Research Article

Phenotypic and Genotypic Characterization of *Pasteurella* Species Isolated from Camels in Egypt

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Abstract | The main aim of this study is to apply phenotypic and genotypic characterization of members of *Pasteurella* species isolated from camel from different governorates in Egypt. Camels with respiratory manifestation as nasal discharge, fever, off food, with ocular discharge and air gasping, paralysis of lips and salivation were subjected to clinical examination and a total of 141 samples were collected as follow; 111 nasopharyngeal swabs, 15 tracheal swabs and 15 lung specimens collected from camels from different governorates (New valley, Giza, Naj Hammadi (Qena governorate), Halaib and Shalateen (Halaib Triangle) were subjected to isolation and identification of *Pasteurella* species by conventional phenotypic method and genotypically by polymerase chain reaction (PCR). At the level of biochemical characterization, the incidence of *Pasteurella multocida* was 40.4%, and *Mannheimia haemolytica* was 7.1%. Using KMT1 gene for identification of the isolates for *P. multocida* and SSE gene for identification of the isolates for *M. haemolytica*, the results revealed that six isolates showing positive PCR for *Pasteurella multocida* and were subject to further phylogenic characterization. *M. haemolytica* could not be detected by PCR. Identification and characterization of *Pasteurella* species isolated from camel improve diagnosis, epidemiology and treatment of the organism causing diseases.

Keywords | Camel, *Pasteurella multocida, Mannheimia haemolytica*, Phenotypes, Genotyping, PCR

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INTRODUCTION

Camels belong to *Camelidae* family of mammals. The *Camelidae* comprised two types (large and small camelids); the large camelids include two domesticated species, the one-humped camel (dromedary) and the two-humped camel (Bactrian camel) (Faye, 2015). The global population of large camelids is estimated to be about 28 million heads, of which 95 percent are dromedary camels (FAO, 2016). More than 60% of the world's camel population is found in the Horn of Africa region (Jores, 2015). Camels are multipurpose animals; they can be used for milk, meat, wool, transport, race tourism, agriculture work and beauty contest (Faye, 2015). Camels' milk filtrate product has antimicrobial activity against different pathogenic microorganisms (Al-Zaiadi, 2016).

The camel studies are still quantitatively marginal compared to other ruminant species (Faye, 2015). Moreover, pathogens and diseases related to camelids are less well known than those of other domesticated species (FAO, 2016). Recent research has shown that camels are indeed susceptible to a large number of pathogenic agents (Abbas and Omer, 2005), especially the respiratory diseases which are common among camels, as shown by the reports of bronchopneumonia and pneumonia lesions at abattoirs.

Yet, little is known of the complex etiology of these diseases. *Pasteurella multocida* type A may be important (Fassi-Fehri, 1987). New epidemics of respiratory disease have caused 29.6% morbidity and 6.4% mortality in camels in the Somalia region of Ethiopia (which may be a cause for the problem in camel that have been retrieved or imported from the endemic regions), that means the carrier state of bacterial infection may be implemented and consequent to the clinical picture moreover.

Camels used to be considered resistant to most of the diseases commonly affecting livestock, but as more research was conducted, they were found to be susceptible to many pathogenic agents (Abbas and Omer, 2005) as the respiratory problems are common among camels. Fassi-Fehri (1987) *Pasteurella multocida* is the most isolated pathogen from pneumonic animals, yet *P. haemolytica* has also been associated with severe pneumonia (Megersa, 2010). Furthermore, *P. multocida* is the main cause of hemorrhagic septicemia (HS) in camels (El-Tawab et al., 2016). Bacteriologically, *Pasteurella* species are spherical, ovoid, or rod-shaped cells that exist singly, or in pairs or short chains. They are Gram-negative, but Bipolar staining may be seen (El-Jakee et al., 2020). Direct and indirect contact with the source of infection are the prime routes of *Pasteurella multocida* transmission mainly via aerosols. *Pasteurella multocida* is divided into the following three subspecies: *P. multocida* subsp. *multocida, P. multocida* subsp. *septica* and *P. multocida* subsp. *gallicida* (El-Jakee et al., 2020). The serological classification of *Pasteurella multocida* can determine five capsular types (A, B, D, E and F), as well as 16 different somatic types (De-Alwis 1999; Setta et al., 2017; El-Hamid et al., 2019). *M. haemolytica* is a part of Pasteurellaceae family, classified among the γ-proteobacteria of pathogens, genera *Mannheimia, Pasteurella* and others (Highlander, 2001). *M. haemolytica* (formerly *Pasteurella haemolytica*) is classified based upon sequence phylogeny of 16S rRNA and DNA-DNA hybridizations (Angen et al., 1999). *M. haemolytica* comprises two biotypes: A and T, based on fermentation of arabinose and trehalose, respectively. Within these biotypes, 17 serotypes were identified on the basis of soluble or extractable surface antigen. Serotypes 1, 2, 5, 6, 7, 8, 9, 11, 12, 13, 14, 16, and 17 belong to the biotype A, which was reclassified as *M. haemolytica*. However, serotype 11 was later reclassified as *Mannheimia glucosida.* The serotypes 3, 4, 10 and 15 belong to biotype T (Abera et al., 2014). Genomic analysis would provide an opportunity to discover features that potentially related to the bacterial virulence as well as differentiation between *Pasteurella* and *Mannheimia* from other *Mannheimia* spp. (Ewers et al., 2004).

Bacterial infection of lung is one of the most common causes of pneumonia, where *Pseudomonas* spp., *E. coli*, *Klebsiella* spp., *Mannheimia haemolytica*, *Pasteurella multocida*, and *Citrobacter* spp. were among the mostly bacteria recovered from pneumonic lungs (Amany, 2000; Azzam and Zaki, 2006). Therefore, the aim of this study was to investigate the phenotypic and genotypic characterization of members of *Pasteurella* species from camels clinically suffered respiratory manifestation from different governorates in Egypt.

MATERIALS AND METHODS

Samples collection

A total of 141 samples were collected from diseased camels suffering from respiratory manifestation. The samples consisted of 111 nasopharyngeal swab samples from clinically diseased camels from different governorates (20 from New valley, 16 from Naj Hammadi (Qena governorate), 36 from Halaib and Shalateen (Halaib Triangle), 39 nasopharyngeal swab from Giza and governmental veterinary clinics include "Met-rahena, El-Badrashin, Mazghona"). As well as samples from slaughtered camels in the abattoir as follow; 15 tracheal swabs and 15 lung specimens from Saqqara abattoir in Giza (Figure 1).

Figure 1: Sampling of suspected diseased camels; A: Nasal swabbing, B: Blood sampling.

All samples collected under complete aseptic conditions. Sterile cotton tipped nasopharyngeal swabs of 15 cm length were directed to the nasopharynx after cleaning the nostril with cotton soaked in 70% alcohol then backed and covered by sterile plastic sheet and kept in ice box. Lung specimens and tracheal swabs were taken immediately after slaughtering. Those specimens were put in separate sterile plastic bags and both swabs and specimens kept in ice box.

Culturing and isolation

Nasopharyngeal and tracheal swabs were inoculated into brain heart infusion (BHI) broth (Oxoid, England) and incubated at 37°C for 24 hrs. Lung specimens were sterilized with hot flamed spatula and incised with sterile scalpel blade. A sterile swab sample from inner tissue was taken and inoculated into BHI broth and incubated at 37°C for 24 hrs. After that, a loopful from that broth was streaked onto 5% defibrinated sheep blood agar plate and incubated at 37°C for 24 hrs. Suspected colonies

were picked up and streaked onto brain heart infusion agar plate to obtain pure culture, the growth subjected to microscopical examination using Gram staining and further sub-cultured on 5% defibrinated sheep blood agar (Oxoid, England) and MacConkey agar (Oxoid, England) plate for colony morphology identification. Furthermore, the characteristic colony was subjected to the biochemical identification using urease, oxidase, and catalase for final identification to species level using biochemical tests include Indole production, hydrogen production on Triple Iron Sugar agar (TSI), fermentation reaction for sugars (glucose, lactose, sorbitol, dulcitol, arabinose, trehalose). It was carried out according to methods described by (Quinn et al., 2011).

Pathogenicity test

A loopful from each suspected isolate was cultured in BHI broth for 24 hrs. at 37°C Then, 0.1 ml from each broth culture was injected subcutaneously into a laboratory mouse. On the other hand, another two mice were inoculated with 0.1 ml of sterile BHI broth as negative control. Deaths were recorded daily for up to 72 hrs. and heart blood smears stained with Leishman's stain were examined for demonstration of bipolarity and then streaked onto 5% sheep blood agar for re-isolation. It was carried out according to methods described by (Ramdani et al., 1990).

Molecular identification

DNA Extraction

Suspected isolates were cultured in BHI broth at 37°C for 24 hrs. Then, 1ml of BHI broth was centrifuged at 5000 rpm for 10 min, the supernatant was discarded, and the pellet was washed by tris acid-EDTA and centrifuged again. This step was repeated three times, then the supernatant was discarded, and the pellet was re-suspended with 200µl of tris acid-EDTA and boiled at 100°C in water bath for 10 min, then cooled in ice and centrifuged at 5000 rpm for 10 min. The supernatant was taken for additional identification by PCR (Queipo-Ortuño et al., 2008).

PCR analysis

The oligonucleotide primers used in conventional PCR (Metabion, Germany) (Table 1) was used for detection of Preparation of PCR Master-mix was carried out according to Emerald Amp GT-PCR master-mix (Takara). The kit consists of 12.5µl of Emerald Amp GT-PCR master-mix (2X premix), 5.5 µl of PCR grade water, 1 µl forward primer (20 pmol), 1 µl reverse primer (20 pmol) and 5µl template DNA (sample) to have total volume of 25 µl per well/tube. The PCR amplification conditions were 94 °C for 5 min., 94 °C for 30 sec., 72 °C for 45 sec., and final extension at 72 °C for 10 min. We used PCR tubes 0.2 ml capacity and T3 Thermal cycler (Biometra, Germany). A DNA ladder of 100 bp with agarose gel electrophoresis was conducted according to procedures stated by (Sambrook et al., 1989). Then, the gel was transferred to UV cabinet for reading.

Sequencing

The PCR product was purified using QIA quick PCR product extraction kit (Qiagen Inc. Valencia, CA), and gene sequencing was performed with a Perkin-Elmer Bigdye Terminator V3.1 cycle sequencing kit from Perkin-Elmer in Foster City, CA, using an Applied Biosystems 3130 genetic analyzer (Hitachi, Japan). On an Applied Biosystems computer, a purified PCR product was sequenced in the forward and/or reverse directions.

To determine sequence identity to GenBank accessions, a BLAST® analysis (Basic Local Alignment Search Tool) (Altschul et al., 1990) was used.

Phylogenetic analysis

A comparative analysis of sequences was performed using the "CLUSTALW" Multiple Sequence Alignment program, version 1.83 of MegAlign module of Laser gene DNA Star software pairwise, designed according to the methods described by (Thompson et al., 1994). Phylogenetic analyses were done using maximum likelihood, neighbour joining and maximum parsimony in MEGA-6 in accordance to methods described by (Tamura et al., 2013).

RESULTS AND DISCUSSION

Clinical presentation and phenotypic characterization

The primary investigations of camels under the study revealed the respiratory manifestation that appears as nasal discharge, fever with signs of off food and may accompanied with ocular discharge in addition to the air gasping manifestation with paralysis of lips and salivation (Figure 2). Thus, the further diagnostic tools were used for the confirming the diagnosis and catch the real cause. Identification of bacterial isolates was made by observation of Gram staining reaction, colonial morphology and biochemical characteristics. The isolates showing Gram negative coccobacilli short rods, round, smooth or mucoid non-haemolytic colony on blood agar and failed to grow on MacConkey agar (40.4%) were suspected to be *P. multocida*. The 7.1% round, smooth pinpoint with zone of β-haemolysis and that grew on MacConkey agar with lactose fermentation were suspected as *M. haemolytica* (Figure 3). Both cultures gave positive results against the oxidase test, nitrate reduction test and catalase test, but were negative to the urease test. The indole test gave positive results for *P. multocida* and negative for *M. haemolytica* (Table 2). The phenotypic characterization declared that

there were 57 isolate of *P. multocida*, while there was only 10 isolates of *M. hemolytica* throughout the study. Also, the isolates were distributed either from camel nasopharyngeal swabs or from tissue from camels slaughtered in the abattoir.

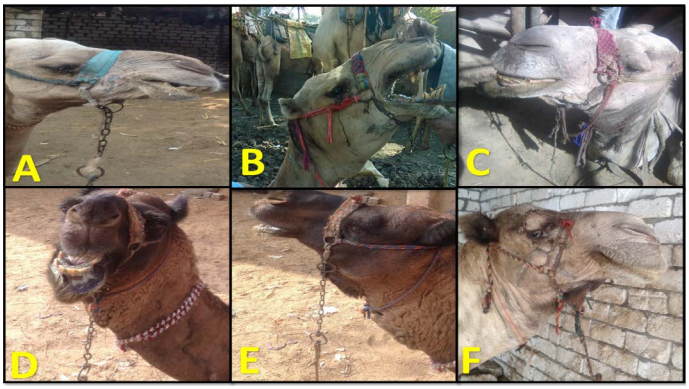

Figure 2: Clinical presentation of the suspected diseased camels suffering respiratory distress; A: lethargy with depressed demeanor, B: oral breathing and ocular discharge, C: nasal discharge with difficult breathing, D and E: salivation and paralysis of lower lip, F: semi-closed eye lids with ocular discharge.

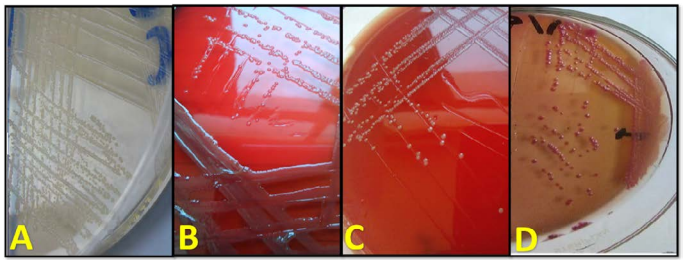

Figure 3: A: Growth of *P. multocida* on BHI agar plate, B: growth of *M. haemolytica* on blood agar plate with β-zone of hemolysis, C: growth of *P. multocida* on blood agar plate with no hemolysis, D: growth of *P. multocida* on MacConkey agar plate with lactose fermentation.

Pathogenicity test

The study of pathogenicity test in challenged laboratory mice for the 67 *Pasteurellaceae* isolates resulted in 100% mortality of mice within 72 hrs., whereas no recorded mortality was recorded in the control mice. Smears of heart blood from dead mice revealed the identification of characteristic bipolarity with Leishman's stain (Figure 4).

Molecular identification and species differentiation

All isolates were subjected to PCR using KMT1 gene primer, PCR analysis for the presence of *KMT1* gene with amplicon size of 460 bp confirmed that six of isolates out of 67 total isolates were belonged to *P. multocida*. However, PCR failed to detect *SSE* gene specific for *M. haemolytica* (Figure 5). The positive *P. multocida* samples were distributed as follow; two nasopharyngeal swabs from Vet. Clinic (Met-Rahena) in Giza, one lung specimen and two tracheal swabs from Saqqara abattoir, Giza, and the last nasopharyngeal swabs from Halaib and Shalateen (Halaib Triangle) locality. In the same way, the *P. multocida* isolated strains were sequenced and subjected to the phylogenic analysis (Neighbor joining tree) based on the amino acid sequences of the common gene with the sequence distancing (identity %) and nucleotide alignment report for detection the genetic variations between the isolated strains and ancestors (Figures 6, 7 and 8). The isolated strains were submitted to the Gene bank for the accession numbers (Table 3).

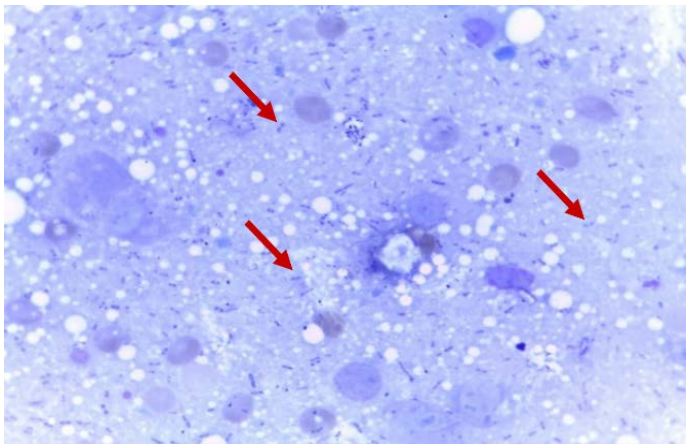

Figure 4: Smears of heart blood from dead mice stained with Leishman's stain revealed the characteristic bipolarity of *Pasteurella* spp.

The sequencing, phylogenetic characterization and nucleotide alignment assisted the correlation of the isolated strains with the circulating bacteria as well as in between the isolated strains, where the neighbor joining tree based on amino acid sequences of *P. multocida* common gene revealed that, the isolates in this study have the same ancestor of *P. multocida*, where they were antigenically similar (sister taxa) (MT263083 and MT263079). The same, MT263078 and MT263081 were a clade from the same ancestor and they were antigenically similar. On the other hand, despite MT263080 and MT263082 have the same ancestor and they were a clade, but they were not antigenically similar. But the three sets of the isolated strains were not antigenically identical or related to each other.

In the present study, 141 samples taken from camels from different governorates as a trail for isolation and identification of *Pasteurella* species as well as genotypic characterization by PCR to characterize the *M. haemolytica* and *P. multocida* from nasal discharge, and lung tissue of pneumonic lung using *KMT1* gene at amplicon size of 460 bp, after that, the positive isolates were subjected to sequencing and further phylogenic characterization.

Table 1: The oligonucleotide primers name, sequences, target genes and the expected amplicon size used in PCR.

Target agent	Target gene	Primers sequences	Ann.	Amplified segment (bp)	Reference
P. multocida	KMT1	ATC-CGC-TAT-TTA-CCC-AGT-GG	55°C 40 sec.	460 bp	OIE (2012)
		GCT-GTA-AAC-GAA-CTC-GCC-AC			
M. haemolytica	SSE	TTCACATCTTCATCCTC	50°C 40 sec.	325 bp	Hawari *et al*., 2008
		TTTTCATCCTCTTCGTC			

Table 2: Samples and the bacterial isolates referred to *P. multocida* and *M. haemolytica* in different localities.

Governorate	Samples		Total isolates		Potential (P. multocida)		Potential (M. haemolytica)	
			No.	%	No.	%	No.	%
New valley	20		10	50	6	30	4	20
Giza Giza city	39	19	0	0	0	0	0	0
Vet. clinic. (Met-Rahena, El-Badarashin and Mazghona)		20	9	23	3	7.7	6	15.4
Qena (Naj Hammadi)	16		11	68.7	11	68.7	0	0
Halayeb w Shalateen	36		11	30.5	11	30.5	0	0
Saqqara abattoir (tracheal swabs)	15		13	86.6	13	86.6	0	0
Saqqara abattoir (lung specimens)	15		13	86.6	13	86.6	0	0
Total	141		67	47.5	57	40.4	10	7.1

Table 3: The accession number of the isolated strains of *P. multocida* on the Gene bank.

Strain	Accession number	Strain name	Isolation source	Country
P. multocida	MT263078	*P._multocida*_EGY_15	Camel	Egypt
P. multocida	MT263079	*P._multocida*_EGY_17	Camel	Egypt
P. multocida	MT263080	*P._multocida*_EGY_42	Camel	Egypt
P. multocida	MT263081	*P._multocida*_EGY_45	Camel	Egypt
P. multocida	MT263081	*P._multocida*_EGY_48	Camel	Egypt
P. multocida	MT263082	*P._multocida*_EGY_61	Camel	Egypt

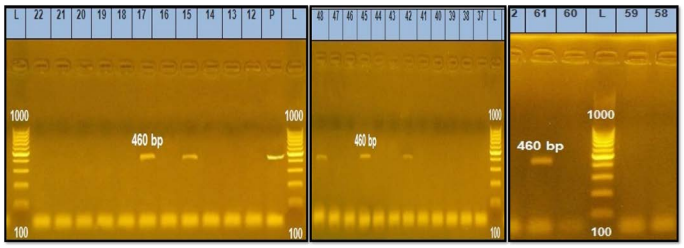

Figure 5: Agarose gel photo-documentation of conventional PCR on genetic material extracted from *P. multocida* strains as a molecular typing for detection of *KMT1* gene. Lane L, Ladder (Molecular weight marker, 100-1000 bp); Lane P: positive control, Lanes 15, 17, 42, 45, 48, 61 positive samples with amplicon size of 460 bp, the rest lanes are negative samples.

The major clinical signs observed were fever of 40 - 41.5°C, depression, cough, loss of appetite with watery nasal discharge that became mucopurulent at a later stage. Finally, the camel would become recumbent with extended neck straight along the ground with some dying within 8-9 days (Bekele, 1999).

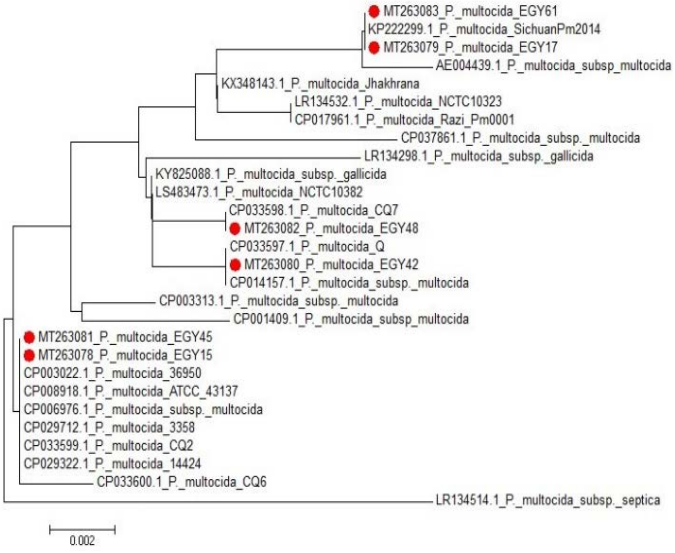

Figure 6: The Phylogenic tree (Neighbor joining tree) of the isolated *P. multocida* strains versus the ancestors.

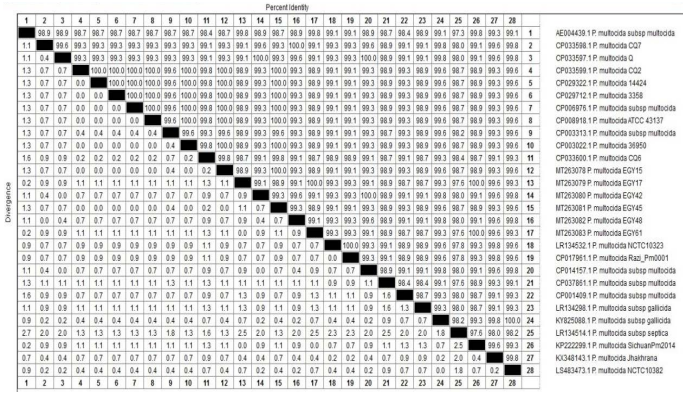

Figure 7: The Sequence distance and the identity percentage between the isolated strains (*P. multocida*) and the common referral ancestors.

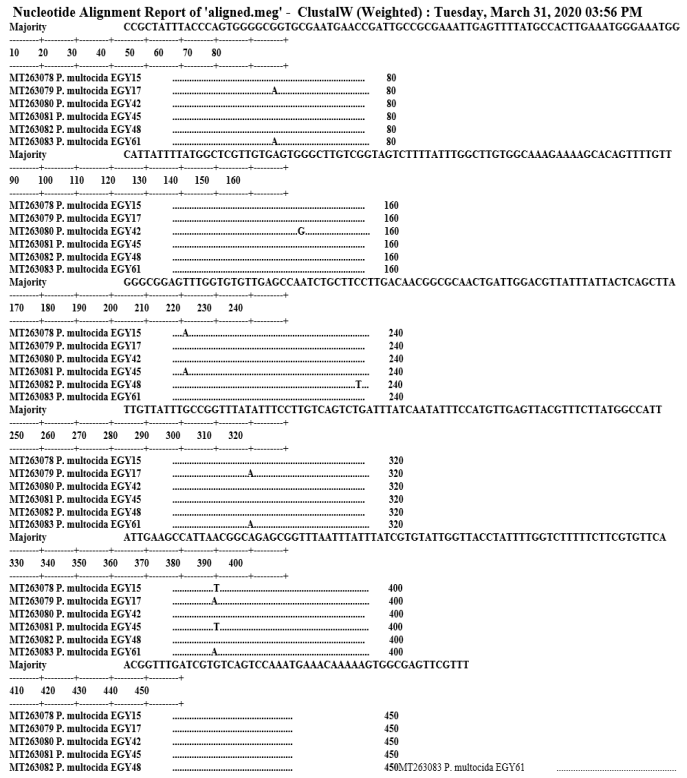

Figure 8: The Nucleotide Alignment Report of the isolated strains (Clustal W).

Our findings from bacteriological examination revealed isolation percentage of 36.9% (41 out of 111 swabs) from nasopharyngeal swabs and 86.6% (26 out of 30 tracheal swabs andtissue samples). Our results were not aligned with Abo-Elnaga and Osman (2012) who found that, the incidence rate of *P. multocida* was 2.9% in the examined camel lungs, Wareth et al. (2014) who recorded the isolation rate was 2,85% also from lung samples, Kibruyesfa (2015) who isolated *Pasteurella* spp. from lungs with incidence of 5.7% as well as El-Tawab et al. (2016). The variation in the isolation percentage may be due the management and biosecurity measures as well as the sampling time.

Concerning the pathogenicity test, our results were agreed with OIE Terrestrial (2008, 2012) and El-Tawab et al. (2016) who report that all isolates of *P. multocida* were highly pathogenic and causing severe clinical disease and manifestation.

A study carried out in northern Jordan for examination of 284 lungs from slaughtered camels (age range from 6 months to 10 years old) revealed that *M. haemolytica* and *P. aeruginosa* were the most frequent organisms from cases of chronic proliferative bronchopneumonia and chronic pleuropneumonia, while *E. coli* and *Klebsiella* spp. were the most frequent isolates from cases of interstitial pneumonia. *S. aureus*, *A. pyogenes* and *hemolytic streptococci* were the most frequent isolates from lung abscess cases (Al-Tarazi, 2001). Examining the bacteria of internal organs from 14 dead camels with severe respiratory symptoms in that study revealed the presence of *P. multocida* in 86.6% of the sampled organs. *P. multocida* subspecies multocida (serotype B) represented 85% of the isolates, while *P. multocida* subspecies septica (serotype A) was isolated only from one lung specimen (1.7%) (Seleim et al., 2003). In the same way, a parallel study in goats suffering respiratory manifestation, (Mousa and Soliman, 2016) isolated *P. multocida* and *M. haemolytica* at a rate of 18.1% and 81.8%, respectively.

Identifying bacterial species involved in lung lesions of camels slaughtered at Addis Ababa abattoir enterprise (2009–2010), Ethiopia revealed a total of 54 bacterial species were isolated and identified. These included coagulase negative staphylococci (21.1%), *Streptococcus* species (19.3%), *Escherichia coli* (17.5%), *Francisella tularensis* (5.3%), *Flavobacterium* species (5.3%), *Rhodococcus equi* (5.3%), *Bordetella bronchoseptica* (3.5%), *Aeromonas hydrophila* (3.5%), *Neisseria* species (3.5%), *Streptococcus agalactia* (1.8%), *Staphylococcus aureus* (1.8%), *Pasteurella trehalosi* (1.8%), *Pasteurella anatipestifer* (1.8%), *Pseudomonas aeruginosa* (1.8%), *Micrococcus* species (1.8%) and *Mycobacterium* species (5.3%) (Awol et al., 2011). Moreover, other studies focus the possible causes of respiratory manifestation in camels that need comprehensive diagnostic procedures, where the examination of lung samples from camels of different ages and different parts of the Sudan, revealed that only one sample was found positive for parainfluenza 3 (PI3), adenovirus, respiratory syncytial virus (RSV) and Bovine viral diarrhea (BVD) as a mixed infection (Muna et al., 2015). A study targeting the isolation and molecular characterization of hemorrhagic septicemia in camels (2014 – 2016), where nasopharyngeal swab s from 30 camel in Mersa-Matruh province and lung samples of 120 camel (70 slaughtered in Basateen abattoir in Giza Governorate and 50 slaughtered in Al-Shohada abattoir at Al-Menofia Governorate), where *P. multocida* was isolated only from the examined lung samples with percentage of 4.2% and

conversely, all the 30 nasopharyngeal swabs were negative (El-Tawab et al., 2016).

Molecularlley, all isolates were subjected to PCR for detection of KMT1 gene with amplicon size of 460 bp, PCR analysis confirmed that only six isolates out of 67 as a total isolates number at a rate of 9% were belonged to *P. multocida*. However, PCR of *M. haemolytica* failed to detect SSE specific gene. Our findings were nearly agreed with that reported by Kasivalu et al. (2021), where a total of 16% of samples showed amplification product with *KMT1* primers and disagree with that reported by Tahamtan et al. (2016) as *KMT1* gene was observed for all isolates of *P. multocida*.

Also, KMT1 was also used in the *P. multocida* multiplex capsular PCR typing assay (Townsend et al., 2001; Eid et al. 2019; Hemeg et al., 2020).

A total of 69 nasopharyngeal swabs and blood samples from clinical diseased camels, 115 nasopharyngeal swab s from apparently healthy camels, and lung samples from 10 dead camels were collected for bacteriological isolation and PCR assay. *P. multocida* was isolated from 80% of the internal organs of the dead camels, while 68% and 7% from the clinical cases and healthy camels, respectively (Tahamtan et al., 2016). In the same way, some reports recorded that the *Pasteurella* spp. were the main isolates from pneumonic lungs of camel, where the prevalence was high as 56% (Al-Rawashdeh et al., 2000); low as 1.07 and 2.85% (Tigani et al., 2007; Wareth et al., 2014, respectively) and may be very low as 0.04% (El-Deeb, 2015). Owing to *P. multocida*, the recovery rate from pneumonic lung lesions were 2.9% (Abo-Elnaga and Osman, 2012), 4.4% (Abubakar et al., 2010) and 10.7% (Chitgar et al., 2014), while *M. haemolytica* was 0.3% (Abubakar et al., 2010), 1.4% (Abo-Elnaga and Osman, 2012), 6.6% (Al-Tarazi, 2001) and 7.4% (Mahmoud et al., 2005).

A review article consolidates the respiratory problem in camels, where they found that the most common bacterial species isolated from lesions of pneumonic camels were *Staphylococcus aureus, Corynebacterium pyogenes, Streptococcus pyogenes, Escherichia coli, K. pneumonia, Pseudomonas aeruginosa, Arcanobacterium pyogenes, M. haemolytica* and *P. multocida*. The most common viral causes of pneumonia were PI3, Adenovirus, Respiratory Syncytial Virus (RSV), Bovine Herpes Virus-1 or Infectious Bovine Rhinotracheitis (IBR) and Pestivirus or Bovine Viral Diarrhea Virus (BVD) (Ismai, 2017; Hemeg et al., 2020). The six *P. multocida* isolated in the present work located in three sets of strains that were not antigenically identical or related to each other.

CONCLUSIONS AND RECOMMENDATIONS

Respiratory problem/ diseases are the main problem that affects camels, little was known about the causes of this problem, where many causative agents such as viruses, bacteria, fungi and parasites may be incriminated in the respiratory problems in camels. *P. multocida* was found as a common detectable isolate from the most camel cases as well as the apparently healthy ones. Also, Camels should also be vaccinated every six months, in tandem with other contact animal species, to ensure full and limited control measures against pasteurellosis in Egypt. Further studies are needed to fulfill the gab in the camel researches to know the ecology of the disease differences between camels and other allied ruminants and food animals.

NOVELTY STATEMENT

Common camel problems and shortage of papers on camel diseases made this work shed light on the Pasteurella and their importance in camels as has become a serious respiratory threat as well as detection of their virulence genes and the differentiation between Mannheimia and Pasteurella owing to the associated problem either on the phenotypic and genotypic levels. Also, Phytogenic tree for the isolated P. multocida strains.

AUTHOR'S CONTRIBUTION

All authors share in the work design, practical section as well as, analysis of the results, writing and revising of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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