



Multi-Drug Resistant *Pasteurella multocida* and *Mannheimia haemolytica* Strains Isolated from Different Hosts Affected by Pneumonic Pasteurellosis in Egypt

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Abstract | *Pasteurella multocida* (*P. multocida*) and *Mannheimia haemolytica* (*M. haemolytica*) are widespread pathogens, resulting in economically significant animal diseases. This study aimed to investigate some of the epidemiological aspects of *P. multocida* and *M. haemolytica* infections in Egypt, proper diagnosis of *P. multocida* and *M. haemolytica* infections, describe the distribution of capsular types of *P. multocida* isolates and measure the prevalence of multi-drug resistance in bacterial isolates recovered from cattle, buffaloes, sheep, and goats (ruminants) suffering from respiratory manifestations. A total of 155 deep nasal swabs were collected from 20 cattle, 37 buffaloes, 80 sheep, and 18 goats. Detection of 24 *P. multocida* and 12 *M. haemolytica* isolates from tested samples was carried out by bacteriological isolation, then identified by biochemical tests, and confirmed by polymerase chain reaction (PCR). The highest rate of infection with *P. multocida* and *M. haemolytica* has been found in young males (0-6 months age group). *P. multocida* capsular group A was found in the majority of the *P. multocida* strains (87.5%), while group D bacteria were identified in only three samples. Capsular groups B, E, and F have not been detected. The antimicrobial susceptibility pattern of *P. multocida* and *M. haemolytica* isolates indicated a high prevalence of multi-resistance to the majority of antimicrobials used as high resistance was detected against ampicillin, amoxicillin, penicillin-G, tetracycline, streptomycin, cefotaxime and chloramphenicol, however, 100% sensitivity was demonstrated by *M. haemolytica* isolates to gentamicin. Therefore, continuous monitoring of antimicrobial resistance is important to prevent the dissemination of resistant bacteria.

Keywords | Antimicrobial resistance, Capsular groups, *M. haemolytica*, *P. multocida*, Ruminants

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INTRODUCTION

Pneumonic pasteurellosis is among the most economically important infectious diseases of ruminants with a high prevalence all over the world. *P. multocida* and *M. haemolytica* are the causative agents of the disease. They are commensals in the upper respiratory tract of clinically healthy ruminants, they can gain access to the lungs and are able to induce disease in animals with impaired pulmonary defense mechanisms. Physical

or physiological stress induced by unfavourable climatic and ecological conditions, including severely bad weather, improper management, co-mingling, overcrowding, shipping or prior infection with mycoplasma, respiratory viruses or certain other pathogens, may therefore contribute to pneumonic pasteurellosis (Tadesse *et al.*, 2017).

The acute febrile course of pneumonic pasteurellosis is characterized by death of infected animals within few days after the onset of clinical signs if they are not properly

diagnosed and treated, while animals that withstand the acute attack can get chronically infected (Tadesse et al., 2017; Jesse et al., 2019).

The animal breed, sex, age, and season can be effective in the occurrence of pasteurellosis (Karimkhani et al., 2011).

In 1881, Louis Pasteur showed that *P. multocida* was the cause of fowl cholera. Since then, it has been known that several other economically significant diseases in different animal species are caused by this Gram-negative bacteria (Harper et al., 2006). *P. multocida* is the cause of pneumonic pasteurellosis in cattle, water buffaloes, sheep, and goats (E-Kobon et al., 2017; Homayoon et al., 2018) and haemorrhagic septicemia in cattle and buffaloes (Quinn et al., 2002). Five *P. multocida* capsular serogroups (A, B, D, E and F) and 16 somatic serotypes (1 to 16) have been identified by serotyping techniques. *P. multocida*-PCR and capsular PCR are effective methods for early identification as well as serogrouping of *P. multocida* isolates, particularly in epidemiological studies compared to traditional serogrouping methods, which is time-consuming and involves the development and maintenance of a hyperimmune sera battery (Townsend et al., 2001; Kumar et al., 2009).

M. haemolytica formerly *P. haemolytica* biotype A comprises serotypes 1, 2, 5-9, 12-14, 16, and 17 (Angen et al., 1999). Serotyping methods are not specific enough for *M. haemolytica* reliable identification (Jaramillo-Arango et al., 2007). *M. haemolytica* was known to be the most significant pathogen causing severe pneumonia in bovine respiratory disease (Confer and Ayalew, 2018) and ovine pneumonic pasteurellosis (Tabatabaei and Abdollahi, 2018).

The most effective method for managing *Pasteurella* and *Mannheimia* infections is using antimicrobial agents. However, imprudent antimicrobial usage dramatically increases the risk of selecting resistant bacteria, enabling the dissemination of resistance genes located on plasmids and transposons, thereby reducing the effectiveness of presently approved antimicrobials for the treatment of food-producing animals (Kehrenberg et al., 2001).

The purpose of this study was to explore certain aspects of *P. multocida* and *M. haemolytica* epidemiology, diagnosis of *P. multocida* and *M. haemolytica* infections using bacterial isolation and identification, apply molecular diagnosis of *P. multocida* and *M. haemolytica*, describes the distribution of capsular types of *P. multocida* isolates and measures the prevalence of multi-drug resistance in bacterial isolates recovered from cattle, buffaloes, sheep, and goats suffering from respiratory manifestations.

ETHICAL APPROVAL

Animal ethical approval was obtained from the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Cairo University.

ANIMALS AND SAMPLES

A total of 155 deep nasal swabs were collected from 20 cattle, 37 buffaloes, 80 sheep, and 18 goats of different ages and sexes from different localities in the Giza Governorate during the period from October 2018 to December 2019. The animals were suffering from respiratory manifestations.

CLINICAL EXAMINATION

Animals showed clinical signs of coughing and copious nasal discharge in association with rectal temperatures above 39.5°C, congested mucous membranes, and respiratory discomfort were examined.

EPIDEMIOLOGICAL STUDIES

Prevalence of *P. multocida* and *M. haemolytica* isolation in the examined animals was reported by species, ages, sexes, and season of the year.

ISOLATION AND IDENTIFICATION OF *P. MULTOCIDA* AND *M. HAEMOLYTICA*

Sterile cotton swabs were used to collect the deep nasal swab samples from the nostrils of affected animals. After sampling, the tips of each nasal swab were placed into individual tubes containing Amies transport medium (Oxoid, UK) and immediately transported on ice to the laboratory. Upon arrival at the laboratory, the swabs were removed from the transport media and placed individually into brain heart infusion (BHI) broth (Oxoid, UK) and incubated aerobically at 37°C for 6-8 hours.

A loopful from the broth was streaked onto blood agar media supplemented with 7% defibrinated sheep blood and MacConkey agar media (Oxoid, UK) and incubated aerobically for 24 hours at 37°C. The suspected isolates of *P. multocida* and *M. haemolytica* were biochemically tested and microscopically examined after staining with Gram's stains (Quinn et al., 2002).

MOLECULAR IDENTIFICATION OF *P. MULTOCIDA* AND *M. HAEMOLYTICA*

Whole cells obtained from single colonies grown on 7% sheep blood agar were used for DNA extraction as the template in the amplification reactions. DNA was extracted from bacterial cultures with the QIAamp DNA Mini Kit (Qiagen GmbH, Germany).

For identification of *P. multocida* (*KMT1* gene) and *M.*

haemolytica (*Rpt2* gene), a uniplex PCR assay was applied for each species separately in a 25 µl PCR amplification mixture containing 12.5 µl of 2X *Taq* PCR Master Mix (Qiagen, Germany), 1 µl of each primer (Metabion company, Germany), 5 µl of DNA template and 5.5 µl of PCR grade water (Jena Bioscience, Germany).

The amplification condition was applied according to (Townsend et al., 2001) for the identification of *P. multocida* and according to (Deressa et al., 2010) for the identification of *M. haemolytica*.

MOLECULAR CAPSULAR SEROGROUPING OF *P. MULTOCIDA* STRAINS

All genomic DNA of the identified *P. multocida* strains were subsequently PCR tested for different capsular serogroups A, B, D, E and F based on capsule biosynthesis genes (*hyaD-hyaC*, *bcbD*, *dcbF*, *ecbJ*, and *fcfD*, respectively) using multiplex PCR according to (Townsend et al., 2001). The PCR reaction was performed in a final volume of 50 µl PCR amplification mixture using 25 µl of 2X *Taq* PCR Master Mix, 1 µl of each primer, 10 µl of DNA template, and 5 µl of PCR grade water.

CONFIRMATORY UNIPLEX PCR

Based on the findings of the multiplex PCR, the existence of certain capsular serogroups in the isolates under investigation was confirmed by uniplex PCR according to (Townsend et al., 2001). All amplifications were performed with the (Biometra, Germany) thermal cycler. Target genes, oligonucleotide primer sequences, the expected product size in different PCR assays with references are illustrated in Table 1.

Gel Analysis of the PCR Products: Aliquots of 7µL of the

amplified PCR products were separated by electrophoresis on 1.5% agarose gel stained with ethidium bromide in 1x TAE EDTA [pH 8.0] buffer at room temperature. Finally, results were visualized under UV light and documented with a GelDoc 1000 fluorescent imaging system (Bio-Rad).

ANTIMICROBIAL RESISTANCE TESTING

Antimicrobial resistance test was conducted on *P. multocida* and *M. haemolytica* isolates using the Kirby Bauer disk diffusion method (Bauer et al., 1966). The isolates were tested to 12 different antimicrobials across 7 antimicrobial classes: (I) beta lactams (ampicillin, amoxicillin, penicillin-G); (II) tetracyclines (tetracycline); (III) fluoroquinolones (ciprofloxacin, enrofloxacin); (IV) aminoglycosides (gentamicin, spectinomycin, streptomycin); (V) cephalosporins (cefotaxime); (VI) folate pathway antagonists (trimethoprim-sulfamethoxazole), (VII) phenicols (chloramphenicol). The interpretations were carried out according to (NCCLS, 2002; CLSI, 2006, 2008).

RESULTS

EPIDEMIOLOGICAL STUDIES

Isolation findings for *P. multocida* and *M. haemolytica* in the examined cattle, buffaloes, sheep, and goats regarding sex, season, and age are shown in Tables 2 and 3.

ISOLATION AND IDENTIFICATION OF *P. MULTOCIDA* AND *M. HAEMOLYTICA*

On blood agar, *P. multocida* appeared as moderate size, round, greyish mucoid colonies, non-haemolytic, and has a characteristic sweetish odour while *M. haemolytica* is beta-haemolytic and has no odour. On MacConkey agar, *M. haemolytica* grew as pinpoint red colonies while *P. multocida* did not grow on MacConkey agar.

Table 1: Primer sequences for detection of *P. multocida* and *M. haemolytica* genes.

Bacterial species	Gene	Name	PCR primer sequence (5'-3')	Amplicon size (bp)	References
<i>P. multocida</i>	<i>KMT1</i>	<i>KMT1T7</i>	ATCCGCTATTTACCCAGTGG	460	(Townsend et al., 2001)
		<i>KMT1SP6</i>	GCTGTAACGAACCTCGCCAC		
	<i>hyaD-hyaC</i>	CAPA-FWD	TGCCAAAATCGCAGTCAG	1044	
		CAPA-REV	TTGCCATCATTTGTCAGTG		
	<i>bcfD</i>	CAPB-FWD	CATTTATCCAAGCTCCACC	760	
		CAPB-REV	GCCCCGAGAGTTTCAATCC		
<i>dcbF</i>	CAPD-FWD	TTACAAAAGAAAGACTAGGAGCCC	657		
	CAPD-REV	CATCTACCCACTCAACCATATCAG			
<i>ecbJ</i>	CAPE-FWD	TCCGCAGAAAATTATTGACTC	511		
	CAPE-REV	GCTTGCTGCTTGATTTTGTC			
<i>fcfD</i>	CAPF-FWD	AATCGGAGAACGCAGAAATCAG	851		
	CAPF-REV	TTCCGCCGTCAATTACTCTG			
<i>M. haemolytica</i>	<i>Rpt2</i>	<i>Rpt2</i> -FWD	GTTTGTAAGATATCCCATTT	1022	(Ryan and Lo, 1999; Deressa et al., 2010)
		<i>Rpt2</i> -REV	CGTTTCCACTTGCGTGA		

Table 2: Number and percentage of positive *P. multocida* samples in the examined animals regarding sex, season and age.

Species	Sex		Season		Age				Total	
	Male	Female	Winter	Summer	0-6 m	7-12 m	13-24 m	>24 m	No.	Percentage
Cattle	6/11	1/9	4/14	3/6	5/5	0/3	1/8	1/4	7/20	35%
Buffaloes	6/23	0/14	5/5	1/32	6/12	-	0/15	0/10	6/37	16.22%
Sheep	6/54	5/26	11/75	0/5	-	-	11/79	0/1	11/80	13.75%
Goats	0/11	0/7	0/15	0/3	-	-	0/18	-	0/18	-
Total	18/99	6/56	20/109	4/46	11/17	0/3	12/120	1/15	24/155	15.48%
Percentage	18.18%	10.7%	18.35%	8.69%	64.7%	-	10%	6.66%	15.48%	-

Table 3: Number and percentage of positive *M. haemolytica* samples in the examined animals regarding sex, season and age.

Species	Sex		Season		Age				Total	
	Male	Female	Winter	Summer	0-6 m	7-12 m	13-24m	>24 m	No.	Percentage
Cattle	4/11	2/9	5/14	1/6	0/5	1/3	5/8	0/4	6/20	30%
Buffaloes	5/23	1/14	0/5	6/32	6/12	-	0/15	0/10	6/37	16.22%
Sheep	0/54	0/26	0/75	0/5	-	-	0/79	0/1	0/80	-
Goats	0/11	0/7	0/15	0/3	-	-	0/18	-	0/18	-
Total	9/99	3/56	5/109	7/46	6/17	1/3	5/120	0/15	12/155	7.74%
Percentage	9.09%	5.36%	4.59%	15.22%	35.29%	33.33%	4.17%	-	7.74%	-

Table 4: Antimicrobial resistance test result of the *P. multocida* and *M. haemolytica* isolates.

Antimicrobials tested	<i>M. haemolytica</i>			<i>P. multocida</i>		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Ampicillin	-	-	12(100%)	-	-	24(100%)
Amoxicillin	-	-	12(100%)	-	-	24(100%)
Penicillin-G	-	-	12(100%)	-	-	24(100%)
Tetracycline	1(8.3%)	1(8.3%)	10(83.3%)	-	-	24(100%)
Ciprofloxacin	7(58.3%)	2(16.7%)	3(25%)	7(29.2%)	2(8.3%)	15(62.5%)
Enrofloxacin	6(50%)	1(8.3%)	5(41.7%)	3(12.5%)	3(12.5%)	18(75%)
Gentamicin	12(100%)	-	-	8(33.3%)	2(8.3%)	14(58.3%)
Spectinomycin	5(41.7%)	3(25%)	4(33.3%)	5(20.8%)	12(50%)	7(29.2%)
Streptomycin	1(8.3%)	2(16.7%)	9(75%)	1(4.2%)	1(4.2%)	22(91.6%)
Cefotaxime	3(25%)	2(16.7%)	7(58.3%)	1(4.2%)	2(8.3%)	21(87.5%)
Trimethoprim-Sulfamethoxazole	10(83.3%)	-	2(16.7%)	6(25%)	8(33.3%)	10(41.7%)
Chloramphenicol	3(25%)	2(16.7%)	7(58.3%)	6(25%)	-	18(75%)

On TSI agar, both *P. multocida* and *M. haemolytica* fermented all sugars and produced yellow slant and butt without H₂S or gas production. In the Indole reaction, *P. multocida* gave red ring at the interface while *M. haemolytica* gave yellow ring. Both *P. multocida* and *M. haemolytica* are catalase, oxidase-positive, and citrate negative.

Both *P. multocida* and *M. haemolytica* appeared as small, Gram-negative rods or coccobacilli in Gram's stained slides.

The frequency of micro-organism detection in this study

was 15.48 % (24/155) for *P. multocida* and 7.74 % (12/155) for *M. haemolytica*.

MOLECULAR IDENTIFICATION OF *P. MULTOCIDA* AND *M. HAEMOLYTICA*

PCR using the *KMT1* gene confirmed the 24 culture-positive *P. multocida* isolates, which developed specific 460 bp molecular size bands (Figure 1). Also, the 12 culturally positive *M. haemolytica* isolates have been confirmed using the *Rpt2* gene, which developed specific 1022 bp molecular size bands (Figure 2).

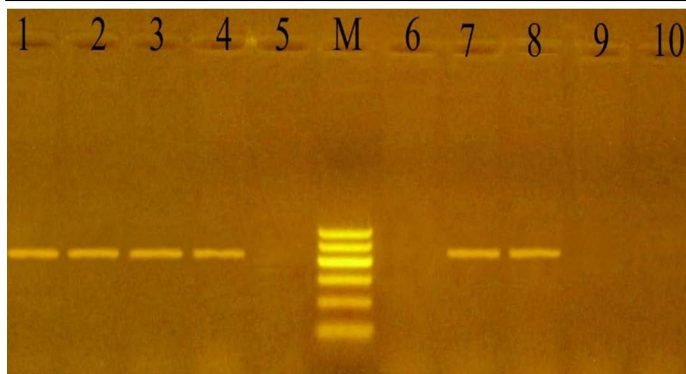


Figure 1: PCR for detection of *Kmt1* gene in *P. multocida* isolates at amplicon size 460 bp. Lane M: Molecular weight marker, 100-600 bp (100bp plus DNA ladder, Vivantis, Malaysia). Lanes 1-4 and 7-8: positive samples. Lanes 5, 6 and 9-10: negative samples.

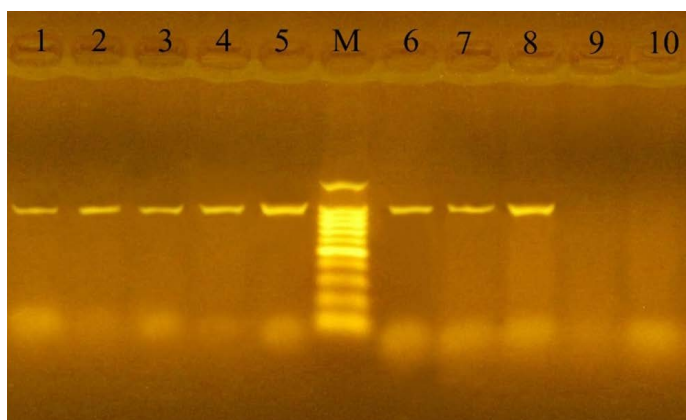


Figure 2: PCR for detection of *Rpt2* gene in *M. haemolytica* isolates at amplicon size 1022 bp. Lane M: Molecular weight marker, 100-1500 bp (100bp plus DNA ladder, Vivantis, Malaysia). Lanes 1-8: positive samples. Lanes 9-10: negative samples.

MOLECULAR CAPSULAR SEROGROUPING OF *P. MULTOCIDA* STRAINS

Capsular serogrouping of the 24 confirmed *P. multocida* strains revealed that 21 out of the 24 strains belonged to serogroup A (11 from sheep, 7 from cattle, and 3 from buffaloes) with an approximate molecular size of 1044 bp (Figure 3). The isolation percentages of *P. multocida* serogroup A were, therefore, estimated to be 87.5%. The other 3 *P. multocida* isolates (isolated from buffaloes) belonged to serogroup D with an approximate molecular size of 657 bp (Figure 4). The isolation percentages of *P. multocida* serogroup D were, therefore, estimated to be 12.5%. Serogroups B, E, and F have not been identified in the isolated *P. multocida*.

ANTIMICROBIAL RESISTANCE TESTING

P. multocida (n= 24) and *M. haemolytica* (n= 12) isolates were subjected to a panel of 12 antimicrobials. The antimicrobial resistance pattern of the isolates indicated a high prevalence of multi-resistance to the majority of antimicrobials

commonly used to control or treat respiratory diseases as illustrated in Table 4.

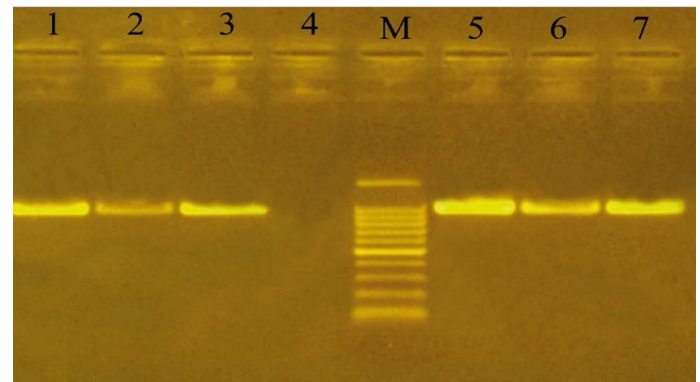


Figure 3: Uniplex PCR for detection of cap-A (*hyaD-hyaC* gene) in *P. multocida* isolates at amplicon size 1044 bp. Lane M: Molecular weight marker, 100-1500 bp. Lanes 1-3 and 5-7, positive samples. Lane 4: negative sample.

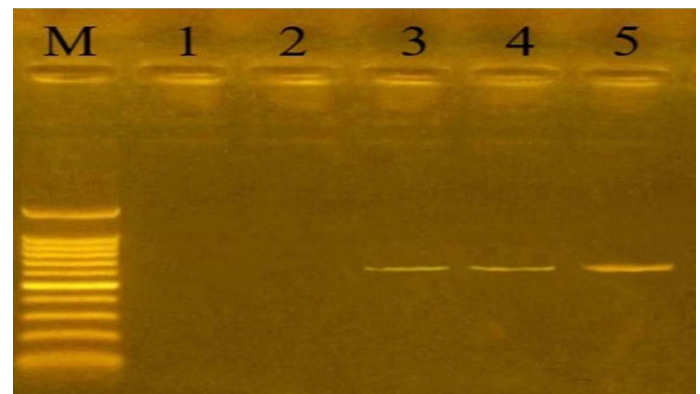


Figure 4: Uniplex PCR for detection of cap-D (*dcbF* gene) in *P. multocida* isolates at amplicon size 657 bp. Lane M: Molecular weight marker, 100-1500 bp. Lanes 1-2: negative samples. Lanes 3-5: positive samples.

For *M. haemolytica*, 100% of the isolates were resistant to ampicillin, amoxicillin, and penicillin-G. Resistance to tetracycline (83.3%) and streptomycin (75%), followed by cefotaxime and chloramphenicol (58.3%) while they were 100% sensitive to gentamycin followed by 83.3%, 58.3% and 50% sensitivity to trimethoprim-sulfamethoxazole, ciprofloxacin, and enrofloxacin respectively.

Similarly, *P. multocida* isolates showed 100% resistance to ampicillin, amoxicillin, penicillin-G, and tetracycline. Resistance to streptomycin (91.6%), cefotaxime (87.5%), and (75%) of the isolates were resistant to both enrofloxacin and chloramphenicol.

DISCUSSION

Pneumonic pasteurellosis is a very complex multifactorial disease with a widespread prevalence in ruminants. It is often caused by a combination of stress, immunity, and the causative bacteria (*P. multocida* and *M. haemolytica*)

that commensally occur in the upper respiratory system of susceptible animals (Tadesse et al., 2017).

This study used 155 deep nasal swabs for isolation, identification, and detection of antimicrobial resistance of *P. multocida* and *M. haemolytica* which were obtained from cattle, buffaloes, sheep, and goats in Egypt; *P. multocida* was isolated from 24 samples and *M. haemolytica* was isolated from 12 samples.

The findings of this study revealed that *P. multocida* was more prevalent in samples (15.48 %) compared to *M. haemolytica* (7.74 %); (El-Seedy et al., 2019) has reported similar results in Egypt, probably the lack of effective vaccines, contribute to the increased prevalence of *P. multocida* (Guo et al., 2020).

P. multocida is an important micro-organism related to pneumonia in cattle. This study has isolated *P. multocida* from 35% of the samples collected from cattle in accordance with (França Dias de Oliveira et al., 2016) who found that *P. multocida* (15.50 %) was the most prevalent bacterial species in healthy and unhealthy cattle suffering from respiratory diseases and not isolated *M. haemolytica*. Also (Nefedchenko et al., 2016) analysis revealed 50% of *P. multocida* and 11.2% of *M. haemolytica* in all examined samples of biological material from the infected animals.

In our study, *M. haemolytica* has not been isolated from sheep which is inconsistent with (Legesse et al., 2018) who demonstrated that *M. haemolytica* has been correlated with one-third of pneumonic cases suggesting its major role in sheep pneumonia in central Ethiopia, which may be due to annual vaccination using a monovalent vaccine (inactivated *P. multocida* biotype A) there.

No isolation of either *P. multocida* or *M. haemolytica* was carried out from goats in contrast to (Rawat et al., 2019) who identified (7) *M. haemolytica* and (5) *P. multocida* isolates out of 10 clinical cases of goat pneumonia.

Young animals especially (0-6 months age group) were found to be at risk for developing pneumonic pasteurellosis. This may be due to the fact that the animal's immune status is capable of predisposing to bacterial infection and many other predisposing etiological agents as mentioned by (Abera et al., 2014).

Colonization by *P. multocida* was high in the winter, this is because of environmental stresses such as cold weather and overcrowding as recorded by (Karimkhani et al., 2011) in contrary to *M. haemolytica* which is higher in the summer.

Males were found to be more vulnerable to respiratory tract infections than females as mentioned by (Karimkhani

et al., 2011). This may be because males are used for meat production, which renders them susceptible to the stress of transportation, the stress of repeated unloading, loading, handling, lack of food and water, exposure to inclement weather during transportation, and co-mingling with other animals from multiple sources that may be diseased or latent carrier of respiratory pathogens and shed these pathogens which can cause respiratory diseases in animals (Taylor et al., 2010).

The used PCR protocol identified the *P. multocida* and *M. haemolytica* isolates accurately, hence it should be used as an easy, available, and highly repeatable serological typing analogue in veterinary laboratories that enables the identification and genotyping of *P. multocida* and *M. haemolytica* strains at every step of bacteriological surveys and, thereby reduce the time needed to approach a diagnosis, making it possible to improve epizootic measures (Nefedchenko et al., 2016).

P. multocida serogroup A was present in the majority of samples (87.5%); in only three cases, group D bacteria were identified. Identification of the *P. multocida* bacteria of capsule group A in a larger number of samples can indicate that it played a more significant role in developing various diseases in domestic animals than the *P. multocida* bacteria of capsule group D did as mentioned by (Nefedchenko et al., 2016; Cucco et al., 2017). Bacterial distribution of *P. multocida* capsule groups B, E, and F among affected animals has not been determined.

In sheep, all *P. multocida* isolates are of capsular type A, this is similar to the results obtained by (Kumar et al., 2009) indicating the role of this capsular type in the development of sheep bronchopneumonia.

Data analyzed in the present study indicate that multiple antimicrobial resistance against *P. multocida* and *M. haemolytica* in cattle, buffaloes, and sheep has been established (Table 4) as stated by (Klima et al., 2014) who found that *M. haemolytica* and *P. multocida* isolates exhibited a high rate of antimicrobial resistance, with 45% exhibiting resistance to three or more antimicrobials. Antimicrobials impose selection pressure on bacterial populations that can contribute to the emergence of antimicrobial resistant organisms. Bacterial pre-exposure to antimicrobials was also involved as a risk factor for antimicrobial resistance evolution during subsequent antimicrobial treatments (Gould and MacKenzie, 2002).

The in-vitro sensitivity testing of *P. multocida* isolates showed 100% resistance to ampicillin, amoxicillin, penicillin-G, and tetracycline, and high resistance to streptomycin, cefotaxime, enrofloxacin, and chloramphenicol. Meanwhile, the in vitro antimicrobial susceptibility

testing of *M. haemolytica* isolates demonstrated 100% resistance to ampicillin, amoxicillin, and penicillin-G and high resistance to tetracycline, streptomycin, cefotaxime, and chloramphenicol. On the other hand, *M. haemolytica* isolates were 100% sensitive to gentamicin followed by 83.3%, 58.3%, and 50% sensitivity to trimethoprim-sulfamethoxazole, ciprofloxacin, and enrofloxacin respectively.

Tetracycline resistance was prevalent in our study in accordance with (El-Seedy et al., 2019; Guo et al., 2020), this may be due to increased administration of this drug for the treatment of respiratory diseases in animals. Resistance to aminoglycoside in *P. multocida* isolates is also documented by (Wang et al., 2017) who found that all of the isolates were resistant to at least two types of aminoglycosides.

Resistance to β -lactams among *P. multocida* and *M. haemolytica* isolates detected in this study was agreed with (El-Seedy et al., 2019) which is mostly mediated by β -lactam resistance plasmids (Livrelli et al., 1988; Schwarz et al., 1989).

Pasteurella infections are widely managed at the group level with fluoroquinolones and cephalosporins as important antibiotics in human medicine, leading to their overall use in food-producing animals (Vasseur et al., 2017) which contributes to the emergence of resistant strains against these antibiotics. Fluoroquinolone resistance can also be mediated by plasmid (Rodríguez-Martínez et al., 2011).

High resistance was detected against chloramphenicol which disagreed with (Choudhary et al., 2019) who demonstrated that 100% of *P. multocida* isolates were responsive to chloramphenicol. (Vassort-Bruneau et al., 1996) reported that chloramphenicol resistance is typically attributed to plasmids that encode inactivating enzymes; the chloramphenicol acetyltransferases.

All of *M. haemolytica* isolates were sensitive to gentamicin, likely due to decreased use of gentamicin in the treatment of animals and 83.3% of the isolates were sensitive to trimethoprim-sulfamethoxazole which is nearly similar to the results obtained by (De Jong et al., 2014).

CONCLUSION

In conclusion, the current investigation indicated that animal age and sex, along with the season of the year, play an important role in the occurrence of pneumonic pasteurellosis. The high prevalence of *P. multocida* serogroup A indicates its role in the pathogenesis of respiratory diseases in ruminants. This study provided unique information on resistance development in *P. multocida* and

M. haemolytica isolates to most of the antimicrobials used for the treatment of ruminants. Continuous monitoring of antimicrobial resistance is therefore important to assist practitioners in the appropriate selection of antimicrobial agents and the prudent use of these drugs. This also includes the development of new vaccines to effectively prevent pneumonic diseases in food-producing animals and thus reduce the overall use of antimicrobial agents.

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AUTHOR'S CONTRIBUTION

Amin MM and Salib FA designed the experiment protocol and the study. Bahr AD and Soliman YA collected and analyzed the samples. All authors were involved in data analysis, scientific discussion, and writing of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

- Abera D, Sisay T, Birhanu T (2014). Isolation and identification of *Mannheimia* and *Pasteurella* species from pneumonic and apparently healthy cattle and their antibiogram susceptibility pattern in Bedelle District, Western Ethiopia. *J. Bacteriol. Res.*, 6(5): 32-41.
- Angen O, Muters R, Caugant DA, Olsen JE, Bisgaard M (1999). Taxonomic relationships of the [*Pasteurella*] *haemolytica* complex as evaluated by DNA-DNA hybridizations and 16s rRNA sequencing with proposal of *Mannheimia haemolytica* gen. nov., comb. nov., *Mannheimia granulomatis* comb. nov., *Mannheimia glucosida* sp. nov., *Mannheimia ruminalis* sp. nov. and *Mannheimia varigena* sp. Nov. *Int. J. Syst. Bacteriol.*, 49: 67-86. <https://doi.org/10.1099/00207713-49-1-67>
- Bauer AW, Kirby WMM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 45(4): 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493
- Choudhary M, Choudhary BK, Ghosh RC, Bhojra S, Chaudhari S, Barbudhe SB (2019). Cultivable microbiota and pulmonary lesions in polymicrobial bovine pneumonia. *Microb. Pathog.*, 134: 103577. <https://doi.org/10.1016/j.micpath.2019.103577>
- CLSI (2006). Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement. *Clin. Lab. Stand. Inst.* 940 West Valley Road, Suite 1400, Wayne, Pennsylvania USA.
- CLSI (2008). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from

- animals. M100-S18. Clinical and Laboratory Standards Institute. Wayne, PA.
- Confer AW, Ayalew S (2018). *Mannheimia haemolytica* in bovine respiratory disease: immunogens, potential immunogens, and vaccines. *Anim. Health Res. Rev.*, 19(2): 79–99. <https://doi.org/10.1017/S1466252318000142>
 - Cucco L, Massacci FR, Sebastiani C, Mangili P, Bano L, Cocchi M, Luppi A, Ortenzi R, Pezzotti G, Magistrali CF (2017). Molecular characterization and antimicrobial susceptibility of *Pasteurella multocida* strains isolated from hosts affected by various diseases in Italy. *Vet. Ital.*, 53(1): 21–27.
 - De Jong A, Thomas V, Simjee S, Moyaert H, El Garch F, Maher K, Morrissey I, Butty P, Klein U, Marion H, Rigaut D, Valle M (2014). Antimicrobial susceptibility monitoring of respiratory tract pathogens isolated from diseased cattle and pigs across Europe: The Vet Path study. *Vet. Microbiol.*, 172(1–2): 202–215. <https://doi.org/10.1016/j.vetmic.2014.04.008>
 - Deressa A, Asfaw Y, Lubke B, Kyule MW, Tefera G, Zessin K-H (2010). Molecular detection of *Pasteurella multocida* and *Mannheimia haemolytica* in sheep respiratory infections in Ethiopia. *Int. J. Appl. Res. Vet. Med.*, 8(2):101–108.
 - E-kobon T, Leenan R, Pannoi S, Anuntasomboon P, Thongkamkoon P, Thamchaipenet A (2017). OmpA protein sequence-based typing and virulence-associated gene profiles of *Pasteurella multocida* isolates associated with bovine haemorrhagic septicaemia and porcine pneumonic pasteurellosis in Thailand. *BMC Vet. Res.*, 13(1): 243. <https://doi.org/10.1186/s12917-017-1157-6>
 - El-Seedy FR, Abed AH, Hassan HM, Nabih AM, Khalifa E, Salem SE (2019). Antimicrobial and immunological studies on *Pasteurella multocida* and *Mannheimia haemolytica* recovered from calves affected with respiratory manifestations. *J. Vet. Med. Res.*, 26(1): 55–63.
 - França Dias de Oliveira BA, Gaeta NC, Ribeiro BLM, Alemán MAR, Marques LM, Timenetsky J, Melville PA, Marques JA, Marville V, Gregory L (2016). Determination of bacterial aetiologic factor on tracheobronchial lavage in relation to clinical signs of bovine respiratory disease. *J. Med. Microbiol.*, 65(10): 1137–1142. <https://doi.org/10.1099/jmm.0.000345>
 - Gould IM, MacKenzie FM (2002). Antibiotic exposure as a risk factor for emergence of resistance: the influence of concentration. *Symp. Ser. Soc. Appl. Microbiol.*, 92: 78S–84S. <https://doi.org/10.1046/j.1365-2672.92.5s1.10.x>
 - Guo Y, McMullen C, Timsit E, Hallewell J, Orsel K, Meer FVD, Yan S, Alexander TW (2020). Genetic relatedness and antimicrobial resistance in respiratory bacteria from beef calves sampled from spring processing to 40 days after feedlot entry. *Vet. Microbiol.*, 240: 108478. <https://doi.org/10.1016/j.vetmic.2019.108478>
 - Harper M, Boyce JD, Adler B (2006). *Pasteurella multocida* pathogenesis: 125 years after Pasteur. *FEMS Microbiol. Lett.*, 265(1): 1–10. <https://doi.org/10.1111/j.1574-6968.2006.00442.x>
 - Homayoon M, Tahamtan Y, Kargar M, Hosseini SMH, Sepahy AA (2018). *Pasteurella multocida* inactivated with ferric chloride and adjuvanted with bacterial DNA is a potent and efficacious vaccine in Balb/c mice. *J. Med. Microbiol.*, 67(9): 1383–1390. <https://doi.org/10.1099/jmm.0.000794>
 - Jaramillo-Arango CJ, Hernández-Castro R, Campuzano-Ocampo V, Suárez-Güemes F, Delgado-González R, Trigo-Tavera F (2007). Characterization of *Mannheimia sp.* and *P. multocida* strains isolated from bovine pneumonic lungs in two slaughterhouses in Mexico. *J. Anim. Vet. Adv.*, 6(12): 1398–1404.
 - Jesse FFA, Amira NA, Isa KM, Maqbool A, Ali NM, Chung ELT, Lila MAM (2019). Association between *Mannheimia haemolytica* infection with reproductive physiology and performance in small ruminants: A review. *Vet. World*, 12(7): 978–983. <https://doi.org/10.14202/vetworld.2019.978-983>
 - Karimkhani H, Salehi TZ, Zali MHS, Karimkhani M, Lameyi R (2011). Isolation of *pasteurella multocida* from cows and buffaloes in Urmia's slaughter house. *Arch. Razi Inst.*, 66(1): 37–41.
 - Kehrenberg C, Schulze-Tanzil G, Martel J-L, Chaslus-Dancla E, Schwarz S (2001). Antimicrobial resistance in *Pasteurella* and *Mannheimia*: Epidemiology and genetic basis. *Vet. Res.*, 32(3–4): 323–339. <https://doi.org/10.1051/vetres:2001128>
 - Klima CL, Zaheer R, Cook SR, Booker CW, Hendrick S, Alexander TW, McAllister TA (2014). Pathogens of bovine respiratory disease in north american feedlots conferring multidrug resistance via integrative conjugative elements. *J. Clin. Microbiol.*, 52(2): 438–448. <https://doi.org/10.1128/JCM.02485-13>
 - Kumar P, Singh VP, Agrawal RK, Singh S (2009). Identification of *Pasteurella multocida* isolates of ruminant origin using polymerase chain reaction and their antibiogram study. *Trop. Anim. Health Prod.*, 41(4): 573–578. <https://doi.org/10.1007/s11250-008-9226-2>
 - Legesse A, Abayneh T, Mamo G, Gelaye E, Tesfaw L, Yami M, Belay A (2018). Molecular characterization of *Mannheimia haemolytica* isolates associated with pneumonic cases of sheep in selected areas of Central Ethiopia. *BMC Microbiol.*, 18: 205. <https://doi.org/10.1186/s12866-018-1338-x>
 - Livrelli VO, Darfeuille-Richaud A, Rich CD, Joly BH, Martel JL (1988). Genetic determinant of the ROB-1 β -lactamase in bovine and porcine *Pasteurella* strains. *Antimicrob. Agents Chemother.*, 32(8): 1282–1284. <https://doi.org/10.1128/AAC.32.8.1282>
 - NCCLS (2002). Performance Standards for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals: Approved standard, second ed., National Committee for Clinical Laboratory Standards. M31-A2, Wayne, USA.
 - Nefedchenko AV, Shikov AN, Glotov AG, Glotova TI, Ternovoy VA, Agafonov AP, Sergeev AN, Donchenko NA (2016). Development of a method for identification and genotyping of *Pasteurella multocida* and *Mannheimia haemolytica* bacteria using polymerase chain reaction and phylogenetic analysis of bacterial cultures isolated from Cattle. *Mol. Gen. Microbiol. Virol.*, 31(2): 75–81. <https://doi.org/10.3103/S0891416816020063>
 - Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC (2002). *Veterinary microbiology and microbial diseases*, 2nd Ed., Blackwell Science Ltd., UK, pp. 137–143.
 - Rawat N, Gilhare VR, Kushwaha KK, Hattimare DD, Khan FF, Shende RK, Jolhe DK (2019). Isolation and molecular characterization of *Mannheimia haemolytica* and *Pasteurella multocida* associated with pneumonia of goats in Chhattisgarh. *Vet. World*, 12(2): 331–336. <https://doi.org/10.14202/vetworld.2019.331-336>
 - Rodríguez-Martínez JM, Cano ME, Velasco C, Martínez-Martínez L, Pascual Á (2011). Plasmid-mediated quinolone resistance: an update. *J. Infect. Chemother.*, 17(2): 149–182. <https://doi.org/10.1007/s10156-010-0120-2>

- Ryan KA, Lo RYC (1999). Characterization of a CACAG pentanucleotide repeat in *Pasteurella haemolytica* and its possible role in modulation of a novel type III restriction-modification system. *Nucl. Acids Res.*, 27(6): 1505–1511. <https://doi.org/10.1093/nar/27.6.1505>
- Schwarz S, Spies U, Reitz B, Seyfert H-M, Lämmner C, Blobel H (1989). Detection and interspecies-transformation of a β -lactamase-encoding plasmid from *Pasteurella haemolytica*. *Zentralbl. Bakteriol. Mikrobiol. Hyg., A* 270(4): 462- 469. [https://doi.org/10.1016/S0176-6724\(89\)80017-3](https://doi.org/10.1016/S0176-6724(89)80017-3)
- Tabatabaei M, Abdollahi F (2018). Isolation and identification of *Mannheimia haemolytica* by culture and polymerase chain reaction from sheep's pulmonary samples in Shiraz, Iran. *Vet. World*, 11(5): 636–641. <https://doi.org/10.14202/vetworld.2018.636-641>
- Tadesse B, Alamirew K, Ketema A, Kiflie W, Endashaw M (2017). Ruminant Pneumonic Pasteurellosis: Review on Epidemiology, Pathogenesis and Virulence Mechanism. *Acad. J. Anim. Dis.*, 6(2): 30-39.
- Taylor JD, Fulton RW, Lehenbauer TW, Step DL, Confer AW (2010). The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors? *Can. Vet. J.*, 51(10): 1095–1102.
- Townsend KM, Boyce JD, Chung JY, Frost AJ, Adler B (2001). Genetic organization of *Pasteurella multocida cap* Loci and development of a multiplex capsular PCR typing system. *J. Clin. Microbiol.*, 39(3): 924–929. <https://doi.org/10.1128/JCM.39.3.924-929.2001>
- Vasseur MV, Lacroix MZ, Toutain P-L, Bousquet-Melou A, Ferran AA (2017). Infection stage adjusted dose of beta-lactams for parsimonious and efficient antibiotic treatments: A *Pasteurella multocida* experimental pneumonia in mice. *PLoS One*, 12(8): e0182863. <https://doi.org/10.1371/journal.pone.0182863>
- Vassort-Bruneau C, Lesage-Descauses MC, Martel JL, Lafont JP, Chaslus-Dancla E (1996). CAT III chloramphenicol resistance in *Pasteurella haemolytica* and *Pasteurella multocida* isolated from calves. *J. Antimicrob. Chemother.*, 38(2): 205-213. <https://doi.org/10.1093/jac/38.2.205>
- Wang Z, Kong L, Jia B, Liu S, Jiang X, Ma H (2017). Aminoglycoside susceptibility of *Pasteurella multocida* isolates from bovine respiratory infections in China and mutations in ribosomal protein S5 associated with high-level induced spectinomycin resistance. *J. Vet. Med. Sci.*, 79(10): 1678–1681. <https://doi.org/10.1292/jvms.17-0219>