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PHENOTYPIC AND GENETIC CHARACTERIZATION OF *PASTEURELLA MULTOCIDA* WITH A REFERENCE TO ITS PATHOLOGICAL CHANGES IN CALVES

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ABSTRACT

This study investigated the presence of *Pasteurella multocida* in Egyptian dairy farm calves with respiratory symptoms. A total of 150 samples were collected aseptically from 133 nasal swabs and 17 pneumonic lung tissues of calves on private farms across different governorates. Samples were tested using three methods: standard bacteriological examination, biochemical identification, and Molecular characterization for *P. multocida*. The *P. multocida* isolates were subjected to multiplex PCR for capsule biosynthesis and 16S rRNA gene. Capsular typing revealed that 60 out of 150 samples (40%) were positive for *P. multocida*. All isolates confirmed as *P. multocida* through PCR were belonged to serogroup A. Antibiotic sensitivity testing of these isolates identified enrofloxacin as the most effective drug. The most detectable resistance gene was *sul II*- in examined isolates with a 100% detection rate, followed by Bla_{TEM} and *tet*A genes (62.5%). Histopathological examination revealed extensive damage across various organs, such as the lungs of calves, including fibrinous exudate inside alveoli and purulent fluid in other alveoli, besides thrombosis of blood vessels in *P. multocida*-infected animals.

Keywords: Pasturella multocida: Molecular Characterization: Serotyping: Histopathology.

INTRODUCTION

P. multocida infection in dairy calves is a major problem. It can cause a wide range of infections in domestic animals and humans, and can even be lethal due to the presence of endotoxins in the blood (Milanov *et al.*, 2017). Antimicrobial treatment is commonly used to control *P. multocida* infections, but the emergence of multidrug-resistant strains is a concern (Becker *et al.*, 2022). The prevalence of *P. multocida* in dairy calves was high, and the bacteria can be detected in the feces of calves less than 24 hours old (Eldesoukey *et al.*, 2022). The close relatedness of *P. multocida* isolates within farms and between farms suggests that the farm-to-farm trade of calves can contribute to the spread of the infection (Bandelj *et al.*, 2018) Therefore, better hygiene and management measures on farms, as well as

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trading older calves, may help decrease the risk of *P. multocida* infection in dairy calves and its dissemination in the community.

P. multocida infection in calves can manifest in different types. One type is pneumonia, which is a common respiratory disease in calves, and can lead to significant economic losses in the cattle industry due to high morbidity and mortality rates. *P. multocida* is often associated with severe infections in calves, causing respiratory manifestations such as fever, nasal discharges, and rapid breathing (Ahmed *et al.,* 2020). Another type of *P. multocida* infection is bovine respiratory disease (BRD), which affects beef cattle calves.

The cranial and middle lobes were affected on both sides of the lungs. They were severely congested, consolidated, and firm in consistency (Praveena *et al.,* 2014).

Previous studies have shown that Pasteurellosis infection in cattle causes pathological alterations in the lungs of infected animals. Fibrinous and suppurative bronchopneumonia was the most common histopathological lesions in pasteurellosis-infected animals (Sharma *et al.,* 2011 and Amin, 2020). broncho-interstitial, mucopurulent, and fibrinous pneumonia were reported (Praveena *et al.,* 2014). Lung lesions consist of acute to subacute bronchopneumonia that may or may not have an associated pleuritis (Dabo *et al.,* 2007).

P. multocida serogroup A-3 is the most common serotype isolated from BRD, and these isolates have limited heterogeneity based on outer membrane protein (OMP) profiles and ribotyping (Abdullah *et al.,* 2013). Overall, *P. multocida* can cause pneumonia and respiratory disease in calves, leading to significant health and economic impacts in the cattle industry.

The present study aims to characterize the phenotypic and genotypic characteristics of *P. multocida* from pneumonic calves in Egyptian dairy farms, focusing on the phylogenetic relations of Egyptian strains to referral strains worldwide.

MATERIALS AND METHODS:

Sample collection:

A total of 150 samples were collected from calves with clinical signs of respiratory illness, including nasal discharge, sneezing, coughing, and conjunctivitis. Sampling occurred on private farms within three Egyptian governorates: Giza, Menofia, Behera, Sharkia, and Ismaelia. Sample was obtained aseptically from individual diseased calves, primarily using nasal swabs and pneumonic lung tissue of dead calves. Samples were collected in sterile containers, labeled, and transported on ice in plastic bags. They were delivered promptly to the microbiology laboratory at the Animal Health Research Institute (AHRI) in Mansoura.

Antimicrobial susceptibility testing:

Antimicrobial susceptibility of all isolates was determined using disk diffusion according to CLSI guidelines (CLSI, 2020) performed against the following antibiotics: ampicillin (10 µg) (AMP), amoxicillin /clavulanic acid (20/10µg) (AMC), gentamicin (10µg) (CN), tetracycline (TE), enrofloxacin (10µg) (ENR), and trimethoprim/sulfamethoxazole (SXT) (1.25/ 23.75 µg). The isolates and antibiotic discs were placed on MH agar and subsequently incubated for approximately 24 h at 37°C. Following incubation, the circular growth inhibition zones (in millimeters, mm) were measured with a manual calliper.

Genomic DNA extraction:

Two loops of bacterial strains were inoculated in 5 ml nutrient broth and incubated for 24 hours at 120 rpm at 37 °C. The bacterial precipitate was suspended in 200µL of TE buffer. The suspension was incubated for 10min at 56 °C and then for 10min at 95 °C before being spun at 16000×g for 2min. After centrifugation, 5µl of the supernatant was used as a template in

a 50 µl PCR reaction (Hamza *et al.,* 2020).

Capsular typing of *P. multocida*:
Capsular typing was done via Polymerase Chain Reaction (PCR). The detection of capsular genes by PCR for all serogroups was done according to the method described by Townsend *et al.* (2001).PCR condition and amplicon size are mentioned in Table (1).

Table 1 shows the capsular typing PCR primers and amplification conditions, according to Townsend *et al.* (2001).

Antimicrobial resistance genes of *P. multocida*:
The bacterial isolates were screened for antibiotic resistance determinants about obtained antimicrobial resistance patterns. The resistant isolates were screened for the presence of AMR genes, including genes represented to ampicillin (bla_{TEM}), tetracycline (*tetA*), and sulfamethoxazole (*sul II*) (Table 1). Conventional PCR was performed to detect most AMR genes, Emerald Amp GT PCR master mix (2x premix) followed the manufacturer's instructions. The PCR products were separated by gel electrophoresis using 1.5% agarose gel in 1X Tris-acetate/EDTA Gels and visualized PCR products under UV light.

Sequence analysis of *P. multocida* isolates by *16srRNA* gene sequencing:
Sequences of 16S rRNA were amplified with universal primers, Univerbac1 (5'-AGGAGGTGATCCAACCGCA-3') as reverse primer, and (5' AACTGGAGGAAGGTGGGGAT 3') as forward primer (Greisen *et al.,* 1994). A PCR master mix was prepared containing 25 µL Dream Taq Master Mix, 5 µL DNA template, 2 µL primer pair, and nuclease-free water to a final volume of 50 µL. The PCR cycling conditions were: initial denaturation at 94 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 52 °C for 1 minute, and extension at 72 °C for 1 minute. A final extension at 72 °C for 5 minutes was also performed. The amplified 16S rRNA gene fragments were visualized by electrophoresis on a 1 % agarose gel (Ibrahim *et al.,* 2023).

PCR products of *P. multocida* were identified by sequence analysis of *16srRNA*gene sequencing and identification of homologies between nucleotide sequences of the detected *P.multocida* strains and other strains published on GenBank was done using BLAST 2.0 search programs (National Center for Biotechnology Information, 'NCBI'; http://www.ncbi.nlm.nih.gov/). The maximum scores designated in the BLAST search have a well-defined statistical interpretation, making matches easier to distinguish from random background hits.

Histopathological examination:
Tissue specimens from the lung, trachea, liver, kidney, and spleen were fixed in 10% neutral buffered formalin, processed, paraffin-embedded, and sectioned at 4-6µ thickness. Tissues were stained with Hematoxylin and Eosin stain according to Bancroft *et al.* (1996).

RESULTS

Recovery rate of *P. multocida:*
In the present work, *P. multocida* was isolated from dairy farms of different localities. The recovery rate was approximately 60 *P. multocida* were identified by standard bacteriological methods (Quinn *et al.,* 1994). The total recovery was 40 % for all examined dairy farms. The highest detection rate was 60% & 40 % from Cairo-Alex road at Giza & Behera respectively in table (2).

Table (2): The total number of nasal samples collected and *P. multocid*a recovery percent from calves:

Molecular capsular typing of *P. multocida* isolates:
Multiplex PCR analysis was performed to identify capsular genes in the isolates,

utilizing the capsule-specific primers CAPA, CAPB, CAPD, CAPE, and CAPF (Townsend *et al.*, 2001). Each 50µl reaction contained 10ng of DNA, Dream Taq PCR master mix (Thermo), and the aforementioned primers. The molecular capsular typing revealed the production of amplicons with an average size of 1044 bp for cap A type, while other capsular serogroups did not produce any product (figure 1).

Fig.(1): Elechropherosis profile of *P. multocida* molecular typing producing 1044 bp of capA serogroup PCR products in examined samples, DNA Marker Gene Ruler DNA Ladder, Thermo

Antimicrobial susceptibility testing and resistance gene determinants:

Sixty bacterial isolates were tested for antimicrobial susceptibility to 6 different antibiotics (Table 3). The highest resistance towards trimethoprim/sulfamethoxazole (88.3 %) was followed by ampicillin (80 %). The most effective antimicrobial therapy was related to fluoroquinolone & Aminoglycoside with sensitivity (66.7 %) & (65 %) to enrofloxacin & gentamicin (Table 3). The results showed that 37 of 60 isolates (61.6 %) were resistant to at least four different antimicrobial classes, which indicates multidrug resistance (MDR). The *P. multocida* isolates from clinical infected calves were harbored *sul* II (100%, *n* = 53 out of 53), followed by *bla*$_{TEM}$ (62.5%, *n* = 30 out of 48), and *tetA* (62.5%, *n* = 15 out of 24) in table 4. The produced amplicons antimicrobial resistance patterns were *tet*A positive results revealed 164 bp amplicon (Figure 2), the *sul* II gene amplicons were 704 (Figure 3) and 964 bp for *bla*$_{TEM}$ (Figure 4).

Table 3: Sensitivity patterns of examined *P. multocida* strains

Table 4: Antimicrobial resistance determinants gene of examined *P. multocida* strains

Figure (2) Elechropherosis profile of *P. multocida* tetracycline resistance gene producing 164 bp of tetA PCR products in the examined samples, DNA Marker 100bp DNA Ladder, Jena Bioscience

Figure (3) Elechropherosis profile of *P. multocida* sulfonamides resistance gene producing 704 bp of *sul*II PCR products in examined samples, DNA Marker 100 bp DNA Ladder, Jena Bioscience

Figure (4) Elechropherosis profile of *P. multocida* Bet-lactam resistance gene producing 964 bp of *bla*$_{TEM}$ PCR products in the examined samples, DNA Marker 100bp DNA Ladder, Jena Bioscience

Nucleotide sequence accession number:

The sequence was deposited in the GenBank database under the accession numbers PP077062- PP077064 (Bovine Lung). The nucleotide sequences of the *16s*rRNA for examined isolates were compared with the sequences available in the public domains using the National Centre for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) server.

Table 5: The source modifier data of 3 sequenced samples in this study.

Histopathological findings:

Trachea:

Intense destruction and thickening of tracheal mucosa and replaced by inflammatory exudate containing necrotic debris, living and degenerated neutrophils, with or without bacterial colonies (suppurative tracheitis) (Fig. 5-A, B). Thickened tracheal submucosa by necrotic proliferative glands with retained secretion, edema, and few leukocytes. Depletion of lymphoid follicles with intense inflammatory fluid in submucosa beside necrotic muscles and recent thrombi in most blood vessels (Fig.5-C).

Lungs:

Fibrinopurulent exudate bronchopneumonia, represented by partial or complete obliteration of the bronchial lumen by fibrin

and mixed with neutrophils together with a destructed wall infiltrated by leukocytes was common (Fig.6-A). The majority of alveoli showed destructed epithelium and were impacted by fibrinous or fibrinopurulent exudate with thickened interalveolar tissue and pulmonary septa (Fig.6-B). The latter was thickened by exuded fluid and dilated blood vessels contain recent thrombi. Pleuritis is characterized by severe thickening of the serous membrane by fibrinous deposits and hyperemic blood vessels were encountered (Fig.6-C). Some alveoli contained serous fluid and neutrophils or mononuclear cells, and sometimes extravasated erythrocytes with proliferative interalveolar capillaries filled with erythrocytes (Fig.6-D).

Liver:

Designated microthrombi containing neutrophils with pressure atrophy and necrosis or dissociation of the hepatic cells were common (Fig.7-A). Portal areas were infiltrated with mononuclear cells and neutrophils, beside proliferative bile duct epithelium and mild periductular fibrosis (Fig.7-B). Some blood vessels had recent thrombi or hyalinized vascular walls. The majority of hepatic cells suffered from degenerative necrosis or necrotic changes with thickened hepatic capsules (Fig.7-C).

Kidneys:

Proliferative and dilated glomerular tufts containing microthrombi with thickened glomerular basement membrane were noticed (Fig.8-A). Focal areas of coagulative necrosis and tubular casts were also detected (Fig.8-B).

Spleen:

Intense depletion and necrosis of the splenic white pulps proliferative of reticuloendothelial cells were common (Fig.9-A&B). Intense hemosiderosis with proliferative septa and a capsule beside dilated red pulps was encountered

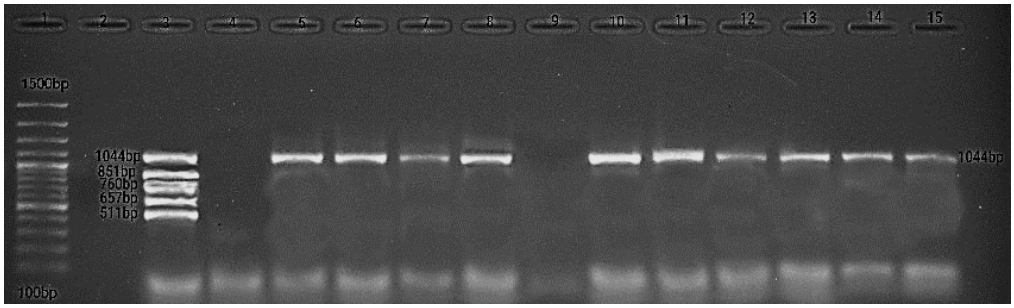

Figure 1: Electrophoresis profile of P. multocida molecular typing producing 1044 bp of capA serogroup PCR products in examined samples, DNA Marker Gene Ruler DNA Ladder, Thermo

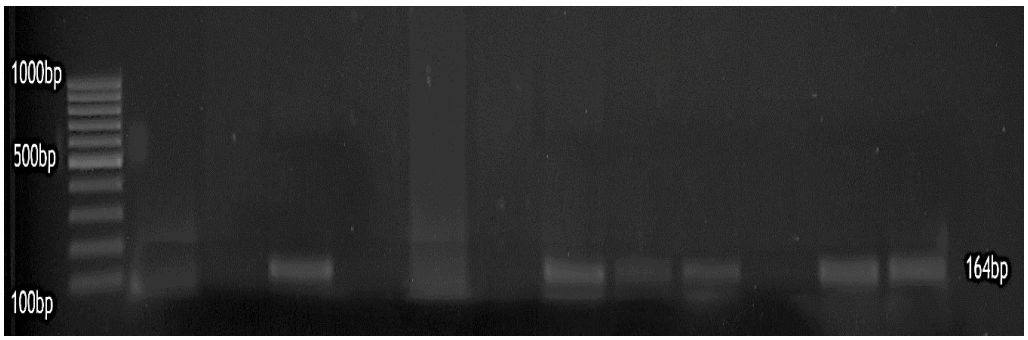

Figure 2: Electrophoresis profile of P. multocida tetracycline resistance gene producing 164bp of *tetA* PCR products in the examined samples, DNA Marker 100 bp DNA Ladder, Jena Bioscience

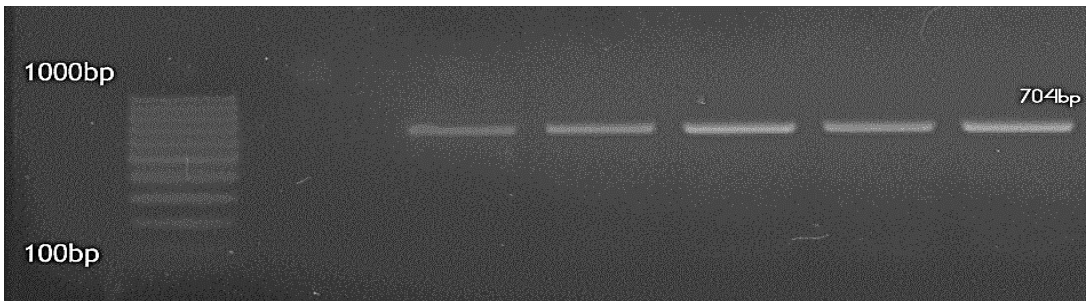

Figure 3: Electrophoresis profile of *P. multocida* sulfonamides resistance gene producing 704 bp of *sul*II PCR products in the examined samples, DNA Marker 100 bp DNA Ladder, Jena Bioscience

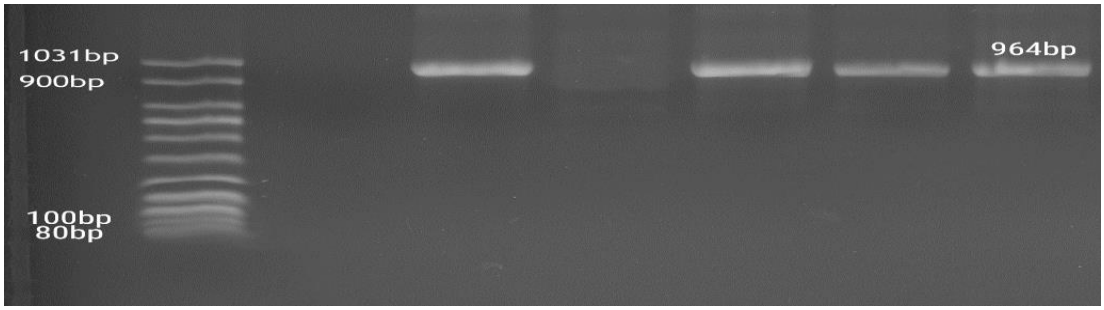

Figure 4: Electrophoresis profile of *P. multocida* Bet-lactam resistance gene producing 964 bp of blaTEM PCR products in the examined samples, DNA Marker 100 bp DNA Ladder, Jena Bioscience

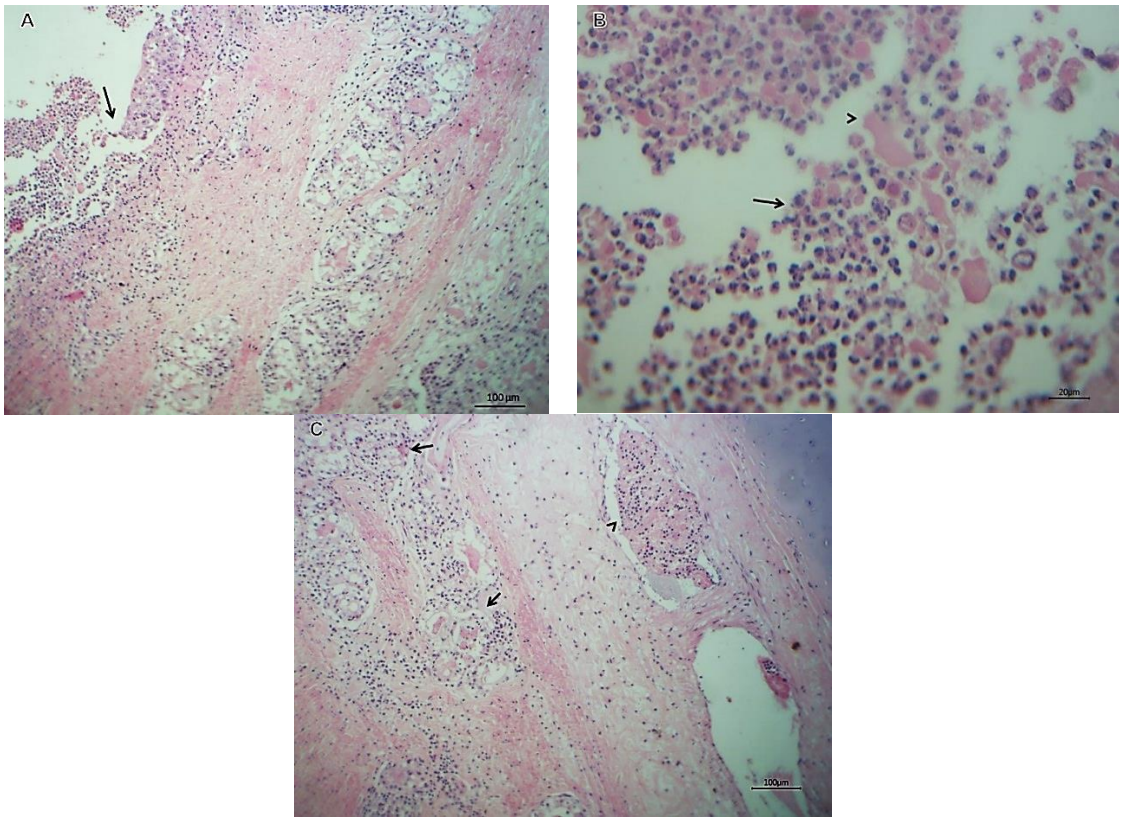

Fig. 5: Photomicrograph of calves' trachea infected with pasteurellosis stained by H & E (A) showing intense destruction of tracheal epithelium and replaced by neutrophils and pus (arrow). (B) Showing the high power of the previous figure to neutrophils (arrow) and necrotic debris (arrowhead). (C) Showing necrotic tracheal glands (arrow) and thrombosis of blood vessels (arrowhead).

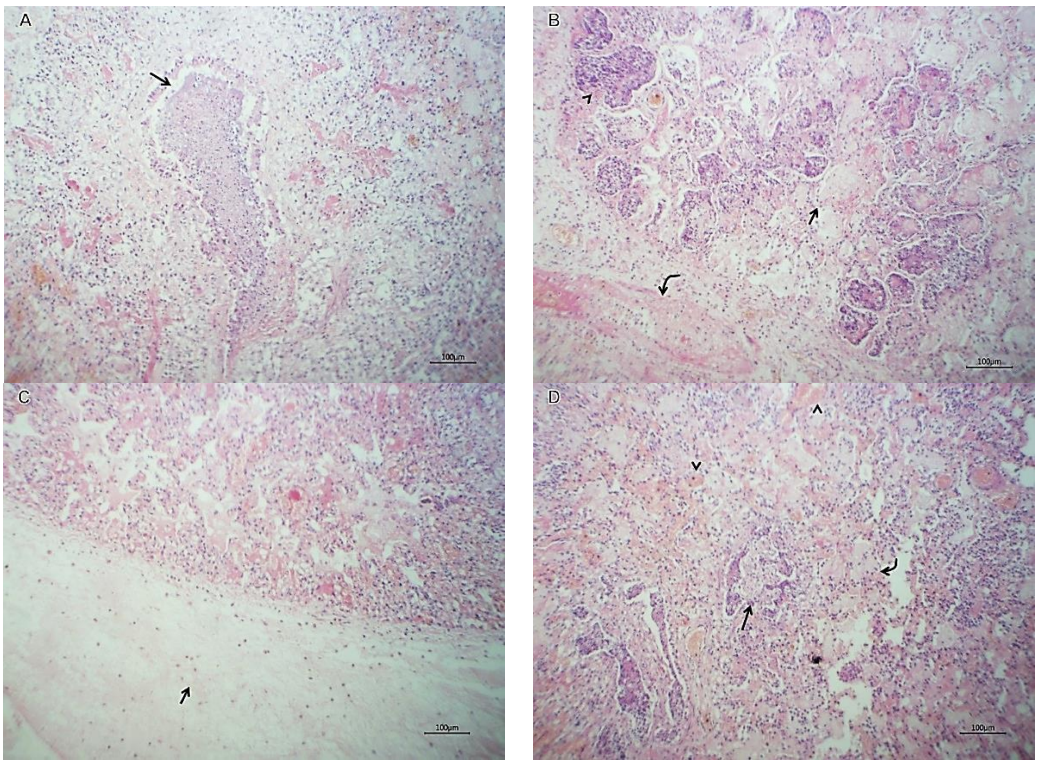

Fig. 6: Photomicrograph of the calves' lung infected with pasteurellosis stained by H&E. (A) showing complete obliteration of bronchiolar lumen by pus (arrow) and adjacent alveoli by exudate. (B) Showing fibrinous exudate (arrow) inside alveoli and purulent fluid in other alveoli (arrowhead) beside thrombosis of blood vessels (curved arrow). (C) Showing thickened pleura by fibrin (arrow). (D) Showing fibrinous exudate inside the bronchiole, proliferative and dilated capillaries (arrowhead) and serous fluid within the alveoli (curved arrow).

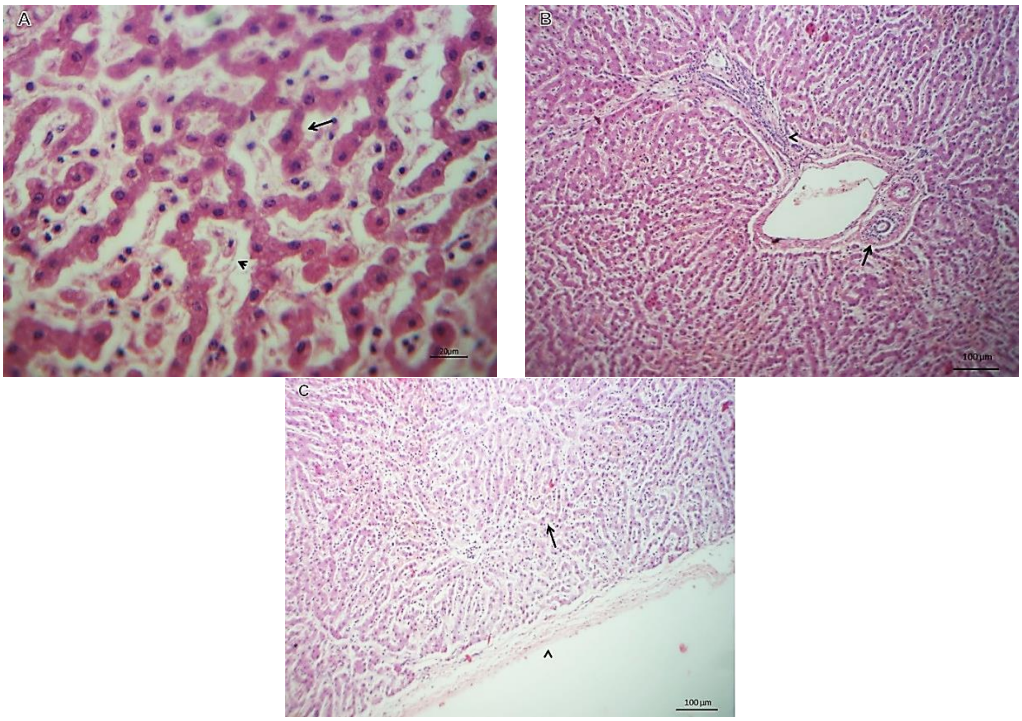

Fig. 7: Photomicrograph of calves' liver infected with pasteurellosis stained by H & E. (A) Showing fibrin threads inside hepatic sinusoids (arrowhead) and pressure atrophy by the hepatic cells (arrow). (B) Liver of cattle (pasteurellosis) showing periductular fibrosis (arrow) and proliferative bile ductules (arrowhead) in the portal area. (C) Showing atrophied and necrotic hepatic cells (arrow) and thickened capsule (arrowhead).

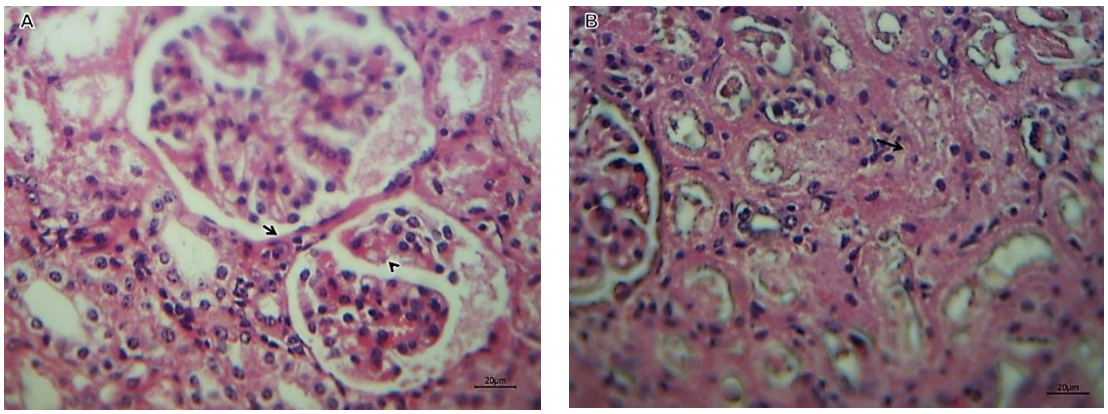

Fig. 8: Photomicrograph of calves' kidney infected with pasteurellosis stained by H&E. (A) Showing dilated glomerular tuft containing microthrombi (arrowhead) and thickened glomerular basement membrane (arrow). (B) Showing focal coagulative necrosis (arrow).

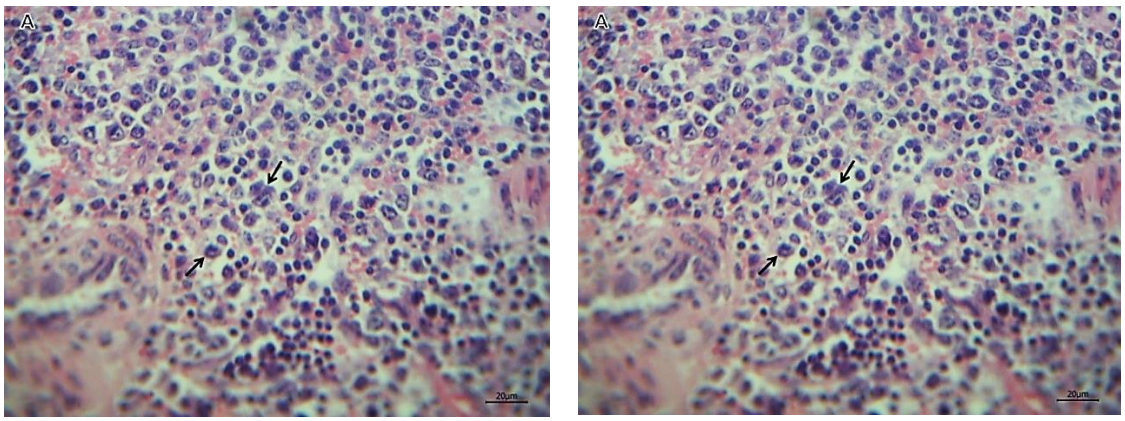

Fig. 9: Photomicrograph of calves' spleen infected with pasteurellosis stained by H & E.(A) Showing proliferative reticuloendothelial cells (arrow) with lymphoid depletion. (B) Showing intense hemosiderosis (arrow) in the depleted splenic white pulp.

Table 1: Oligonucleotide primers encoding for capsular typing & antibiotic-resistant genes PCR primers and amplification conditions.

Gene	PCR Primer Sequence 5′-3′	PCR Product	Amplification condition				
			Initial Den.	Den.	Ann.	Ext.	Final Ext.
				35 Cycles			
CAP*A*[1]	TGCCAAAATCGCAGTCAG TTGCCATCATTGTCAGTG	1044	95 °C 3min	95 °C 30 Sec	55 °C 30 Sec	72 °C 30 Sec	72 °C 5min
CAP*B*[1]	CATTTATCCAAGCTCCACC GCCCGAGAGTTTCAATCC	760					
CAP*D*[1]	TTACAAAAGAAAGACTAGGAGCCC CATCTACCCACTCAACCATATCAG	657					
CAP*E*[1]	TCCGCAGAAAATTATTGACTC GCTTGCTGCTTGATTTTGTC	511					
CAP*F*[1]	AATCGGAGAACGCAGAAATCAG TTCCGCCGTCAATTACTCTG	851					
Tet A F[2] *Tet* A R	GCGCGATCTGGTTCACTCG AGTCGACAGYRGCGCCGGC	164	94 °C 5min	94 °C 30 Sec	52 °C 30 Sec	72 °C 45 Sec	72 °C 7min
Bla_{TEM} F[3] Bla_{TEM}R	ATGAGTATTCAACATTTCCG ACCAATGCTTAATCAGTGAG	964	94 °C 3min	94 °C 1min	50 °C 1min	72 °C 1min	72 °C 7min
sulI, F[4] sulI, R	ACAGTTTCTCCGATGGAGGCC CTCGTGTGTGCGGATGAAGTC	704					

According to Townsend *et al.* (2001), Maynard *et al.* (2003), Naas *et al.* (2001), Kehrenberg, &Schwarz (2001)

Table 2: The total number of nasal samples collected and *P.multocid* recovery percent from calves.

Farm-Region	Governorate	No. of samples			*P. multocida* Recovery	
		Nasal swap	lung tissue	total	No.	%
Farm 1/Alex –Cairo Road	Giza	30	5	**35**	21	60
Farm 2/Alex –Cairo Road	Menofia	32	3	**35**	12	34.2
Farm 3/Alex –Cairo Road	Behera	25	5	**30**	12	40
Farm4/Zagazeg	Sharkia	23	2	**25**	8	32
Farm5/Ismaelia Desert Road	Ismaelia	23	2	**25**	7	28
Total		133	17	**150**	60	40

Table 3: Sensitivity patterns of examined *P. multocida* strains.

Sensitivity Pattern	Penicillin	β-Lactam Combination	Aminoglycoside	Tetracycline	fluoroquinolone	Folate antagonist	MDR
	AMP	AMC	CN	TE	ENR	SXT	
Sensitive	12/60	25/60	39/60	36/60	40/60	7/60	37/60
Resistant	48/60	35/60	21/60	24/60	20/60	53/60	
Resistance%	80	58.3	35	40	33.3	88.3	61.6

Table 4: Antimicrobial resistance determinants gene of examined *P .multocida* strains.

Antimicrobial resistance determinants	**Bla*TEM***	**tet*A***	**sul*I***
Present	30/48	15/24	53/53
Absent	18/48	9/24	0/53
Determinants %	62.5	62.5	100

Table 5: The source modifier data of 3 sequenced samples in this study.

No. of Isolate	Farm Region	Capsular type	Resistance genes by PCR			GenBank Accession No.
			***bla*TEM**	***tet*A**	***sul*II**	
Vet-MU-1	Menofia	A	+	+	+	PP077062
Vet-MU-2	Behera	A	-	-	+	PP077063
Vet-MU-4	Ismaelia	A	-	+	+	PP077064

DISCUSSION

P. multocida infection in dairy calves can have various consequences. It can cause respiratory disorders and is involved in the bovine respiratory disease complex (BRDC), leading to significant economic impact (Su *et al.,* 2020). Mastitis, although rare, can also occur in dairy cows infected with *P. multocida*, resulting in acute inflammation of the mammary gland (Milanov *et al.,* 2017). In addition, *P. multocida* can cause systemic infections in humans, such as pneumonia, lung abscess, peritonitis, endocarditis, meningitis, and sepsis (Abubakr *et al.,* 2020). The bacterium can produce toxins, and some strains of *P. multocida* are toxigenic, which can contribute to the severity of the infection (Gondaira *et al.,* 2022). Overall, *P. multocida* infection in dairy calves can have detrimental effects on both animal health and human health.

This study investigated the potential risk of *P. multocida* infection in young calves on dairy farms in Egypt. We found a 40% prevalence of *P. multocida* recovered from calves, with significant regional variations ranging from 28 % to 60 %. This high prevalence poses a serious threat to dairy farm production chains in Egypt.

Overall, previous investigation about *P. multocida* prevalence in dairy calves has been investigated in several studies. In a large field study conducted on Swiss farms, Becker *et al.* observed that 20 isolates of *P. multocida* exhibited a multidrug-resistant (MDR) profile, including resistance to the macrolide tulathromycin (Becker *et al.,* 2022). Another study in Egypt by (Zareh *et al.,* 2021) found that 6.5 % of the samples collected from calves were positive for *P. multocida*. Milanov *et al.,* (2017) described a rare case *of P. multocida* mastitis in dairy cows, indicating *that P. multocida* can cause infections in this population. (Abubakr *et al.,* 2022) conducted a study in Egypt and identified *P. multocida* in 16.75 % of the samples collected from cows with respiratory manifestations (Abubakr *et al.,* 2022). (Asfour *et al.,* 2016) investigated mastitic milk samples from cows and ewes in Egypt, and found that *P. multocida* was isolated from 15.3% of clinical mastitic milk samples in cows. Overall, these studies highlight the presence of *P. multocida* in dairy calves and its potential role in respiratory infections and mastitis.

Molecular capsular typing of *P. multocida* in the present study revealed that all examined cases harbored *P. multocida* capsular type A (Fig. 1).

P. multocida type A has significant implications in dairy farms. It is one of the primary pathogens causing bovine respiratory disease (BRD) and leads to substantial losses in the cattle industry (Zhan *et al.,* 2021). The pathogen can cause pneumonic pasteurellosis, a common respiratory infection in ruminants, which has major economic and welfare implications worldwide (Livingstone *et al.,* 2022). Studies have shown that *P. multocida* type A is commonly isolated from lung tissues and nasal swabs of cattle, indicating its role in respiratory disease (Tamer *et al.,* 2016). Furthermore, the organism is also known to cause pneumonia and septicaemic pasteurellosis in lambs, with zoonotic potential in humans (Susmitha *et al.,* 2020). Overall, the presence of *P. multocida* type A in dairy farms poses a significant health and economic risk to livestock and humans alike.

Risk factors for multidrug-resistant (MDR) *P. multocida* in dairy cattle include the use of standard antimicrobial agents and critically important antimicrobial agents, such as macrolides, fluoroquinolones, and higher-generation cephalosporins (Becker *et al.,* 2022). Antimicrobial drug (AMD) use for bovine respiratory disease (BRD) is also a risk factor for the development of antimicrobial resistance (AMR) in respiratory isolates of *P. multocida* (Sarah *et al.,* 2022).

The prevalence of MDR *P. multocida* strains in feedlot cattle with bovine respiratory disease (BRD) is high, and using macrolides, tetracyclines, and in-feed supplements containing heavy metals may be selected for MDR isolates (Tamer *et al.,* 2016).

The Multi-Drug Resistance in the present work recorded a high core, reaching 61.6 % with the highest resistance patterns to sulfonamides (88.3 %) and confirmed by antimicrobial resistance determinants for *sul*II gene by (100 %) detection rate. Also, the most powerful active group against *the P. multocida* pathogen was enrofloxacin from the fluoroquinolone group by sensitivity power (66.7 %).

The resistance gene determinants revealed that *sul*II is the most prevalent gene in examined strains (100 %). The bla_{TEM} & *tet*A were detected by (62.5 %), the detection rate for resistance genes is somewhat high.

MDR *P. multocida* can affect the health of dairy cattle by causing respiratory infections and mastitis. In respiratory infections, *P. multocida* is a common commensal bacterium in the upper respiratory tract of calves, but it can also cause respiratory infections with multifactorial etiopathogenesis. Antimicrobial treatment is commonly used to control these diseases, but MDR isolates of *P. multocida* have been observed, which exhibit resistance to multiple antimicrobial agents including macrolides, tetracyclines, and fluoroquinolones (Becker *et al.,* 2022). In the case of mastitis, *P. multocida* is a rare cause, and the source of infection is often unknown.

In our results, the histopathological examination of the trachea revealed destruction and thickening of tracheal mucosa and replaced by inflammatory exudate, lungs showed fibrinopurulent bronchopneumonia. The alveoli showed destructed epithelium and were impacted by fibrinous exudate. Pleuritis was also seen. These results are in agreement with (Elbatawy *et al.,* 2022) who revealed extensive damage to alveoli, bronchioles, interstitial tissue, and pleura was expanded in many pulmonary tissues due to mononuclear inflammatory cellular infiltration. Similar results were also reported by (Abubakar and Zamri-Saad 2011 and Praveena *et al.,* 2014) who stated fibrino-necrotizing bronchopneumonia and pleuritis. Pasteurella endotoxins induce intravascular thrombosis of pulmonary blood vessels resulting in severe intra-alveolar inflammation (Jones *et al.,* 1997). These histopathological changes may be due to *P. multocida* endotoxins and toxic proteins such as leukotoxin, lipopolysaccharide and polysaccharide (Hodgson, 2006) and also due to the inflammatory factors produced by neutrophils and other inflammatory cells. Leukotoxin has a toxic effect on leukocytes, leading to fibrin deposition on pulmonary tissue and pleural surfaces that could clarify the occurrence of fibrinous pleuritis and pneumonia. The pulmonary tissues were infiltrated with different inflammatory cells due to the release of cytokines (Locksley *et al.,* 2001). These cytokines enhance leukocytic cellular infiltrations at the infection site (Yoshi *et al.,* 2001).

Portal areas in our results were infiltrated with mononuclear cells and neutrophils. The majority of hepatic cells suffered from necrotic changes with thickened hepatic capsules. The kidney showed proliferative and dilated glomerular tufts containing microthrombi. Focal areas of coagulative necrosis were seen. The spleen, in our results showed intense depletion and necrosis of the splenic white pulps. Intense hemosiderosis beside dilated red pulps. These results were agreed with (Aziza Amin, 2020) who found peri-ductal leukocytic cellular infiltrations, as well as multiple focal areas of coagulative necrosis were detected in the hepatic parenchyma. An extensive hemorrhage in the red pulp of the spleen with marked lymphoid depletion. Hemorrhages and necrotic changes were demonstrated in the glomerular tuft with the widening of the

Bowman's space and renal tubules.

CONCLUSION

In conclusion, this study investigated *P. multocida* prevalence and MDR profiles in Egyptian dairy calves, highlighting potential threats to animal and human health. Overall, this study emphasizes the need for comprehensive *P. multocida* management strategies in dairy farms, including judicious antibiotic use and effective vaccination programs, to safeguard animal health and reduce zoonotic risks.

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التوصيف المظهرى و الوراثى للباستريلا مالتوسيدا مع الاشارة الى التغيرات الباثولوجية فى العجول

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هدفت هذه الدراسة إلى التحقق من وجود بكتيريا الباستريلا مالتوسيدا في عجول مزارع الألبان المصرية المصابة بأعراض تنفسية. تم جمع ١٥٠ عينة بطريقة معقمة من ١٣٣ مسحة أنفية و١٧ نسيج رئوي للعجول من المزارع الخاصة في مختلف المحافظات. تم اختبار العينات باستخدام ثلاث طرق: الفحص البكتريولوجي القياسي، وتحديد الكيمياء الحيوية، والتوصيف الجزيئي للباستريلا مالتوسيدا. تم تعريض عزلات الباستريلا مالتوسيدا إلى PCR المتعدد من أجل التخليق الحيوي للكبسولة وجين S rRNA١٦. كشف تصنيف المحفظة البكتيرية أن ٦٠ من أصل ١٥٠ عينة (٤٠٪) كانت إيجابية بالنسبة للباستريلا مالتوسيدا. جميع العزلات التي تم التأكد من أنها بالباستريلا مالتوسيدا من خلال تفاعل البوليميراز المتسلسل تنتمي إلى المجموعة المصلية A. وقد حدد اختبار الحساسية للمضادات الحيوية لهذه العزلات أن الإنروفلوكساسين باعتباره الدواء الأكثر فعالية. وكان جين المقاومة الأكثر قابلية للاكتشاف هو sul II- في العزلات المفحوصة بنسبة اكتشاف ١٠٠% يليه جين BlaTEM وtetA (٦٢,٥%). كشف الفحص النسيجي المرضي عن أضرار واسعة النطاق في مختلف الأعضاء في الحيوانات المصابة بالباستريلا مالتوسيدا