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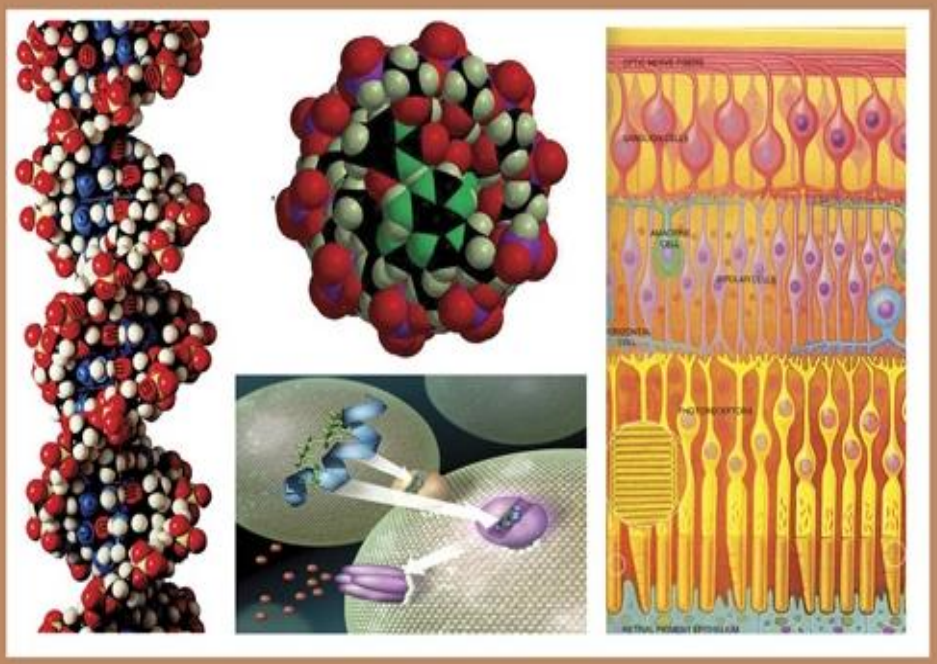

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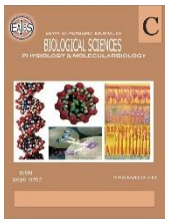

Evaluating the Antimicrobial Susceptibility of *M. haemolytica* and *P. multocida* isolated From Dairy Calf Pneumonia

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ABSTRACT

On dairy farms, bovine respiratory disease (also known as BRD) is still considered to be one of the most serious infectious diseases. The main bacteria that cause BRD are *Pasteurella multocida* and *Mannheimia hemolytica*. **Objectives:** This research evaluated antibiotic resistance prevalence. *M. hemolytica* and *P. multocida* were isolated from bovine lung samples at Veterinary Farms and investigated for antibiotic resistance. **Methods:** Samples were taken from the lungs of both sick and healthy cattle. Using bacteriology culture tests, testing of biochemicals, and the polymerase chain reaction (PCR) method, both *Pasteurellaceae species* were separated from the samples. The isolates were identified using the PCR methodology. The Kirby–Bauer disc diffusion method was used to find out how susceptible the isolates are to antibiotics. **RESULTS:** 120 samples were obtained and *P. multocida* 60 (50%) and *M. hemolytica* strains 30 (20%) were found in the samples' bacteriological analysis. *M. haemolytica* from pneumonia samples differed from those from apparently healthy lung tissues. Penicillin and erythromycin were the most resistant, while all isolates were sensitive to tulathromycin. *Pasteurellaceae* species were resistant to multiple drugs. **Conclusions**: BRD pathogens must be monitored for antibiotic resistance patterns to update treatment strategies. To establish resistance-limiting measures, surveillance studies are needed.

INTRODUCTION

Bovine respiratory disease (BRD) is still considered one of the infectious diseases most likely to devastate dairy farms. It is the primary reason for the economic loss that results from the mortality of animals, poor milk output, a drop in weight gain and development rates, as well as an increase in the expenses of treatments and other economic factors (Anholt *et al.*, 2010; Haji *et al.*, 2010). Dairy calf pneumonia is complex. This illness complex may be caused by stress, housing, ventilation, colostral immunity, and other viral pathogens. *Mannheimia haemolytica* and *Pasteurella multocida* are two bacteria this kind of are quite often identified from purulent bronchopneumonia lung, illness. Calves' upper respiratory flora includes these DCP-causing bacteria. (Abbas Panah *et al.*, 2010). BRD's major pathogen is *Pasteurella haemolytica* (Klima *et al.*, 2014). *P. multocida* causes BRD. This Gram-negative zoonotic bacterium can infect livestock and cause significant economic losses. The primary goal of BRD treatment on large commercial farms is to treat sick animals with metaphor-lactic antibiotic therapy to prevent the spread of the disease. In most cases, a different antimicrobial antibiotic is administered to sick animals that did not react to the first one.

Antimicrobial resistance has been discovered, particularly multi-drug resistance (MDR) in cattle isolates, prompting the study of potential treatment methods for BRD (Ashrafi *et al.,* 2022). Researchers have found an increase in antimicrobial resistance, particularly in MDR isolates (Nefedchenko *et al.,* 2019). It is essential to collect additional data on the frequency of BRD-causing agents as well as their antimicrobial resistance because of the impact BRD has on animal husbandry economics and animal health (Noyes *et al.,* 2015; Sebbar *et al.,* 2019). Antimicrobial susceptibility testing is crucial because the distribution of resistant microorganisms is always altering. It helps identify the best appropriate empirical antimicrobial treatment based on the pathogenic bacteria recovered from DCP (Mohammadi *et al.,* 2006; Valadan *et al.,* 2014). The goal of this research was to identify which antimicrobials were effective against *M. haemolytica* and *P. multocida* that were recovered from calves with dairy calf pneumonia.

MATERIALS AND METHODS

During 2021 and 2022, 100 calves from two weeks to six months old in Veterinary Hospital farms with dairy calf pneumonia had been observed. The Case calf one in which the physician confirmed pneumonia. Each sample was placed in a sterile plastic bag with a zipper and transported to the microbiology lab at Abasyn University, main branch, within two hours of the animal's death and stored cold at 4 ° c until analysis. Hyperemia, a rough surface, and consolidation symptoms were observed in samples ranging from 2-3 cm3 that were taken from sick cranioventral lung lobes (Ashrafi *et al.,* 2022).

Isolation and Identification of Bacteria:

The materials were homogenized in a sterile physiological solution, and the resulting suspension was cultured at 37°C for 24 hours on a blood agar medium containing 5% defibrinated sheep blood. Colonies were chosen based on whether or not they resembled *M. haemolytica* (white-gray, spherical, non-mucoid, and -hemolytic) or *P. multocida* (non-hemolytic, greyish, spherical, non-mucoid). The viable colonies were scattered over blood agar and McConkey agar, and the temperature was kept at 37°C for twenty-four hours. Following that, the samples were examined to determine whether or not they produced catalase and oxidase and whether or not they were able to produce a Gram stain. In addition to TSI, SIM, urea agar, MRVP, nitrate, gelatin, Simmons citrate agar, and other culture media, sugar-containing culture media such as glucose, arabinose, mannose, trehalose, ONPG, and NPG were utilized in the process of identifying microorganisms (Lubbers *et al.,* 2013). The cultures were incubated at 37°C for 24 hours. In two separate studies (Khalili *et al.,* 2016; Nefedchenko *et al.,* 2019), this is the case. *M. hemolytica* grew on McConkey agar and had a limited hemolysis zone, but no indole synthesis (Al-Haj Ali and Al-Balla, 2019). Even though Gram-negative *coccobacilli,* catalase, oxidase, and indole positivity, citrate utilization, methyl red, and VP tests, gelatin hydrolysis negativity, and non-growth on McConkey agar with no hemolysis on blood agar were diagnostic of *P. multocida,* it was determined in a study that non-growth on McConkey agar with no hemolysis on blood agar was (Khamesipour *et al.,* 2014).

PCR-Species Confirmation:

Cultural and biochemical studies identified the isolates. The PCR test verified the presence of the bacterial isolate, which was then placed in BHI broth and kept at 37°C between 18 and 24 hours. Genomic DNA was extracted using a system called the GenoPlus TM Genomic DNA Extraction Miniprep System from a culture that had been kept in isolation overnight (Viogene, China (Ashrafi *et al.,* 2022. Agarose gel electrophoresis (1%) assessed DNA quality and quantity. Table 1 shows the species-detecting primers

(Rawat *et al.,* 2019). Amplification was performed in a 25-µL total reaction volume with 0.5-µLTaq DNA polymerase, 5 µL of 5× reaction buffer (Bioline, UK), 1 µL of each primer, 1 µL of template DNA, and 16.5 µL of DEPC water. The KMT1 gene was amplified using 35 cycles of denaturation at 95°C for 45 s, annealing at 56°C for 45 s, and elongation at 72°C for 1 min. The initial denaturation was 95°C for 3 min and the final extension was 72°C for 5 min (Rawat *et al.,* 2019). A Gel Red-stained 1.5% agarose gel electrophoresed the PCR products PHSSA gene amplified. Amplification of the PHSSA gene was carried out using 35 cycles consisting of denaturation at 95 ° C. for one minute, annealing at 48 ° C. for one minute, and elongation at 72 ° C. for 30 seconds. The first denaturation took 3 min at 95°C and the final extension took 5 min at 72°C (Yatsentyuk *et al.,* 2022).

Antimicrobial Susceptibility:

Antimicrobial susceptibility testing of isolates was done using the Kirby-Bauer disc diffusion technique according to Clinical and Laboratory Standards Institute (CLSI, 2018) guidelines. After 18 hours of incubation at 37°C. The results were interpreted based on the measurements of the inhibition zone against each isolate. Antimicrobial agents include floorfenicol (30 µg), chlorampheniicol (30 µg), oxytetraacycline (30 µg), amoxiicillin (25 µg), gentaamicin (30 µg), cepahalothin ((30 µg), lincomyciin (30 µg), Erythrromycin (30 µg), sulphamethoxzol (30 µg), and peniciillin (30 µg) (Ashrafi *et al.,* 2022).

Research Statistical Analysis:

The Chi-square test was used to determine differences in bacterial isolation, and a P-value of less than 0.05 was declared statistically significant.

RESULTS

A total of 120 isolates from nasopharyngeal swabs from 120 calves were cultured. *P. multocida* 60 (50 %) and *M. haemolytica* 29 (24.9 %) caused the most cases. Additional isolates were *Bacillus spp.* 6 (5.1%), *Staphylococcus spp.* 7 (5.8%), *Streptococcus spp.* 8 (6.6%), *Pseudomonas, Proteus,* and *E. coli* 5 (4.1%). *M. haemolitica* and *P. multocida* were examined for their antibacterial susceptibilities. 100% of *M haemolitica* isolates from nasopharyngeal swabs (n= 60) were susceptible to Cepahalothin, Penicillin, and Erythromycin. On the other hand, similar isolates showed both sensitivity to and resistance to oxytetracycline (25.2% sensitivity, 10.75% bacterial resistance), gentamicin (36 % sensitivity, 18.80% bacterial resistance), and lincomycin (18.90% sensitivity, 19.18% bacterial resistance). *P. multocida* isolates were 100% sensitive to florfenicol, chloramphenicol, and cephalothin, but only 81.35% susceptible to penicillin. *P. multocida* isolates were resistant to oxytetracycline, amoxicillin, gentamicin, lincomycin, enrofloxacin, and trimethoprim-sulfamethoxazole in 9.35% of cases. Additional *P. multocida* isolates were resistant to oxytetracycline (16.45%), amoxicillin (15.60%), penicillin (18.80%), gentamicin (10.55%), and lincomycin (17.80%) (Ashrafi *et al.,* 2022).

Table 1. Primers used for the detection of *P. multocidaa* and *M. hemolyticaa*

Primers for *P.multocida* and*M.hemolytica* detection	**Gene detected**	**Primer Sequence**	**Amplicon size (bp)**
M. hemolyticaa	PHSSA	**F 5AACTTAAGAAGTGGGAG3 R 5TTGAATTCTTCACCCTC3**	**275**
P. multocidaa	KMT1	**F 5TTAGGCGATTTACTA3 ` R 5AATCCGCTAAATGAT3`**	**356**

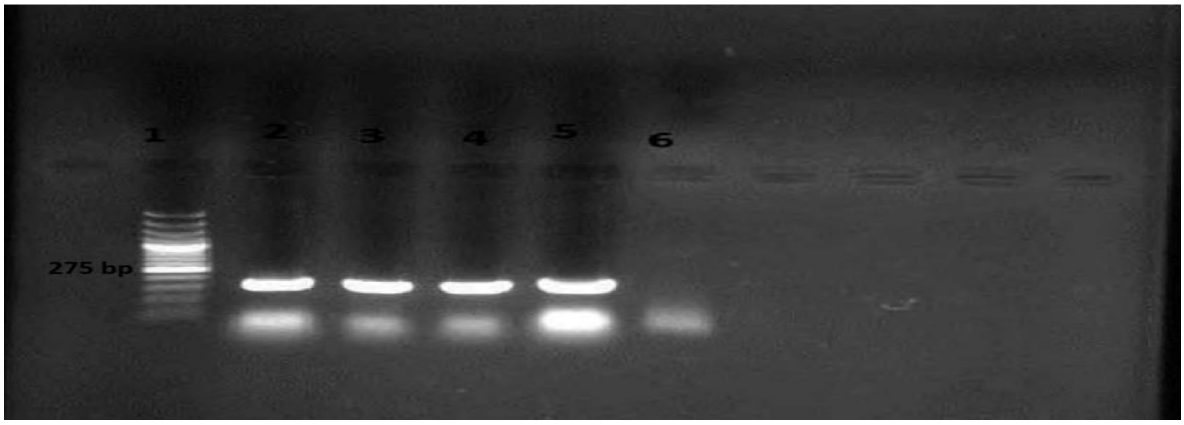

Fig. 1.*P. multocida* KMT1 (457 bp) and *M. haemolytica* PHSSA (327 bp) gene amplification.

DISCUSSION

To treat BRD, a veterinarian must first decide which antimicrobial antibiotic will be most effective. A significant decrease in treatment efficacy, such as increased mortality or the number of animals requiring re-treatment, generally causes the practitioner to submit samples for microbiological culturing and antimicrobial susceptibility testing. Veterinarians use BRD pathogen antimicrobial susceptibility trends to choose the most effective and cost-efficient treatment drugs. Antimicrobial susceptibility among BRD pathogens may be affected by several variables (Lubbers *et al.*, 2013). Although *M. haemolytica* and *P. multocida* are common bovine respiratory tract flora, the susceptibilities of isolates in upper respiratory tract samples may not represent the susceptibility of the causal strain of pneumonia. As seen above, *M. haemolytica* and *P. multocida* prevalence and antibiotic resistance differed greatly between trials. These differences may be attributed to the environment, season, breeding, commercial activities, therapies, and management tactics of affected cattle. (Anholt *et al.*, 2017).

Conclusion

In conclusion, *M. haemolytica* was found more often than *P. multocida* in lung samples from both healthy and sick cattle. Moreover, *M. haemolytica* populations from apparently healthy and sick samples differed. *M. haemolytica* and *P. multocida* from Lung Samples were all susceptible to tulathromycin, while penicillin and erythromycin were the most resistant. Each Pasteurellaceae possesses MDR.

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