



Multi-drug Resistant *Mannheimia hemolytica* and *Pasteurella multocida* in Pneumonic Sheep in Bangladesh

Sonia Akhter¹, Md. Asief Hossain Zihadi², Md Habibur Rahman³, Md Zillur Rahman⁴

¹Senior Scientific Officer, In-Charge, Jessore Regional Station, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh;

²Scientific Officer, Sheep Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh;

³Senior Scientific Officer, In-Charge, Faridpur Regional Station, Bangladesh Livestock Research Institute, Dhaka, Bangladesh;

⁴Chief Scientific Officer (R.C) and Head, Sheep Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh

Abstract

Background: Pneumonia in sheep is a complicated multicausal disease which affects the respiratory system causing increased morbidity and herd mortality rate. The members of Pasteurellaceae family are one of the prime causes of bacterial pneumonia in ruminants. Once infected by these organisms, antimicrobial therapy becomes obligatory. In Bangladesh, causation of pneumonic pasteurellosis and their antibiogram is unknown in sheep which insists the veterinarians to choose empirical antimicrobial therapy.

Objectives: This study was undertaken to identify multidrug resistant *Pasteurella multocida* and *Mannheimia hemolytica* in sheep suspected with pneumonia. **Methodology:** The samples of nasal swab and the lungs were collected aseptically. After overnight incubation in Brain Heart Infusion broth, the samples were streaked onto 5.0% sheep Blood Agar to obtain pure colony with characteristic features which were subjected to biochemical tests, polymerase chain reaction (PCR), and antibiogram study against eight antibiotics. **Results:** A total number of 140 samples were collected of which 120 nasal swabs and 20 lungs were collected aseptically. In total, 16.7% pneumonia cases in sheep were found to be caused by *Pasteurella* species. Out of 140 samples, 43.57% (61/140) were positive for *Mannheimia hemolytica* (68.85%) and 19.67% for *Pasteurella multocida*. The mixed infection by both organisms was 11.48%. The *Mannheimia hemolytica* (MH) was predominant in lungs (100.0%) and nasal swab samples (18.3%) than *Pasteurella multocida* (PM) which were 15% and 7.5% respectively. All the isolates were found to be multi-drug resistant where resistance against Penicillin and Ampicillin was 100% for all isolates. The resistance of *Mannheimia hemolytica* and *Pasteurella multocida* towards sulfonamide, ceftriaxone, oxytetracycline, gentamicin, and streptomycin were 63.33%, 49.44%, 44.44%, 16.67%, 11.11% and 78.22%, 45.46%, 45.45%, 36.36%, 27.27% respectively. Among all antibiotics tested, Chloramphenicol stands out to be the most effective one (100% sensitive). **Conclusion:** This study states the first molecular identification of *Mannheimia hemolytica* and *Pasteurella multocida* from sheep suspected with pneumonia in Bangladesh and their antibiogram pattern against commonly prescribed antimicrobials. Due to their commensalism nature and wide host coverage, these MDR pathogens warrant judicious use of antibiotics in small ruminants to reduce veterinary public health hazard. [*Bangladesh Journal of Infectious Diseases*, June 2023;10(1):16-23]

Keywords: Drug Resistance; Pasteurella Infections; Microbial Sensitivity Tests

Correspondence: Dr. Md. Asief Hossain Zihadi, Scientific Officer, Sheep Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh; **Email:** asief@blri.gov.bd; **Cell No.:** +8801751944071; **ORCID:** <https://orcid.org/0000-0002-1940-3462>

©Authors 2023. CC-BY-NC

Introduction

Livestock is one of the most significant accelerators needed for the growing economy of Bangladesh. The contribution of livestock sub sector to Gross Domestic Product (GDP) is 1.47% (constant price) while in total it shares 3.46% directly in agriculture sector¹. Sheep, comprising of 6.61% of total ruminants, is a promising climate resilient livestock in Bangladesh due to its multifunctionality (meat, milk, skin, wool, and manure), increased disease resistance, well adaptability in resource limited livelihood, and docile animal able to bi-annual lambing with multiple births². Amidst of limited variants of livestock and poultry population in Bangladesh, these 3.752 million thriving sheep populations play a significant role in meeting overall meat demand in the country. Besides the increased growth rate of sheep population (19.37%) compared to goat (5.92%) over the last 10 years is an indication to become a booming small ruminant livestock industry in recent future. There are different well defined native sheep breeds namely Coastal, Barind and Jamuna River Basin currently reared by backyard farmer in Bangladesh which also have the potentiality for commercial production³. But sustainable disease management approach is a prerequisite for the profitability of livestock farming. Besides this era of one health urges the veterinary practitioner to check any zoonotic disease to secure veterinary public health⁴.

The wide variety of infectious and non-infectious diseases in livestock population is a great concern for veterinary public health practitioner due to their multi-species and zoonotic nature. Among them, sheep suffer mostly from gastrointestinal (25.11%) and reproductive diseases (20.52%) whereas multiple systems, respiratory, metabolic, udder, and nervous system account for 8.98%, 7.60%, 6.22%, 5.99%, and 5.06% respectively⁵. Out of 7.60% respiratory diseases, Ovine infectious respiratory diseases (OIRD) produce a serious economic impact upon the sheep industry by increasing mortality, limiting growth rate and increased culling rate of the superior animals⁶. Though the majority of the OIRDs are multicausal, combining adverse climate and stress that favor microbial growth⁷, 77.6% of bacteria harboring the respiratory tract of apparently healthy sheep playing a vital role in producing the infection⁸.

Pneumonic pasteurellosis (PP) is a common OIRD of sheep caused principally by bacteria *Mannheimia hemolytica* (MH) and *Pasteurella multocida* (PM)⁹. These bacteria come out of their commensal state when antimicrobial barrier of the respiratory tract is

lost and once they enter into the lungs, they become opportunistic and infective⁷. The acute signs are typically fever, listlessness, anorexia, and sudden death. The recovered animals either become vulnerable to other infection or chronically affected throughout their life showing decreased lung capacity and weight gain efficiency leading to sporadic death⁷. The common signs and symptoms of this infection is acute fever, respiratory distress, drowsiness, anorexia, and death⁹. Recovery of the animal mainly depends on initiating early treatment with sensitive antibiotics along with other drugs. These bacteria are gram negative and usually sensitive to penicillin, tetracycline, and chloramphenicol. In Bangladesh, veterinarians usually prefer oxytetracycline, gentamicin, amoxicillin, streptomycin, penicillin, ampicillin, ceftriaxone (personal communication) for respiratory tract infection of ruminants as an empirical therapy. Due to the increased antimicrobial resistance, most of the commonly used antibiotics are becoming ineffective against these bacterial infections.

Traditional bacterial culture-based method is performed to identify sensitivity pattern of bacteria against available antibiotics¹⁰. As this is a time-consuming method requiring appropriate standardization, veterinarians tend to prescribe empirical antibiotics for symptomatic infections. Such approach may lead to treatment failure, extended dosage regimen and increased treatment cost¹¹. As culture-antibiogram laboratory activity in livestock sector is a critically troublesome due to limited facility and trained manpower, a representative sampling of an individual disease identifying prime causal agents and their antibiogram pattern would reasonably help veterinarians to choose right antimicrobials during treatment. To the best of our knowledge, there is no study regarding molecular identification of causal agent of PP in Sheep and their antibiotic sensitivity in Bangladesh. Hence, this present study was undertaken for isolation, identification, and antibiotic susceptibility pattern identification of causal agents of PP in suspected population of sheep concentrated areas in Bangladesh.

Methodology

Ethical Consideration: The live animals were handled by trained veterinary attendant for collecting samples humanely. The dead animals were necropsied and disposed by registered veterinarians at Government facilities. Ethical consent was taken from Bangladesh Livestock Research Institute Research Ethics review

committee. All the procedures were performed as per Indian national science academy: Guidelines for care and use of animals in scientific research¹².

Research Area: The study was conducted in the selected six sheep concentrated areas of Bangladesh namely Noakhali (Boshurhat), Nihongchori (Sadar), Tangail (Sadar), Faridpur (Vanga), Rajshahi (Godagari) and Savar (Dhamrai).

Study Animals: The sheep showing the signs of high fever (104-105°C), coughing, sneezing, lethargy, dyspnea, serous or mucopurulent ocular-nasal discharge was selected for sampling irrespective of age, sex, breed, body condition score, and vaccination status⁹. The lungs of slaughtered sheep was visually inspected for hemorrhagic inflamed area with sharp demarcation or consolidation to be sampled as pneumonic lungs¹³.

Research Design: A cross-sectional study was undertaken from June to December 2021 from study areas where suspected ovine pneumonic cases were selected for sample collection. The convenience sampling method was followed due to research specificity¹⁴. The nasal swabs from live animal and affected lung tissue samples were taken from dead animals slaughtered at adjacent Government Veterinary Hospital. The sample size varied according to the availability of disease of interest at a given time for a selected place. In total, 140 samples were taken (120 nasal swabs and 20 lung samples) from the selected site.

Sample Collection: The periphery of nares was decontaminated by 70% alcohol and a sterile cotton swab was inserted into the nostril rotating 360° against the wall of nasal cavity followed by placement in a sterile test tube containing 3 ml of Brain Heart Infusion (BHI) broth (Oxoid, UK)¹⁵. The affected lung was incised at the lesion site with sterile surgical blade and sample was taken from inner surface using a sterile cotton swab that was packed in similar manner as nasal swab¹⁶. All the samples were immediately placed in a cool box containing ice pack and transported to the Small Ruminant Research Laboratory, BLRI for further processing.

Isolation and Identification of Bacteria: The isolation and identification process were performed as directed by the Hardy Diagnostics, Santa Maria, CA, USA through following steps¹⁶.

Firstly, the samples pre-enriched with tryptone soya broth were incubated for 24 hrs. at 37°C for observation of turbidity and then a loopful of the broth was streaked in blood agar-based petri-plate supplemented with 5% sheep blood incubating aerobically for 24 hours at 37°C.

Secondly, the gram's staining was performed with typical colonies from cultured positive plates to study cellular morphology while sub-culturing was performed in case of mixed and gram-negative bacteria on both blood and MacConkey agar for further analysis. The typical colonies were characterized by the presence and the type of hemolysis, colony morphology, shape, size, color, and consistency, and ability to ferment lactose¹⁷.

Thirdly, single colonies of pure culture from both agars were transferred on nutrient agar-slants for preservation and subjected to a series of primary biochemical tests (catalase: Hydrogen peroxide, Fisher Chemical, UK, oxidase: TM phenylenediamine dihydrochloride, Merck Co., Germany, fermentative/oxidative: OF Basal Medium, Titan Biotech Ltd, India).

Final species level identification was performed by secondary test that includes metabolism of sugars (glucose and L-arabinose) and alcohol production of end products like indole test following the standard protocol¹⁸. The biochemical properties of the bacteria were assayed according to Mac Faddin's method¹⁹.

Species Confirmation by PCR: The bacterial DNA was extracted from pure colony following manufacturer's protocol (Qiagen, Hilden, Germany). The extracted DNA was quantified using Nano Drop 2000 (Thermo Scientific, Waltham, USA) and quality assessed on 0.8% agarose gel electrophoresis.

An amount of 100 ng/μl extracted DNA was used as template in PCR mixture of 25 μl that contained 1× hotstart Taq plus master mix (Thermo scientific) and 0.5 μl & 0.3 μl of *Pasteurella multocida* and *Mannheimia haemolytica* primers respectively. Using mastercycler (Eppendorf Ltd., Mississauga, Canada), PCR amplification was performed following initial denaturation at 95°C for 5 min ensuing 30 cycles of 94°C for 30s, annealing at 56°C for 1 min, extension 72°C for 1 min, and final extension at 72°C for 8 min²⁰. Using 1.5% agarose gel, the PCR products were analyzed through electrophoresis. The species-specific primers were used to detect *Mannheimia haemolytica* and *P*

multocida targeting 16S rRNA²⁰ and KMT1²¹ gene respectively (Table 1).

Table 1: List of Primers for Species Identification

| SL | Primer Set | Sequence (5' - 3') | Gene Target | Amplicon (BP) | Reference |
|----------------------------|-------------------|--|-------------|---------------|---------------|
| <i>M hemolytica</i> | | | | | |
| 1 | 16S | (F)GCTAACTCCGTGCCAGCAG (R)CGTGGACTACCAGGGTATCTAAC | 16S rRNA | 304 | ²⁰ |
| <i>P multocida</i> | | | | | |
| 2 | KMT1T7 KMT1SP6 | (F)ATCCGCTATTTACCCAGTGG (R)GCTGTAAACGAACTCGCC | KMT1 | 460 | ²¹ |

Antimicrobial Susceptibility Testing (AST): The PCR positive isolates were used for in vitro AST by following Kirby–Bauer disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) with quality control organism as *Escherichia coli* ATCC 25922²². The eight antibiotic discs were selected based on the prescription pattern of the veterinarians against pneumonic cases which are penicillin (10 IU), ampicillin (10 mcg), streptomycin (10 mcg), tetracycline (30 mcg), oxytetracycline (30 mcg), gentamicin (10 mcg), sulfonamide (300 mcg), chloramphenicol (30 mcg), and ceftriaxone (30 mcg) (Liofilchem, Italy).

Statistical Analysis: The data were analyzed through Microsoft Excel Data Analysis Toolkit (Microsoft Office 2021). Quantitative data were expressed as percentage, mean \pm standard deviation, and P value was obtained through Paired T Test.

Results

Cultural and Biochemical Characteristics of the Bacteria: The isolates were primarily identified according to cultural characteristics where beta hemolysis and growth on McConkey agar was positive for *Mannheimia hemolytica* and negative for *Pasteurella multocida*. A series of biochemical tests revealed *Mannheimia hemolytica* positive in catalase and oxidase whereas negative in indole, urease, citrate, methyl red, lactose, and arabinose fermentation. The isolates showing opposite characteristics were supposed as *Pasteurella*

multocida. Both of the isolates fermented glucose, sucrose, and sorbitol.

Molecular Identification: The positive percentage of OPP was found to be the highest in Noakhali Sadar 21.31 % (13/61) and lowest in Faridpur Vanga 11.48 (7/61) (Table 2).

Table 2: Area -wise Positive Isolate Percentage

| Sampling Place | Total | Positive Isolate |
|-------------------------------|-----------------|------------------|
| Godagari, Rajshahi | 30 | 12(19.7%) |
| Vanga, Faridpur | 20 | 7(11.5%) |
| Sadar, Tangail | 20 | 9(14.6%) |
| Dhamrai, Savar | 30 | 10(16.4%) |
| Sadar, Noakhali | 25 | 13(21.3%) |
| Sadar, Nihongchori | 15 | 10(16.4%) |
| Mean\pmSD | 16.67 \pm 3.5 | |

Species Specific PCR: Out of the 61 samples grew on blood agar, *Mannheimia hemolytica* was confirmed to be predominant (42/61, 68.85%) in ovine pneumonic pasteurellosis (OPP) cases in the study area followed by *Pasteurella multocida* (12/61, 19.67%), and both organisms (7/61, 11.48%) by PCR. The samples collected from nasal cavity were predominantly positive for *Mannheimia hemolytica* 18.3% (22/120), followed by *Pasteurella multocida* 7.5% (9/120) and both organisms 3.3% (4/120). The lung samples were positive towards *Mannheimia hemolytica* 100.0% (20/20), followed by *Pasteurella multocida* 15.0% (3/20) and both organisms 15.0% (3/20) (Table 3).

Table 3: Species Specific PCR Positive Isolates from Different Sample Types

| Sample Types | Total Sample | <i>P multocida</i> | <i>M hemolytica</i> * | Mixed Infection* |
|--------------|--------------|--------------------|-----------------------|------------------|
| Nasal Swab | 120 | 9 (7.5%) | 22 (18.3%) | 4 (3.3%) |
| Lung Tissue | 20 | 3 (15.0%) | 20 (100.0%) | 3 (15.0%) |
| Total | 140 | 12 | 42 | 7 |

*Significant at 5% level

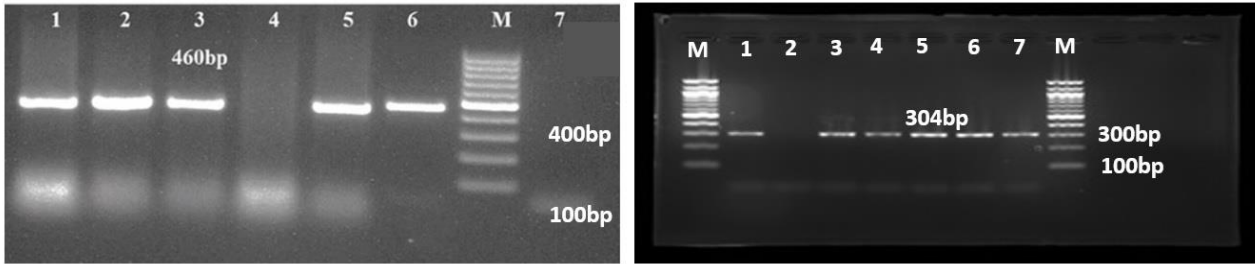


Figure I: Left to Right: PCR amplification for *Pasteurella multocida*, lane 2,3,5,6 (460bp) with lane 1 and 4 positive and negative control respectively. PCR amplification for *Mannheimia hemolytica*, lane 3-7 (304bp) with lane 1 and 2 positive and negative control respectively. 100bp ladder in both case

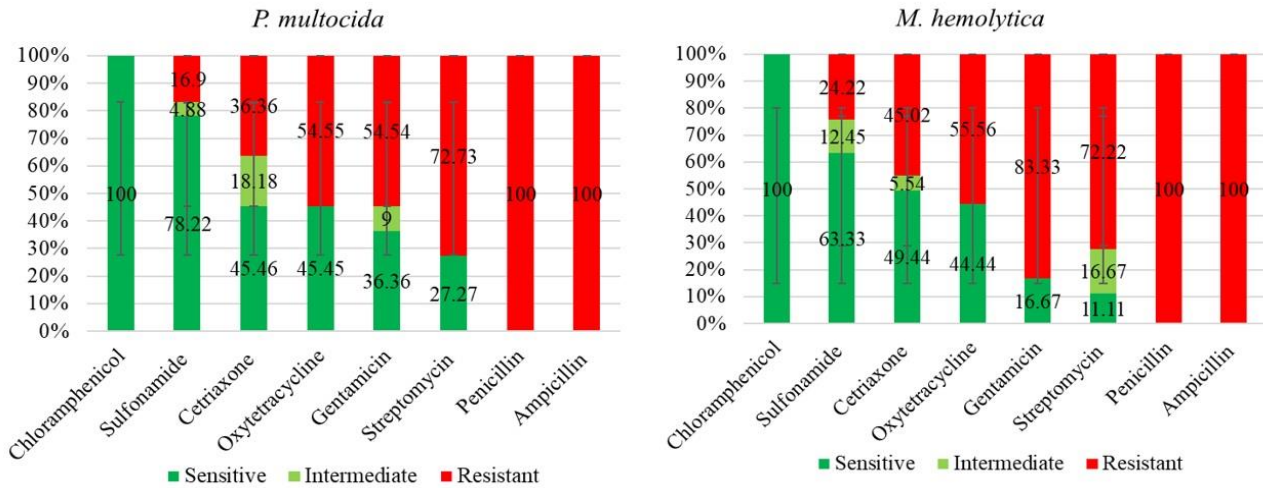


Figure II: Left to Right: Antimicrobial Sensitivity Pattern of *P. multocida* and *M. hemolytica*

In vitro Antimicrobial Sensitivity Test: All the isolates were found to be multi-drug resistant to eight antibiotics tested. Only Chloramphenicol (100.0%) was the most effective antibiotics. Penicillin and Ampicillin were ineffective (100% resistant) drugs against both isolates. Apart from that, *Pasteurella multocida* and *Mannheimia hemolytica* showed the highest sensitivity towards sulfonamide, 78.22% and 63.33% respectively. The sensitivity towards ceftriaxone, and OTC of *Pasteurella multocida* was almost the same, 45.46% while 36.36% to gentamicin. About 72.23% *Pasteurella multocida* isolates were resistant to streptomycin (72.73%). In case of *Mannheimia hemolytica*, gentamicin and ceftriaxone were more sensitive (49.44% and 44.44% respectively) than OTC and streptomycin (83.33% and 72.22% resistant respectively) (Figure II).

Discussion

Small ruminants, goat and sheep, are the major source of income for livestock farmers in Bangladesh. They often suffer from various respiratory diseases and eventually death causing

huge capital loss. Respiratory diseases in livestock and poultry are multifactorial in nature where environmental factors and physiological status play vital role in favoring the disease. Bacterial respiratory infections caused by genus *Pasteurella* is well documented in small ruminants⁸. However, there is variation in species level infection prevalence. This study identified *Mannheimia hemolytica* 30.0% (42/140) rather than *Pasteurella multocida* 8.6% (12/140) as the prime causes of pneumonic pasteurellosis in sheep which is consistent with other studies. A study in India found that *Mannheimia hemolytica* was 55.3% whereas *Pasteurella multocida* was 7.9% in affected lungs of sheep. Such predomination of *Mannheimia hemolytica* species was also observed in nasal swab samples²⁰. Another similar research was conducted in Ethiopia where 25.0% *Pasteurella* species were found where *Mannheimia hemolytica* was 87.5% and *Pasteurella multocida* was 12.5% in apparently healthy sheep²³. A study on Bighorn sheep in USA indicated that beta hemolytic pattern was predominant in respiratory infection in adult sheep but no such correlation was found in case of lamb²⁴. Similarly, a random sampling from veterinary

clinics in Ethiopia confirmed all the lung samples positive for *Mannheimia hemolytica* and *Pasteurella multocida* but only 18.3% and 7.5% nasal swab samples respectively. Evidence of *Pasteurella multocida* causing pneumonic pasteurellosis in sheep is lesser than *Mannheimia hemolytica* nevertheless their strong association in occurring lamb pneumonia²⁵. The difference of positive case percentages might vary due to the types of collected samples. Apparently healthy, suspected, and diseased) and other unknown reasons. However, the variation in disease occurrence couldn't be justified due to the lack of epidemiological data in these mentioned studies.

The choice of antimicrobials is crucial due to the increased wide spread antimicrobial resistance narrowing down the effective antimicrobial spectrum. Despite not as highlighted as other bacteria, *Mannheimia hemolytica* and PM isolated from sheep and goat are gaining attention due to their rising antimicrobial resistance behavior in recent years²⁶. In this study, Chloramphenicol was the only drug that was 100% sensitive to the both isolates followed by Sulfonamide 78.22, 63.33 to *Pasteurella multocida* and *Mannheimia hemolytica* respectively. These findings are corroborated by other studies performed in similar context²⁰⁻²³. Use of sulfonamide in livestock is less practiced in Bangladesh due to its bactericidal impact on gut microbiota which are vital for healthy ruminants. On the other hand, chloramphenicol is not used in food animals.

Hence, these drugs remain sensitive to an optimum level towards *Pasteurella multocida* and *Mannheimia hemolytica*. While investigating respiratory tract infection of sheep, florfenicol was found to be 100% sensitive like chloramphenicol elsewhere²⁷. The use of chloramphenicol in food animal is prohibited by many developed countries whereas florfenicol could be an effective alternative treating pneumonic pasteurellosis in sheep. Besides, injectable sulfonamides can also be practiced where drug resistance becomes evident. The oxytetracycline (OTC) was found to be sensitive in *Pasteurella multocida* (45.45%) than in *Mannheimia hemolytica* (16.67%) but much higher sensitivity was revealed in another study which is 83.9% and 87.5% respectively²³. While ceftriaxone is ~45% sensitive towards both isolates, gentamicin is 49.44% sensitive towards *Mannheimia hemolytica* that gives it upper hand as the drug of choice in clinical cases by *Mannheimia hemolytica* though the isolates of Ethiopia were 100.0% resistant towards gentamicin. The two most important drugs, OTC and Streptomycin showed

the lowest sensitivity, 16.7% and 11.1%, towards *Mannheimia hemolytica* isolates while streptomycin proved to be 27.3% sensitive to *Pasteurella multocida* isolates. While the resistance percentage of streptomycin was similar to one in Ethiopia but widely different from another study in India. The two drugs, Ampicillin and Penicillin, were proved to be 100% ineffective against both isolates which was supported by studies mentioned above with a small percentage of sensitivity shown in study of India.

To the best of our knowledge, molecular investigation of *Pasteurella multocida* and *Mannheimia hemolytica* in sheep and their antibiogram study is first conducted in Bangladesh. Besides this is the first report of molecular identification of *Mannheimia hemolytica* in animals in Bangladesh. The earlier researches regarding *Pasteurella multocida* in domestic animal were only performed to detect the causal agent of Hemorrhagic septicemia (cattle and buffalo), pneumonic pasteurellosis in goat, and fowl cholera in Chicken^{28,26,29}. According to our study, the use of sulfonamide, ceftriaxone, and gentamicin can be considered for pneumonic pasteurellosis cases in sheep and more sensitive antibiotics uncontradictory to public health should be explored.

Besides, other antimicrobial options like phage therapy, nanoparticles, or plant bioactive compounds can also be explored^{30,31,32}. Moreover, elucidation of mechanism of AMR and transmission of Antimicrobial Resistant Genes (ARGs) of *Pasteurella multocida* and *Mannheimia hemolytica* should be studied further for taking effective mitigation approach. As these two bacteria are commensal organisms, their impact in harboring ARGs and subsequent transmission can aggravate the scenario highlighted in this study. It is best to consider prevention of this disease through mitigating risk factors and following strict biosecurity and vaccination by using vaccines produced from local species-specific bacterial isolates³³.

Conclusion

This is the first study to report *Pasteurella multocida* and *Mannheimia hemolytica* in sheep of Bangladesh. It was revealed that both organisms associated with pneumonia are multidrug resistant that creates difficulty for veterinarians to treat the disease efficiently. The most effective drug was found to be chloramphenicol, sulfonamide whereas moderate level of sensitivity was found towards ceftriaxone, oxytetracycline, and gentamicin. As the

use of chloramphenicol in food animal is prohibited, florfenicol can be considered as good choice. The drug streptomycin has the lowest sensitivity while ampicillin and penicillin remain completely ineffective. However, more robust antimicrobial panels should be considered along with Minimum Inhibitory Concentration breakpoints to specify the antimicrobial regimens for future study. For that, CLSI and EUCAST should include more relevant drugs' breakpoints for these two organisms. While considering vaccine seed production for pneumonia cases in ruminants, multicausality of the nature of disease must be noted.

Acknowledgments

This research was extensively supported by Fatema Islam Riya and Sujon Ahmed as Lab Technician and Md Nazmul as Field Assistant.

Conflict of Interest

None

Financial Disclosure

This work was funded by Bangladesh Livestock Research Institute 2021-22 fiscal year revenue project under the title of Investigation of economically important diseases of sheep and their mitigation to develop a model sheep health management package for ideal farming (Memo No. 33.05.2672.304.05.001.21.100)

Contribution to authors:

S Akhter and MH Rahman conceptualized and designed the project, S Akhter and Zihadi MAH conducted the research, Zihadi MAH drafted the manuscript, analyzed the data, and MZ Rahman supervised the project. All the authors reviewed and approved the final manuscript.

Data Availability

The data are property of Bangladesh Livestock Research Institute and anyone needing access to the raw data must apply through proper channel after communicating with corresponding author. Apart from that, any sort of inquiry or question regarding the research should be addressed to the corresponding author.

Ethics Approval and Consent to Participate

The BLRI Research Ethics Committee granted the study approval.

How to cite this article: Akhter S, Zihadi MAH, Rahman MH, Rahman MZ. Multi-drug Resistant *Mannheimia hemolytica* and *Pasteurella multocida* in Pneumonic Sheep in Bangladesh. Bangladesh J Infect Dis 2023;10(1):16-23

Copyright: © Akhter et al. 2023. Published by *Bangladesh Journal of Infectious Diseases*. This is an open-access article and is licensed under the Creative Commons Attribution Non-Commercial 4.0 International License (CC BY-NC 4.0). This license permits others to distribute, remix, adapt and reproduce or changes in any medium or format as long as it will give appropriate credit to the original author(s) with the proper citation of the original work as well as the source and this is used for noncommercial purposes only. To view a copy of this license, please

See: <https://www.creativecommons.org/licenses/by-nc/4.0/>

ORCID

Sonia Akhter: <https://orcid.org/0000-0002-5064-2154>

Md. Asief Hossain Zihadi: <https://orcid.org/0000-0002-1940-3462>

Md Habibur Rahman: <https://orcid.org/0000-0002-3221-2142>

Md Zillur Rahman: <https://orcid.org/0009-0003-2278-335X>

Article Info

Received on: 17 March 2023

Accepted on: 2 May 2023

Published on: 3 June 2023

References

- Salim HM. Livestock Economy at a Glance 2021-22. 2022. Available from: <http://www.dls.gov.bd/site/page/22b1143b-9323-44f8-bfd8-647087828c9b/Livestock-Economy>
- Bhuiyan AKFH. Livestock genetic resources in Bangladesh: Preservation and Management. In: International conference on livestock services, Chinese Academy of Agricultural Science (CAAS), Beijing, China 2006.
- Ahmed S, Rakib MRH, Yesmin M, Sultana N, Jahan N, Ershaduzamman M. Evaluation of lamb production potentiality of the Barind, Jamuna river basin and coastal region sheep of Bangladesh under intensive management. Journal of Advanced Veterinary Animal Research 2018;5(1):37-43.
- Espinosa R, Tago D, Treich N. Infectious Diseases and Meat Production. Environmental and Resource Economics 2020;76(4):1019-44.
- Alekish M, Ismail ZB. Common diseases of sheep (*Ovis aries* Linnaeus) and goats (*Capra aegagrus hircus*) in Jordan: A retrospective study (2015-2021). Open Veterinary Journal 2022;12(6):806-14.
- Lacasta D, González JM, Navarro T, Saura F, Acín C, Vasileiou NGC. Significance of respiratory diseases in the health management of sheep. Small Ruminant Research 2019;181:99-102.
- Brogden KA, Lehmkuhl HD CR. *Pasteurella haemolytica* complicated respiratory infections in sheep and goats. Veterinary Research 1998;29 (3-4):233-54.
- Kaur A, Singh G, Taku A, Bhatt A, Sharma R. Exploration of microbiome in the respiratory tract of sheep using whole metagenomic sequencing. The Pharma Innovation Journal 2022;11(10):310-5.
- Akane AE, Alemu G, Tesfaye K, Ali DA, Abayneh T, Kenubih A, et al. Isolation and Molecular Detection of *Pasteurellosis* from Pneumonic Sheep in Selected Areas of Amhara Region, Ethiopia: An Implication for Designing Effective Ovine *Pasteurellosis* Vaccine. Veterinary Medicine Research Reports 2022;13:75-83.
- Antibiotic susceptibility diagnostics for the future. Nature Microbiology 2019;4(10):1603.
- Chowdhury S, Ghosh S, Aleem MA, Parveen S, Islam MA, Rashid MM, et al. Antibiotic usage and resistance in food animal production: What have we learned from Bangladesh? Antibiotics 2021;10(9):1-14.
- Sahni SK. Indian national science academy: Guidelines for care and use of animals in scientific research 1994.
- Herenda D, Chambers P, Ettriqui A, Seneviratna PDST. Manual on meat inspection for developing countries. Vols. M-25 ISBN, FAO 2000.
- Chandler J, Shapiro D. Conducting Clinical Research Using Crowdsourced Convenience Samples. Annual Review of Clinical Psychology 2016;12(January):53-81.
- GR Carter. Diagnostic procedures in Veterinary Bacteriology and Mycology. Charles. 4th edition. USA: Thomas; 1984. 3-19.
- Quinn PJ, Carter ME, Markey BCG. Bacterial pathogens: microscopy, culture and identification. In Clinical Veterinary

- Microbiology. London, England: Wolfe publishing 1994. 20–60.
17. Sharma SN and Adhaka SC. Text book of veterinary Microbiology. Masjid: Vikas publishing house PVT LTD 1996.
 18. Carter GR. Isolation and identification of bacteria from clinical specimen Edition., In Diagnostic Procedures in Veterinary Bacteriology and Mycology. 4th edition, Edited by Charles C. USA: Thomas 1984. 19–30.
 19. MacFadinn JF. Biochemical tests for identification of medical bacteria. 3rd edition. New York: Williams and Wilkins Lippincott 2000. 5318–5183.
 20. Sahay S, Natesan K, Prajapati A, Kalleshmurthy T, Shome BR, Rahman H, et al. Prevalence and antibiotic susceptibility of Mannheimia haemolytica and Pasteurella multocida isolated from ovine respiratory infection: A study from Karnataka, Southern India. *Veterinary World* 2020;13(9):1947–54.
 21. Townsend KM, Frost AJ, Lee CW, Papadimitriou JM, Dawkins HJ. Development of PCR assays for species- and type-specific identification of Pasteurella multocida isolates. *Journal of Clinical Microbiology* 1998;36(4):1096–110.
 22. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a Standardized single disk method. *American Journal of Clinical Pathology* 1966;45(4):493–6.
 23. Marru HD, Anijajo TT, Hassen AA. A study on Ovine pneumonic pasteurellosis: Isolation and Identification of Pasteurellae and their antibiogram susceptibility pattern in Haramaya District, Eastern Hararghe, Ethiopia. *BMC Veterinary Research* 2013;9(1):1–8.
 24. Miller DS, Weiser GC, Ward ACS, Drew ML, Chapman PL. Pasteurellaceae isolated from bighorn sheep (*Ovis canadensis*) from Idaho, Oregon, and Wyoming. *American Journal of Veterinary Research* 2012;73(7):1024–8.
 25. Ayalew S, Blackwood ER, Confer AW. Sequence diversity of the immunogenic outer membrane lipoprotein PlpE from Mannheimia haemolytica serotypes 1, 2, and 6. *Veterinary Microbiology* 2006;114(3–4):260–8.
 26. Ahmed SJ, Hasan MA, Islam MR, Ali Khan Shawan MM, Uddin MF, Rahman MN, et al. Incidence and Antibiotic Susceptibility Profile of Pasteurella Maltocida Isolates Isolated from Goats in Savar Area of Bangladesh. *Agricultural Science Diges* 2019;39(04):357–60.
 27. Berge ACB, Sisco WM, Craigmill AL. Antimicrobial susceptibility patterns from sheep and goats. *Journal of American Veterinary Medical Association* 2006;229(8):1279–81.
 28. Ara M, Rahman M, Akhtar M, Rahman M, Nazir K, Ahmed S, et al. Molecular detection of Pasteurella multocida Type B causing haemorrhagic septicemia in cattle and buffaloes of Bangladesh. *Progressive Agriculture* 2016;27(2):175–9.
 29. Hossain MR, Meher MM, Afrin M. Epidemiological Investigation of Pasteurella Multocida Infection in Poultry in Gazipur District of Bangladesh. *Bangladesh Journal of Veterinary Medicine* 2018;15(2):91–5.
 30. Jamil T, Mehmood A, Farhan MHR, Kalim F, Hadi F, Younas K et al. Bacteriophage Therapy: Effective Antibiotic Replacer Against Emerging Ghost of Antimicrobial Resistant Bacteria. *One Health Triad* 2023;1:158–67.
 31. Altaf S, Alkheraije KA. Cell membrane-coated nanoparticles: An emerging antibacterial platform for pathogens of food animals. *Frontiers of Veterinary Science* 2023;10(5).
 32. Zihadi MAH, Rahman M, Talukder S, Hasan MM, Nahar S, Sikder MH. Antibacterial efficacy of ethanolic extract of Camellia sinensis and Azadirachta indica leaves on methicillin-resistant Staphylococcus aureus and shiga-toxigenic Escherichia coli. *Journal of Advanced Veterinary Animal Research* 2019;6(2):247–52.
 33. Zihadi MAH, Vahlenkamp TW. Short Review on Vaccination and Surveillance on Avian Influenza in Bangladesh: Existing Gaps and Recent Insights. *Bangladesh Journal of Infectious Diseases* 2018;4(2):48–51