

Epidemiological Study of *Pasteurella Multocida* Isolated from Sheep

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Annotation: The objectives of this study were to determine the epidemiology and laboratory diagnosis of pneumonic pasteurellosis due to *Pasteurella multocida* in sheep at Al-Qasim, Babylon Governorate. A total of 168 samples were obtained from sheep that had exhibited respiratory signs, comprising 80 nasal swabs, 70 oral swabs, and 18 lung tissues from slaughtered suspected pneumonia cases. Isolates were cultured on several selective and differential media, and bacterial identification was done with the VITEK 2 system.

The results showed that *P. multocida* was the predominant isolate (41.10%), followed by *Klebsiella* (19.64%), *Staphylococcus* (15.47%), and *E. coli* (9.52%). Antibiotic susceptibility testing revealed high sensitivity to amoxicillin (87%), ceftiofur (84%), and florfenicol (76%), whereas high resistance was recorded against penicillin G (80%), meropenem (76%), and gentamicin (73%).

A significantly higher prevalence was observed in males (47.91%) than females (38.33%) and in the 1–3-year age group (45.79%) compared to younger

animals (32.78%). These findings highlight the major role of *P. multocida* in ovine respiratory infections and emphasize the importance of continuous antimicrobial monitoring and rational antibiotic use to control multidrug-resistant strains in livestock populations.

Keywords: lung; Pneumonia; sheep; *Pasteurella multocida*.

Introduction:

Sheep pneumonia is a common and economically important disease in the sheep farming sector, causing high morbidity and mortality (1). Although many bacteria can cause pneumonia, some of the most studied include *Chlamydia abortus*, *Mycoplasma ovipneumoniae*, *Mannheimia haemolytica*, *Pasteurella multocida*, and *Bibersteinia trehalosi* (2).

P. multocida is classified into five groups: A, B, D, E, and F, based on the capsular structure (3). Group A represents the most predominant of the five major capsular types and has the highest public health, veterinary, and economic implications (4). The importance of investigating *P. multocida* in relation to bacterial diseases in ovine species focuses on their susceptibility to respiratory pathogens causing Pasteurellosis, which results in significant economic losses by impairing the industry's performance (5).

The main antibiotic groups that have been successful are penicillins and Amphenicols, but many other true positive in vitro drugs have also been recommended (6). Selection of a drug should, however, be made when the nature of the infection can be predicted and knowledge obtained from local antibiograms whenever available (7). Dosage regimen, fat solubility, frequency, route of administration, and side effects are the main points that need to be known before commencing therapy (8). When using the long-acting injectable or oral preparations, care should be taken in accurately fixing the dose calculations (9). It must be remembered that with a general *Pasteurella* infection, antibiotic treatment alone is of no value without also treating the underlying causes of the infection (10).

Materials and methods:

Collection of samples

A total of 168 samples were collected from sheep during the period from September 2024 to April 2025 this included 80 nasal swabs and 70 oral swabs, obtained from live sheep exhibiting respiratory signs (cough and nasal discharge) at participating veterinary clinic. Additionally, 18 lung tissue samples were collected during postmortem examination at local slaughterhouses from sheep with suspected pneumonia lesion. The collected samples were transported to the laboratory of the College of Veterinary Medicine, University of Al-Qasim Green, under aseptic conditions in sterile containers.

In the laboratory, samples were cultured on different selective and differential media including Nutrient agar, Blood agar, Mannitol Salt agar, MacConkey agar, Chocolate agar, and Eosin Methylene Blue (EMB) agar to isolate potential bacterial pathogens. All media were incubated at 37°C for 24 hours. Colonies with distinct morphological features were subjected to further biochemical identification tests to determine the bacterial species involved in respiratory infections in sheep.

Bacterial isolation

Samples from infected cases were cultured on 3%-5% Sheep Blood Agar , MacConkey Agar , Chocolate Agar , Mannitol Salt Agar , EMB Agar , and Nutrient agar . The cultures were incubated at 37°C for 24 hours, and the plates were subsequently examined for growth. The following characteristics were observed: no growth on MacConkey Agar. Biochemical identification was performed using Vitek technology, which showed catalase and oxidase positivity, indole production, and characteristics of non-motility, non-hemolysis on Sheep Blood Agar (SBA), and acid production from glucose (11) (12) (13).

Antibiotic Sensitive by VITEK 2 System

Vitek 2 System was employed to authenticate the diagnosis of *P.multocida*, this system is specific to bacteria that are Gram-negative and has 64 wells that are dried and color-coded. These indicators respond to the sample provided, and the system will document the changes that occur as a result of bacterial growth in the wells. Based on the color alteration, the system is capable of recognizing the bacterial sample according to the advice of bioMeri's (14).

Results and Discussion:

Bacterial Isolation and Identification

A total of 168 samples were collected from sheep exhibiting respiratory symptoms, including 80 nasal swabs (47.6%), 70 oral swabs (41.7%), and 18 lung tissue samples (10.7%).

Table (1) Distribution of Nasal, Oral, and Tissue Samples According to Number and Percentage.

| Sample Type | Number of Samples | Percentage (%) |
|---------------------------|---------------------------------------|----------------|
| Nasal swab | 80 | 47.6% |
| Oral swab | 70 | 41.7% |
| Tissue sample | 18 | 10.7% |
| Total | 168 | 100% |
| Calculated P value | <0.0001(Highly significant) | |

There was a highly significant difference in the distribution ($p < 0.0001$), reflecting that nasal and oral swabs were much more frequent findings than tissue samples. This overrepresentation of the upper respiratory tract samples is consistent with several studies on SRD in sheep. For instance, in (15) and (16) nose swabs represented also the principal means of detecting *P. multocida*; these authors gave this preference due to the fact that sampling can easily be performed while animals are still alive and it is less invasive than collecting samples of tissues. (15) reported 28 of 90 (31.1%) and (16) found *P. multocida* in 42 of 120 (35%) nasal swabs, respectively. These results are consistent with the findings of the present study in which swabs were by far the most common sample. Heterogeneity does exist with regard to studies that have had more lung included. (17), however, observed that 61.6% (53/86) of isolates were from lung tissue, which is much higher compared with the current study. This discrepancy could reflect the different sampling design: (17) worked on abattoir and necropsy samples, which made lung tissue more easily accessible, contrasting with this study that had a major field-restricted nature such limiting tissue collection. In addition, (18) reported higher detection rates on lung samples than nasal swabs with presence of *P. multocida* in 21 out of 84 lung tissues (25%), and thus highlighting their diagnostic role when available. Thus, the concordance with swab dominant studies(15),(16) supports the feasibility of testing upper airway samples in field conditions while discordance with tissue rich studies(17), (18) group differences on study context and access to necropsy material. These comparisons highlight the need for sampling both swabs and lung tissue where possible, as a balance between practical considerations and diagnostic accuracy.

Oral and nasal swabs of sheep showing respiratory distresses were subjected to the isolation of

bacterial isolates. *P. multocida* (69 isolates, 41.10%) was the most prevalent isolate followed by *Klebsiella* species (33) at 19.64%, *Staphylococcus spp* (26) indicating a prevalence of 15.47% and finally *E.coli* took third position with 16 isolates (9.52%). Furthermore, 24 samples (14.27%) were negative for subjected bacteria growth.

Table (2) Distribution of bacterial isolates recovered from oral, nasal swabs and lung tissue samples of sheep.

| Type of bacteria | Oral swab sample | Nasal swab sample | Lung tissue sample | Total number of sample | Percentage % |
|---------------------------|------------------|-------------------|--------------------|-----------------------------|--------------|
| <i>P.multocida</i> | 23 | 31 | 15 | 69 | 41.10% |
| <i>Klebsiella</i> | 14 | 16 | 3 | 33 | 19.64% |
| <i>Staphylococcus</i> | 11 | 15 | 0 | 26 | 15.47% |
| <i>E.coli</i> | 9 | 7 | 0 | 16 | 9.52% |
| No growth | 13 | 11 | 0 | 24 | 14.27% |
| Total | 70 | 80 | 18 | 168 | 100% |
| Calculated P value | | | | <0.0001(Highly significant) | |

In this study *P. multocida* was the most frequent isolate (41.1%), followed by *Klebsiella spp.* (19.6%), *Staphylococcus spp.* (15.5%), and *Escherichia coli* (9.5%). These observations highlight the importance of *P. multocida* in ovine respiratory disease. Similar outcomes were reported in (19) (34.8%) and (20) (38%), by both of whom *P. multocida* was found to be the dominant causative agent for pneumonic pasteurellosis in sheep. In the same vein, (21) reported a preponderant rate of 45% in Bangladesh comparable to isolation in this study. Nevertheless, higher as well lower isolation frequencies were described in some studies. For example, (22) reported an incidence of 28% in Egypt, while *P. multocida* was detected in only 25% of the pneumonic lung samples from sheep in Sudan (23). The lower detection could be due to a variable selection of sampling or geographical factors and management practices. In contrary, an overall prevalence of more than 50% has been reported (24) in ovine flocks from Pakistan indicating that outbreak conditions or stress factors might increase pathogen recovery. This variation between studies can be attributed to methodological as well as ecological factors. Although swab-based surveys of bacterial prevalence may be an underestimate compared with lung tissue-sampling, environmental stress and seasonality, as well as flock density, can also influence pathogen distribution. Accordingly, the 41.1% prevalence observed in our survey is consistent with most global reports and highlights regional heterogeneity in pathogen detection rates.

Culture characteristic of *P. multocida*

69 isolates (41.10%) were confirmed as *P. multocida*. Colonies showed typical growth on sheep blood agar (SBA) without hemolysis (Fig. 4.1A) and on Chocolate agar (Fig. 4.1B), while negative was detected on MacConkey agar. These results are consistent with the classical cultural characteristics of *P. multocida* (25). In the laboratory, lung samples were cultured on different selective and differential media including Nutrient agar, Blood agar, Mannitol Salt agar, MacConkey agar, Chocolate agar, and Eosin Methylene Blue (EMB) agar to isolate potential bacterial pathogens. All media were incubated at 37°C for 24 hours. Colonies with distinct morphological features were subjected to further biochemical identification tests to determine the bacterial species involved in respiratory infections in sheep.



Figure (1) *P. multocida* growth on (A) Blood agar non-hemolysis and (B) Chocolate agar.

The present study demonstrated that *P. multocida* isolates exhibited typical growth on sheep blood agar without hemolysis, small, circular, convex and smooth while on chocolate agar appears large and more mucoid, whereas no growth was observed on MacConkey agar. These findings are in line with the classical description of *P. multocida* as a non-hemolytic, non-lactose fermenting bacterium that requires enriched media for optimal growth (25),(26),(27).

The lack of growth on MacConkey agar is attributable to the organism's inability to tolerate bile salts and crystal violet, which inhibit the growth of fastidious Gram-negative bacteria. Comparable findings have been reported in isolates from small ruminants, reinforcing the observation that *P. multocida* requires enriched conditions for successful isolation (28),(29).

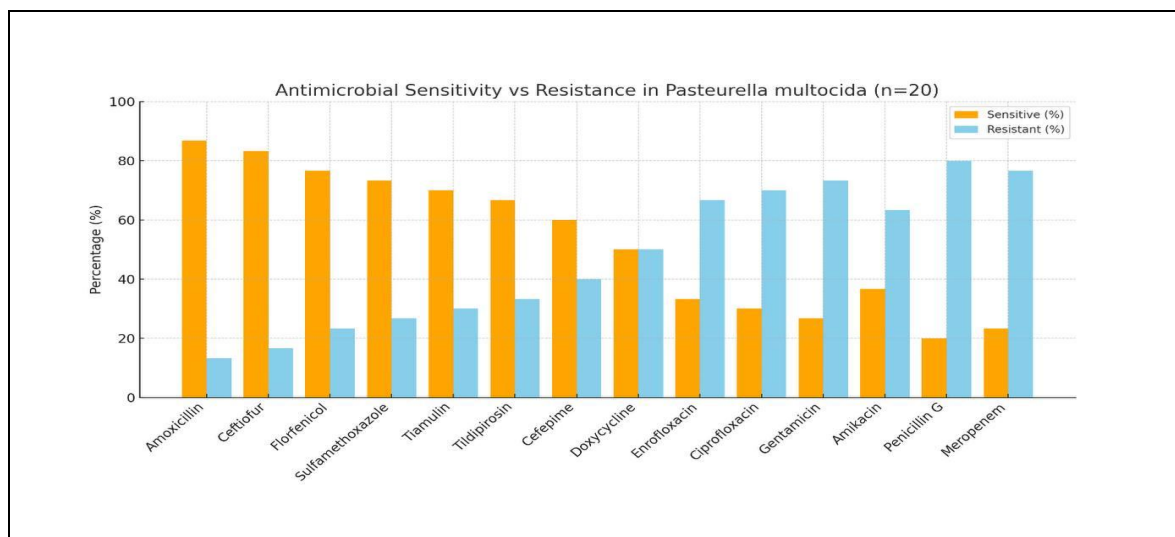


Figure (2) Antibiotic sensitivity test of 20 *P. multocida* isolates.

The antimicrobial susceptibility profile of *P. multocida* isolates in this study revealed high sensitivity to amoxicillin (87%), ceftiofur (84%), and florfenicol (76%). The results of the current study confirm that certain β -lactam antibiotics and florfenicol appear to be useful choices for therapy, which is similar to observations made in isolates from livestock sources (30),(31). Saving (32) also described similar effects regarding the long acting efficacy of amoxicillin clavulanate and cephalosporins against *P. multocida*. A high level of resistance was seen, however, against penicillin G (80%), followed by meropenem (76%), gentamicin (73%), ciprofloxacin (70%) and

amikacin (69%). This multi-drug resistance profile is alarming and in accordance with previous studies, which reported an increasing trend of *P. multocida* to fluoroquinolones, aminoglycosides and carbapenems in both small animals and cattle (33),(34). Resistance phenomena could occur through different mechanisms such as β -lactamase production, efflux pump activity or mutations in the quinolone-resistance-determining regions (QRDRs) as reported for clinical isolates (30). Doxycycline (50.0%) and cefepime (60.0%) showed a moderate susceptibility, but response to macrolides including tilmicosin and tulathromycin varied as observed in similar studies (32). Variation in resistance could also be due to variation in antibiotic use, selection forces, and regional practices for the treatment of veterinary medicine. These findings emphasize the necessity of on going monitoring of antimicrobial resistance in veterinary pathogens. The detected sensitivity to β -lactams and florfenicol provides a reason for their therapeutic use in sheep pasteurellosis supporting the goals of this study to determine suitable treatments. Nevertheless, the high percentage of resistance to multiple antibiotic groups could be a potential arriving smart MDR-*P. multocida* strains and points out economic losses, emphasizing either judicious use of antibiotics or application of antimicrobial stewardships in livestock production. systems.

Prevalence of *P. multocida* according to sex and age

P. multocida isolates were analyzed by age group of the examined sheep to investigate whether there was an effect of age on rates of infection. The prevalence rate in young aged sheep (less than 1 year) was found to be the lowest, with a percentage of 32.78% (20/61). On the other hand, a greater percentage prevalence of 45.79% was obtained in 1–3 years' group when 49 positive samples out of 107 were analysed. Out of 168 sheep tested, a total of 69 samples were positive, with an overall prevalence rate of 41.10%.

Table (3) Distribution of *P. multocida* isolates according to age interval of sheep

| Age group interval | Total examined samples | Positive samples to <i>P. multocida</i> | % Positive |
|---------------------------|------------------------|---|------------|
| Less than 1 year | 61 | 20 | 32.78% |
| 1–3 years | 107 | 49 | 45.79% |
| Total | 168 | 69 | 41.10% |
| Calculated P value | | <0.0001(Highly significant) | |

Out of 168 examined sheep, 69 (41.1%) were positive for *P. multocida*. The prevalence was significantly different among age classes ($P < 0.0001$), and therefore, the age represented also a most significant potential risk factor for infection. In particular, the infection rate was highest in animals aged 1–3 years (45.79%) and lower than those less than one year old (32.78%). These results are consistent with those of (35) who found more prevalence *P. multocida* in young sheep (1–2-year-old), while they mentioned the role of age exposed to stressors due to management factor such as weaning, and transportation as a risk factor. Likewise, (36) reported that the prevalence of pneumonic pasteurellosis was significantly higher in sheep from 1 to 3 years old than lambs, according with the present risk factor found for age. On the other hand, (37) reported higher prevalence of infection among lambs younger than one year old, because the immature immune system and poor maternal antibody protection in some flocks made young animals more susceptible. Similarly, (20) found that one year old lambs of age showed a significant increase in rate of *P. multocida* infection compared to older sheep, but in this study the higher prevalence was recorded among older age group (1-3 years). Collectively, the statistically significant difference in table ($P < 0.0001$) implies that age is an important factor for *P. multocida* epidemiology. However, some of the studies are in agreement with the results observed in this study and demonstrate elevated rates of prevalence among young adults(11,15) while others have considered neonates to a greater extent at risk. This difference may be attributed to variation in maternal immunity, management system and environmental condition of the farm. Recently,

epidemiological studies have demonstrated that *P. multocida* is an opportunistic pathogen and commonly colonizes the upper respiratory tract of clinically healthy animals, only to become pathogenic following a reduction in host immunity. Therefore, age-related development of immunity and stress-induced immunosuppression are important in determining rates of infection (38), (39).

In all, the strong association between age and the prevalence of *P. multocida* adds further relevance to such published results and highlights the need for focused prevention measures (vaccination and better management), particularly in young adult flocks. Sex factor was analyzed to assess the distribution of *P. multocida* isolates among sheep and its effect on infection rates. Results The prevalence among males was 47.91% (23 positive samples out of examined 48 samples). Contrastingly, the throw of positivity among females was 38.33%, with 46 positive samples out of 120 included in the study. In total, 69 sheep samples were tested positive out of the 168 tested with an overall prevalence of 41.10%.

Table (4) Distribution of *P. multocida* isolates according to sex of sheep.

| Sex | Total examined samples | Positive samples to <i>P. multocida</i> | % Positive |
|---------------------------|------------------------|---|------------|
| Male | 48 | 23 | 47.91% |
| Female | 120 | 46 | 38.33% |
| Total | 168 | 69 | 41.10% |
| Calculated P value | | <0.0001 (highly significant) | |

Of 168 sheep screened, 69 (41.1%) of the sheep were found to be positive for *P. multocida*. The overall infection rate was greater in the males (47.91%) than in females (38.33%). Statistical analysis showed that sex was closely associated with prevalence ($p < 0.0001$), therefore, it is an important epidemiological factor which affects the incidence of pneumonic pasteurellosis in sheep. The results were in agreement with (36) that documented a significantly higher *P. multocida* prevalence in males compared to females and explained the phenomenon by management linked and behavioral reasons, such as mating stress, aggressive contacts, and fighting which might weaken the immune defense enabling respiratory colonization. This supports the present findings and is consistent with a previous report that also, (35) found *P. multocida* more frequently in males. In an (20) Iraq study, however documented a slightly higher prevalence of *P. multocida* in females, this probably may be due to physiological and hormonal factors especially during peak pregnancy and lactation predisposes females for infection. In addition, no significant difference was observed between the two genders by (37) and they speculated that environmental factors and farm management strategies may play a more important role for susceptibility to infection than sex. In any case, significance of the observed differences between male and female horses ($P < 0.0001$) indicates that sex may have a role in modulating the epidemiological profile of *P. multocida*. However, the discrepancies among different studies suggest that the effect of sex is not uniform and may vary depending on management systems, physiological status, and environmental conditions prevailing in the study area.

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