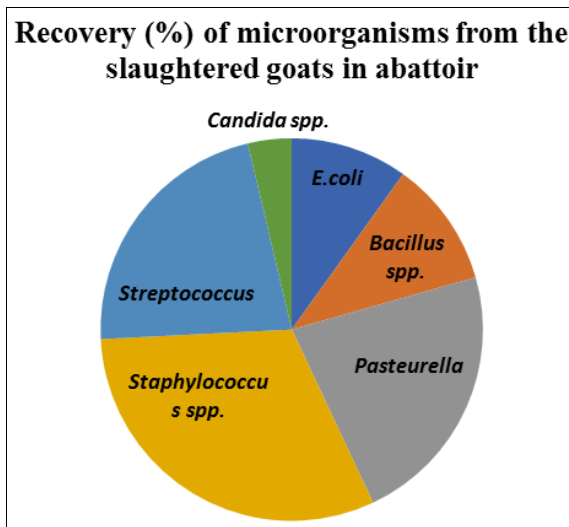
The pure cultures of single colony type, from both blood and MacConkey agar, were transferred onto nutrient agar-medium for a series of primary tests such as Catalase, Oxidase, Motility, and Fermentative-Oxidative and secondary tests including triple sugar iron agar, citrate utilization test, methyl red test and in dole test, (Hi media, Mumbai-Biochemical kit Catalogue No. KB 002Hi-MediaAssortd Bio Chemical kit & KB 005 A Hi Strep Identification kit).

## 3. Results

The result of 178 various organs collected from nasal cavity, liver, trachea and lung (18 from each site), 160 (83.3%) harboured bacteria; animal infection rate was 100%. In general, 5 different bacteria were isolated from 178 infected specimens, accounting for 1.46 bacteria per infected sample; a corresponding bacterial burden per animal was 4 (194/48). The relative proportion of bacterial species identified is indicated on Table 1 and proportion in Figure 1. The predominant species among the isolates were *Staphylococcus* spp (29.2%), *Pasteurella multocida* (22.8%), *Streptococcus pyogenes* (18.5%) and *E.coli* (9.98%), *Bacillus* species (9.9% each) with a lesser contamination of *Candida* spp.(3.7%).

**Table 1:** Bacteria isolated and identified by biochemical tests of pneumonic lungs of slaughtered goats in Thanjavur Dt.

Isolated bacteria	Organs subjected for isolation	Recovery (%) of microorganisms
<i>E. coli</i>	Lungs, liver (a part)	9.9
<i>Bacillus spp.</i>	Intestine, liver, Trachea	13.7
<i>Pasteurella</i>	Lungs, Trachea	12.4
<i>Staphylococcus spp</i>	Intestine, liver	31.2
<i>Streptococcus</i>	Lung, liver	22.1
<i>Klebsiella spp</i>	Lungs, Trachea	10.50
<i>Candida spp</i>	Trachea	0.7



**Fig 1:** Proportionary distribution of isolated bacteria recovered from pneumonic lungs and trachea of slaughtered goats

## 3.2 Enumeration of bacterial isolates

Out of 178 specimens collected from trachea and the corresponding pneumonic lung, five different bacterial species were isolated from 150 infected specimens in aerobic condition and no bacterial growth in anaerobic media. The proportion of Gram positive bacteria were dominated over Gram negative bacteria accounting about 62.8% and Gram negative were 37.2%.

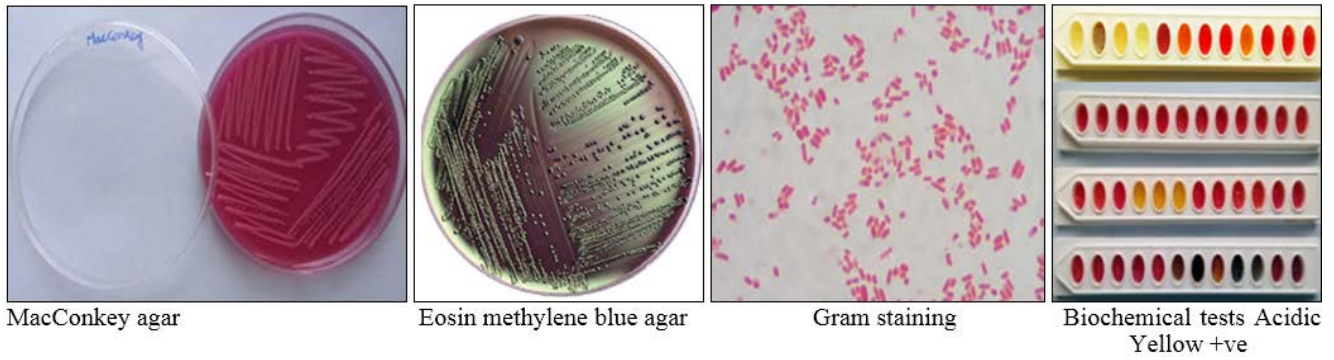
As shown in Table 1, the majority of the isolates colonized both trachea and descended to the lung with the exception of *Micrococcus*, *Klebsiella* and *Archanobacterium* species which were absent from trachea but were isolated from the corresponding lungs, whereas *Klebsiella pneumoniae* absent from lung but isolated from trachea. Among Gram negative bacteria the most frequently isolated and the least encountered bacteria were the *P. multocida* and *Klebsiella* species respectively which are considered risk and dangerous in humans.

The *Staphylococcus* species with proportion of 25.75% was isolated from the lungs (69%) and the trachea (21%) followed by *Bacillus* species (8.5%), *Pasteurella multocida* (2.11%) and *Mannheimia haemolytica* which accounted for 1.5% and *Candida* spp. was the least encountered bacterial genera in aerobic state (0.7%).

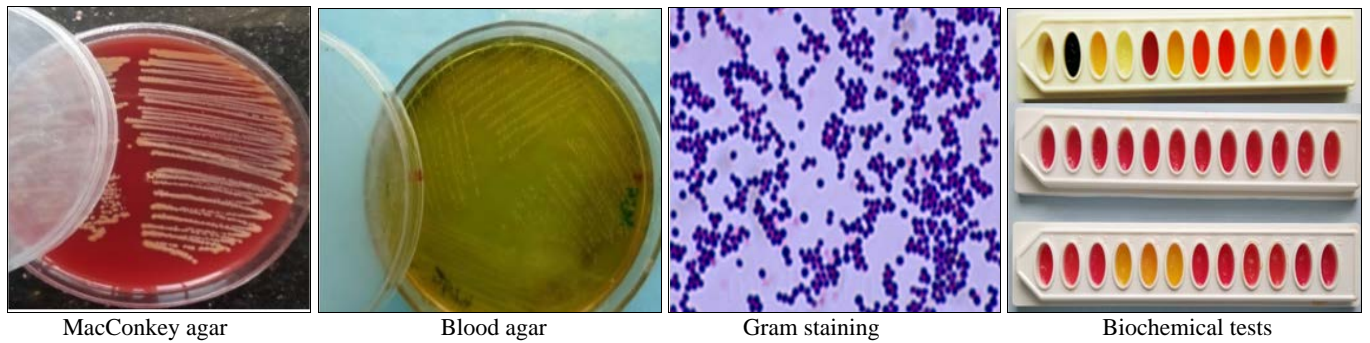
## 3.3 Bacteria isolates cultured in anaerobic medium

According to our study, there was absence of strict anaerobic bacteria (Cat.no.KB 003 Hi Assorted bio chemical kit, Hi Media, Mumbai). The identified and isolated bacteria were subjected to bio chemical characterisation studies and are *E. coli* (Fig 2), *Staphylococcus aureus* (Fig 3), *Klebsiella* spp., (Fig 4) *Pasteurella multocida* (Fig 5), *Streptococcus* spp (Fig 6) and *Pseudomonas* spp (Fig 7)

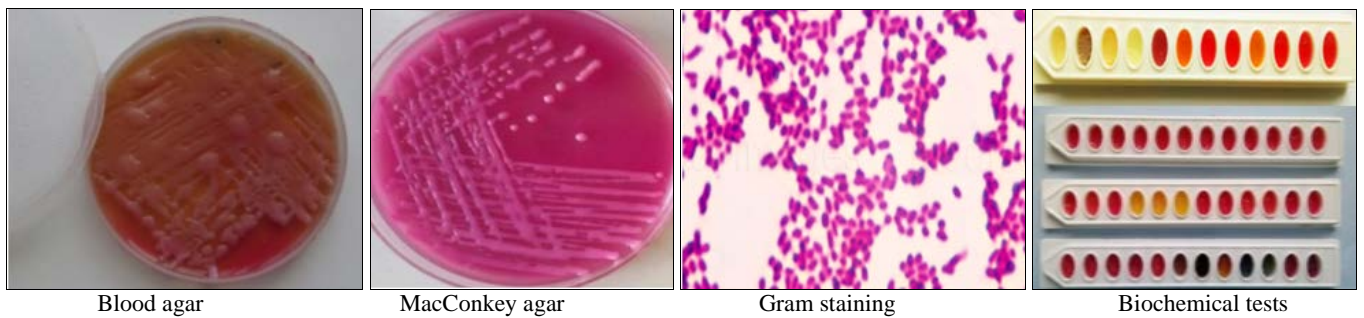
In general, there was a general increment in the isolation rate as one goes down the trachea toward the lung with the highest and lowest infection rate being the lung and trachea, respectively in both aerobic and anaerobic conditions. Most of the bacteria that were isolated have a larger frequency of isolation in anaerobic condition



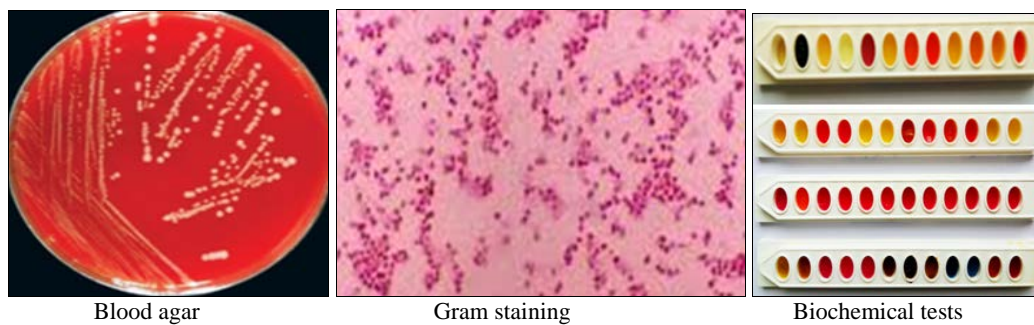
**Fig 2:** Cultural, staining and biochemical characteristics of *E. coli*



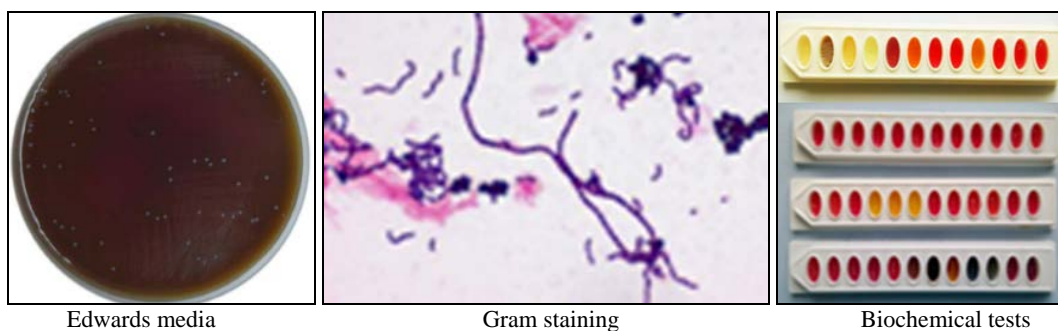
**Fig 3:** Cultural, staining and biochemical characteristics of *Staphylococcus*



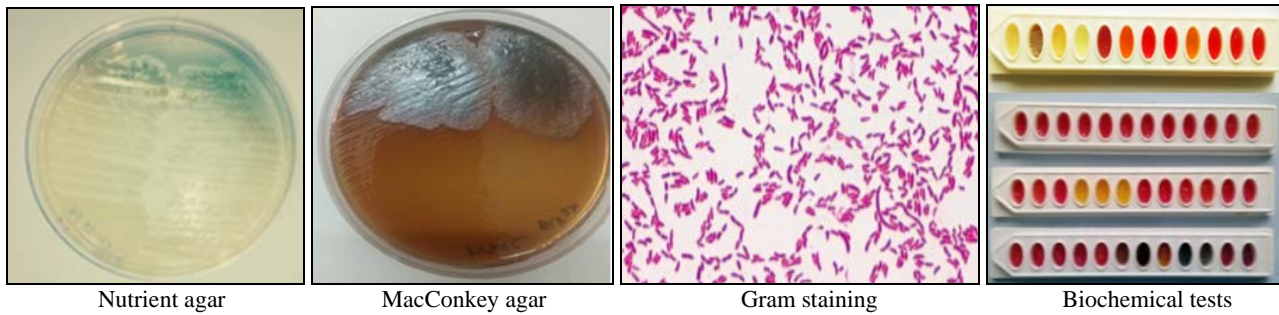
**Fig 4:** Cultural, staining and biochemical characteristics of *Klebsiella*



**Fig 5:** Cultural, staining and biochemical characteristics of *Pasteurella multocida* Fig



**Fig 6:** Cultural, staining and biochemical characteristics of *Streptococcus* spp



**Fig 7:** Cultural, staining and biochemical characteristics of *Pseudomonas*

#### 4. Discussion

In the present study, aerobic and anaerobic growing bacteria from the respiratory tract of 178 goats slaughtered at Thanjavur Abattoir. Five different bacterial species were isolated from both tracheas and lung aerobically. The isolation of Gram positive bacteria in higher proportion than the corresponding Gram negative bacteria in this study agrees with studies of few workers [14] with slighter modification who isolated from pneumonic goats.

In parallel with aerobic isolates, all the bacteria recovered from the anaerobic culture were facultative anaerobes and were similar to those bacteria isolated from aerobic condition. A total of 135 facultative bacteria were isolated and in contrast with the aerobically isolated, the frequency of isolation of some of these bacteria increased comparing to anaerobically isolated bacteria. In line with this study [5] reported similar facultative bacteria from pneumonic lung of cattle in Canada and strictly anaerobic bacteria were not isolated in goats.

Among the total isolates, the *Staphylococcus* species were the predominant bacteria in both conditions and have high proportions in the lung and trachea. In agreement with this study, *Staphylococcus* species were isolated and reported in higher proportion in the previous few studies aerobically. The dominance of these bacteria is also in line with the study conducted by [13, 20] who isolated aerobic bacteria flora of the respiratory passage of healthy goats. These indicate there is a likelihood of association between these bacteria and pneumonic syndrome of lung. The bacteria are commensally living in the mucous membrane of the upper respiratory tract of animals and are opportunistic pathogens [11].

*Bacillus* species were the second abundant bacteria harvested with higher frequency in both situations. The high localization of these bacteria in the lung may play an important role in the progress of bovine pneumonia.

In the present study, *M. haemolytica* and *P. multocida* isolated with a relatively higher proportion and the result is similar to the report obtained from [9] who isolated these bacteria from Maedi-Visna affected sheep in Ethiopia with the rate of 7.89% and 7.02% of *M. haemolytica* and *P. multocida*, respectively. Ng in the upper respiratory tract and all *Pasteurella* species are probably extracellular parasites with various stresses, including concurrent viral infections predisposing to infections as in shipping fever. They play a part in shipping fever and cause pneumonia as a primary or a secondary etiology. *M. haemolytica* produces a soluble cytotoxin (leukotoxin) that has a role in breaching the lung's primary defense mechanism by its action on the alveolar macrophage and other leukocytes of ruminants [12] A proportion of 10.50 % of the pneumonic lung samples were positive for *K. pneumoniae*, this was lower than the results reported by previous researchers [7, 17] but lower than the results reported by [19].

An isolation rate of 8.2% from caprine pneumonic lungs which has a relative correlation with our study as they exist on the upper respiratory tracts [11, 15]. There was a mixed pneumonic pathogens isolated during the study and perfectly agrees with the findings [18] who reported that pneumonic mixed pathogens are mainly caused by *S. aureus*, *E. coli*, *Pasteurella multocida* and other organisms and clearly explains the complexity of the disease where *S. aureus* may predispose the animals to infection by Coliform organisms or other pathogens.

The finding of *E. coli* from pneumonic lungs suggests that *E. coli* is considered as a secondary invader in the bovine pneumonia and contribute to the pneumonic progress. The pneumonic lesions noticed in lungs were variable during the winter season of October to December 2019 with multifocal distribution which coincides without the findings of [10].

#### 5. Conclusions

The study revealed a number of bacterial pathogenic organisms associated with pneumonia in goats slaughtered over a period of six month (Aug 2019-Feb 2020) and gross pathology indicated the types of agent involved and responsible for the development of different pneumonia. The identified and isolated bacteria through agar morphology and bio chemical characterisation studies were *E. coli*, *Staphylococcus aureus*, *Klebsiella* spp., *Pasteurella multocida*, *Streptococcus* spp and *Pseudomonas* spp. The *Staphylococcus* species with proportion of 25.75% was isolated from the lungs (69%) and the trachea (21%) followed by *Bacillus* species (8.5%), *Pasteurella multocida* (2.11%) and *Mannheimia haemolytica* which accounted for 1.5% and *Candida* spp. was the least encountered bacterial genera in aerobic state (0.7%). Hence, the type and extent of distribution of bacteria and the major type of pneumonia (Bronchopneumonia and Verminous pneumonia) explores the organisms responsible for causing disease in goats and needs attention to prevent the pathological and microbial disease occurrence in future for the farmers who are largely involved in this type of farming business.

#### 6. Acknowledgement

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