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Virulence Genes and Antimicrobial Resistance Profile of *Pasteurella multocida* Strains Isolated From Buffaloes

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Abstract : Aim: Isolation, identification, antibiogram pattern and PCR for serotyping as well as detection of virulence genes of *Pasteurella multocida* from diseased and apparently healthy animals.

Methods and results: A total of 270samples were collected from clinically healthy (70), and from diseased buffaloes (50) from each were taken nasal and nasopharynx swabs, in addition (30) lung samples were collected from different localities of El-Sharqia province and Cairo abattoir were examined. Forty three isolates of *P. multocida* were recovered. All isolates were found pathogenic to mice by mouse pathogenicity test. Capsular typing of (43) isolates of *P. multocida* revealed that (30) isolates were associated with serogroup A and (13) isolates were identified under serogroup D by multiplex PCR. Antibiogram assay of all the field isolates detected the highest sensitivity to ciprofloxacin, Gentamicin, Ceftriaxone and Cefotaxime *P. multocida* type A was sensitive, while moderate sensitive with Norfloxacin, Enrofloxacin, Ceftazidime, Cefotaxime. (*P. multocida* type D) while sensitive with Flumequine, Cloxacillin, Cefpodoxine, Cephalothin and Ampicillin were resistant. For detection of virulence gene, all isolates were found to be positive for *toxA*(toxigenic) gene by PCR, indicating the isolates as toxigenic while all isolates were negative for *tbpA*(transferrin binding protein encoding) gene.

Conclusion: Due to *Pasteurella multocida* showed increasing level of resistance to the antibiotics, therefore recommended to monitor the antibiotic sensitivity to design the effective regimen for treatment of the disease. This infections with *P.multocida* were the causes of many disease conditions in buffalo in Egypt and were usually present concomitantly with different bacterial agents. Poor environmental conditions probably served as predisposing stress factors that may result in outbreaks among buffalos.

Significance and impact of the study: As all the field isolates were similar in cultural, morphological, biochemical and two capsular typing can be used for development of HS vaccine to control the disease.

Keywords : Buffaloes - *Pasteurella multocida* - Virulence gene - Antimicrobial resistance - toxA - Hemorrhagic septicemia.

Introduction:

Bovine respiratory disease is an economically important disease. worldwide losses of the feedlot industry are rated to be $3 billion/year **(Watts and Sweeney,[1]).** *Pasteurella multocida* is a commensal in the

upper respiratory tract of many animals **(Mutters et al. , [2])**. The organism also causes diseases in domestic animals, being responsible for pneumonia among cattle(**Frank, [3])** and hemorrhagic septicemia among cattle and buffaloes (**Bosch et al., [4])**. *Pasteurella multocida* present as a natural inhabitant of the mucosal surfaces of upper part of the respiratory tract of buffaloes, and under predisposing environmental or management conditions which constitute stress for the animals such as transport (shipping fever), marketing, change of feed, climate or ventilation **(Radostits etal., [5]).** The disease is per acute, having a short clinical course, involving severe depression, pyrexia, submandibular edema, and dyspnea, followed by recumbency and death **(Horadagoda et al., [6])..**Diagnosis of the Pasteurellosis based on the clinical symptoms, isolation and identification of the causative organism. Also serological identification of five capsular types (A, B, D, E, and F) have been recognized in *P. multocida* based on IHA test and multiplex PCR system (**Harper et al.,[7]).***P. multocida* type A is one of the bacteria associated with bovine pneumonia. It has been frequently isolated from pneumonic and healthy calves. By itself, this bacterium does not usually cause serious disease, but it can be a significant pathogen if associated with other bacteria, viruses, or Mycoplasma as predisposing factor when calves are stressed **(Ishiguro,[8]).**

The PCR-based capsular typing assay was reliable and rapid assay for determining the capsular types of a large number of *P. multocida* isolates (**Townsend et al.,[9]).**

Analysis of the susceptibility status of bovine *P. multocida* isolates from respiratory tract infections conducted in different European countries during the ARBAO-II study 2003–05 revealed, in general, low percentages of resistance, which proved to be particularly true for newer antimicrobial agents such as ceftiofur, cefquinome, florfenicol and fluoroquinolones (**Hendriksen and Mevius,[10]).**

In the last two decades, shift in antibiotic sensitivity spectrum of *Pasteurella* is well evident from observed clinical resistance against conventional antibiotics, which lead to problems in treating the animals suffering from Pasteurellosis. This warrants the need for pre-testing of antibiotics sensitivity against *Pasteurella*, to find out an effective antimicrobial agent to be used by the veterinarian (**Varte et al.,[11]).**

Antibiotics are used for treatment of pneumonia and hemorrhagic septicemia. However, the prolonged and indistinctive use of antibiotics likely to cause multi-drug resistant (MDR) forms of *P.multocida* have emerged **(Arora et al.,[12]**). Antibiotic resistance of *Pasteurella* isolates varies according to time, the host animal species, geographical distribution and antibiotic pretreatment of the animals **(Caprioli et al., [13]). Rind and Shaikh [14]** reported that *P. multocida* was highly sensitive to neomycin and tetracycline (100%), followed by chloramphenicol and tetracycline and their effects on the species were recorded as 93.3% and 96.6%, respectively**.Khamesipour et al., [15]**concluded that all the *P. multocida* isolates were susceptible to ciprofloxacin, co-trimoxazole, doxycycline, enrofloxacin, nitrofurantoin, and tetracyclines.

Ewers et al., [16]showed that some genes, including *PfhA*, *tbpA* and dermonecrotoxin encoding gene (*toxA*), as well as capsule biosynthesis genes, can be important epidemiological marker genes for characterizing *P. multocida* field strains. Moreover, some of these genes play a role in the virulence of bacteria. Among these genes, it was reported that *toxA* alone is associated with the disease status in swine and *PfhA* and *tbpA* are associated with bovine diseases**.** In addition **(Shayegh et al.,[17] and Ranjan et al., [18])** found a high prevalence of *tbpA* and *toxA* among ovine population.

Also, many studies have reported the presence of *tbpA* in bovine isolates of *P.multocida* which is associated with pneumonia and hemorrhagic septicemia **Ewers *et al.*,[16].**

The PCR assay was highly specific and sensitive and provided rapid detection of *P.multocida*, regardless of the purity of the samples by other research groups in India and abroad. In routine diagnostic procedure, it is difficult to obtain a pure culture of *P. multocida* from clinical samples because of contaminants and/or death of organisms therefore, only PCR positive samples could yield isolation of the organism (**Kalorey et al., [19]).**

The aim of the present study is directed for the isolation and identification of the pathogen from diseased and apparently healthy animals and complete identification of these isolates, Antibiogram pattern and PCR for serotyping as well as detection of virulence genes.

Materials and methods:

Samples:

A total of 270samples were examined one hundred and twenty samples from each of nasal and nasopharynx swabs from buffaloes were collected from different localities of El- Sharqia province, there were clinically healthy (70) and diseased buffaloes (50) were examined. in addition of (30) lung samples were collected from Cairo abattoir .

Isolation:

All the samples were plated onto 10% sheep blood agar (SBA), nutrient agar and MacConkey's agar. All plates were incubated at 37°C overnight. The isolates have small glistening mucoid dewdrop-like colonies on blood agar while no growth on Mac Conkey's agar. They were Gram-negative coccobacilli. Morphological identification of suspected *P.multocida* isolates were carried out according to the Cambridge standard biochemical tests **Barrow and Feltham [20].**The cells were stored in brain heart infusion (BHI) with30% glycerol at-70 °C.

Biochemical characterization:

Biochemical tests for all the isolates were performed, the peptone water grown culture of each isolate was inoculated into 1% glucose, sucrose, sorbitol, manitol, fructose, dulcitol, lactose, silicin, arabinose and maltose and incubated aerobically at 37°C for 72 hours. Indol, oxidase, catalase, urease production and nitrate reduction tests were carried out according to their standard bacteriological procedure **(Carter, [21]).**

Pathogenicity test:

Experimental infection complied with relevant professional and institutional animal welfare policies

Eight to ten-week-old male mice were purchased from laboratory animal house of hospital, Giza, Egypt. The mice were provided with clean water and solid feed.

All animal studies were performed in accordance with the guidelines and permission of the animal experiment care and general good health was observed regularly

Pathogenicity of each isolate was tested in six weeks old Swiss albino mice. Three mice were used for each isolate. Mice were inoculated intra-peritoneally with 0.1 ml of inoculum containing $0.3x10^8$ organisms per ml in sterile normal saline. Control mice were injected with 0.1 ml of sterile saline. All the mice were kept under observation and mortality was recorded. Blood smears were prepared from the heart blood of dead mice and stained with Giemsa stain. Re-isolation of *P. multocida* from heart blood of the deadmice was carried out on sheep bloodagar **(Buxton, and Fraser, [22]).**

Antibiogram assay:

Each of the isolate was tested for its sensitivity against 13 different antibiotics (Ciprofloxacin, Flumequine, Norfloxacin, Enrofloxacin, Gentamicin, Cloxacillin, Ceftriaxone, Cefpodoxine, Cephalothin, Ceftazidime,Cefotaxime , Ampicillin and Trimethoprim) using disc diffusion method **(CLSI., [23])**. An eighteen hours culture of each isolate in Brain Heart Infusion broth was plated on Muller-Hinton agar medium enriched with 5% sheep blood. The culture was allowed to adsorb for 10 minutes and then the antibiotic discs (Oxoid) were placed on the plate at an appropriate distance from each other. The plates were incubated aerobically at37°C for 24 hours. The diameters of inhibition zones surrounding the antibiotic discs were measured and subsequently matched with the standard inhibition zone diameters of respective antibiotic discs. On the basis of size of inhibition zones of variousantibiotics, the isolates were classified assensitive, intermediately sensitive or resistant.

Capsular typing by multiplex-PCR according to(Shayegh et al., [24]):

The capsular types were determined by multiplex PCR. The PCR amplification was conducted directly on bacterial culture stock composed of BHI (70%) and glycerol (30%) without genomic DNA extraction step.

Each 25 µl of PCR reaction contained 0.4 µl of bacterial glycerol stock as DNA template, 1 U of DNA *Taq*-polymerase, 3.2 mM from each primer, 200 µM of each dNTP, 1x PCR buffer, and 2 mM MgCl2. The PCR reactions were initiated by an initial denaturation at 94°C for 5 min followed by 35 cycles, each cycle consisting of DNA denaturation at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 30 s. The cycles were followed by a final extension at 72°C for 5 min. Amplified PCR products were separated on 2% agarose gel using electrophoresis, and finally stained with ethidium bromide and photographed.

Virulence gene detection using PCR according to (Tang et al.,[25]):

Capsular typing and Virulence gene detection were applied on RLQP (Reference Laboratory for Veterinary Quality Control on Poultry Production)

DNA extraction: DNA extraction from samples was performed using QIAamp DNA Mini Kit (Qiagen,Germany,GmbH) with modifications from the manufacturers recommendations. Briefly, 20 µl of the suspected colonies was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{o}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturers recommendations. Nucleic was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer: primers used were supplied from **Metabion(Germany)** are listed in Table (1).

Table 1: Primers sequences, target genes, amplicon sizes and cycling conditions

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification(35cycles)			Final extension
				Secondary denaturation	Annealing	extension	
tox*A*	CTTAGATGAGCGACAAGG GAATGCCACCTCTATAG	864	94^{o}C 10 min.	94^{o}C 1min.	48^{o}C 1min.	72^{o}C 1min.	72^{o}C 1min.

PCR Amplification: Primers were utilized in a 25 µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmpl concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR products:

The products of PCR were separated by electrophoresis on 1% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5 V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A 100 bp plus DNA ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a Gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software

Results:

1. Bacteriological finding:

Out of 270 samples collected from buffaloes, forty three samples were positive for *Pasteurellamultocida* as shown in Table (2)

All the isolates exhibited smooth glistening, translucent colonies on nutrient agar, failed to grow on MacConkey's agar and produced non hemolytic dewdrop like colonies on sheep blood agar. Grams stained smears from all isolates revealed microscopically gram negative bipolar coccobacilli and typical biochemical for *P.multocida*.

Table 2: Prevalence of *Pasteurella multocida* isolated from clinically healthy and diseased buffaloes

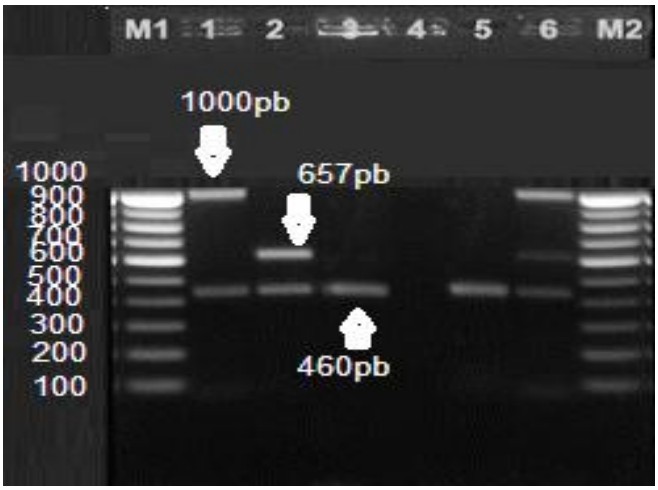
Samples		Clinically healthy Buffaloes (70)		Diseased buffaloes (50)		Slaughtered buffaloes (30)		Total	
Type of samples	No	No	%	No	%	No	%	No	%
Nasal swab	120	3	4.28	10	20	0	0	13	10.83
Nasopharynx swabs	120	9	12.85	13	26	0	0	22	18.33
lung	30	0	0	0	0	8	26.66	8	26.92
Total	270	12	8.6	23	23	8	26.66	43	15.92

2. Pathogenicity:

All the field isolates killed mice within 24 to 36 hours post inoculation. Giemsa stained smears prepared from heart blood of dead mice revealed bipolar organisms. Also, from heart blood of injected mice colonies representative of *P.multocida* were isolated on Sheep blood agar.

3. Capsular typing:

Capsular genotyping was conducted based on amplification of five different Capsular groups using multiplex PCR in the presence of each Capsule specific primers, A pair of *P.multocida* specific primers was also added into the reaction for PCR confirmation of the isolates. The presence of DNA band, about 460 bp size, further established the identification of the isolates as *P.multocida*. All isolates were typed by PCR amplification. Amplified DNA products of 1000 and 657 bp corresponding to *P.multocida* Capsular group A and D respectively were observed. Of the (43) *P.multocida* isolates (30) were identified as Capsular type A and (13) Capsular type D respectively Table (3) and (Fig. 1).



(Figure 1): Capsular typing of *Pasteurella multocida* by multiplex PCR Lane M1 and M2: 100 bp DNA ladder, Lane 1: Capsular type A, Lane 2: Capsular type D, Lane 3: *P. multocida*, Lane 4: Negative control, Lane 5: *P. multocida*and Lane 6: Positive control for capsular type A and D.

Table3: Results of Capsular typing of *Pasteurella multocida* isolated from clinically healthy and diseased buffaloes:

Type of Samples	Clinical status	Results of *Capsular* gene PCR	
		A	D
Nasal swab	Clinically healthy	2	1
Nasopharynx swabs	Clinically healthy	6	3
Nasal swab	Diseased buffaloes	7	3
Nasopharynx swabs	Diseased buffaloes	7	6
lung	Diseased buffaloes	8	0
Total		30	13
%		69.76	30.23

4. Antibiogram assay:

Forty three isolates of *P.multocida* showed different results to 13 used chemotherapeutic agents as shown in Table (4).

Table 4: Antibiogram pattern of forty three isolates of *Pasteurellamultocida* isolated from clinically healthy and diseased buffaloes

Chemotherapeutic agents	Conc. µg	*Pasteurellamultocida* (A)	*Pasteurellamultocida* (D)
Ciprofloxacin	CFR_5	S	S
Flumequine	UB_{30}	R	R
Norfloxacin	NX_{10}	R	M
Enrofloxacin	ENR_5	M	R
Gentamicin	G_{10}	S	S
Cloxacillin	$O\ B_5$	R	R
Ceftriaxone	CRO_{30}	S	S
Cefpodoxine	CPD_{10}	R	R
Cephalothin	KF_{30}	R	R
Ceftazidime	CAZ_{30}	M	M
Cefotaxime	CTX_{30}	S	M
Ampicillin	AMP_{10}	R	R
Trimethoprim	Tr_5	M	M

Virulence genes detection using multiplexPCR analysis:

Virulence genes detection was conducted based on amplification of two virulence factor genes using multiplex PCR in the presence of specific primers. Amplification of DNA bands with about 864bp sizes were addressed to the presence of *toxA* gene with all isolated strains of *P.multocida* with capsular type A and D ,while with *tbpA* gene was absent in isolated strains of *P.multocida* type A and D. as shown in Table (5) and Fig. (2).

Table 5: Virulence genes detection using PCR analysis

Samples	*ToxA* Results	*PtfA* Results
(1) capsular type A	Positive	Negative
(2) capsular type D	Positive	Negative

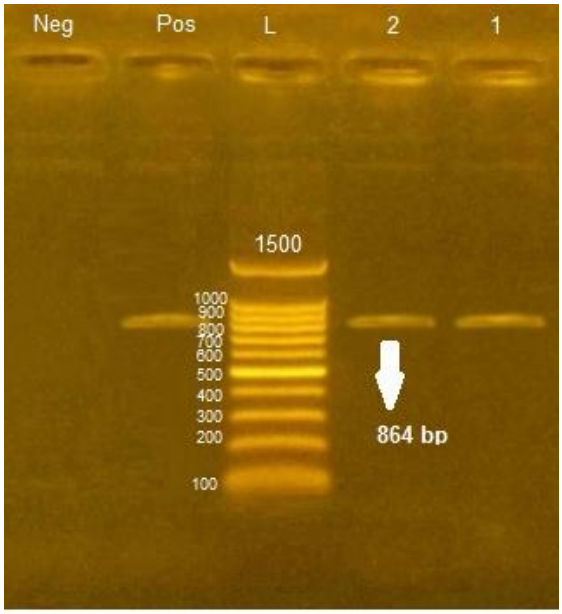


Fig 2: Virulence *ToxA* gene of *P.multocida* detection using multiplex PCR analysis, negative control, positive control for *ToxA* gene 864 bp , lane L 100-1500 Marker lane 2 capsular type D, lane 1 capsular type A.

Discussion:

Bovine respiratory disease (BRD) due to *P.multocida* is a multifactorial and multiagents disease whose colloquial designation, 'shipping fever', refers to some of the factors that play a relevant role in the development of the disease. Transportation over long distances, often associated with exhaustion, starvation, dehydration, chilling or overheating, depending on weather conditions serves as a stressor. Additional stressors include passage through auction markets, commingling of animals from different herds, dusty environmental conditions in the feedlot and nutritional stress associated with changes in diet **Naz et al., [26].**

The present study revealed that isolation of 43 strains of *P.multocida* out of 270 examined samples and relatively low recovery of *P. multocida* from nasal swabs collected from buffaloes. This might be due to the random sampling of the animals. Although the sample size might be low for any conclusive remarks, it revalidates the fact that *P. multocida* is a common inhabitant of buffaloes in the nasopharyngeal areas. Isolation of *P. multocida* from apparently healthy buffaloes indicated that these animals were prone to disease under stress conditions these results matched with those reported by **Kumar et al., [27]** the isolation of *P.multocida* from the lung samples and nasopharynx swabs with a relative high percentage reported by **(OIE [28]; Mark et al., [29])** they mentioned that *P. multocida* were isolated from lung and lymph nodes with high level than liver, brain, samples. In addition **Shayegh et al., [30]** isolated *P.multocida* from lung tissue of cattle and buffaloes suffering of pneumonia in a percentage of (3.7%).

Meanwhile, All the field isolates of *P.multocida* were found pathogenic for mice and killed the mice inoculated within 6-24 hours post-infection these results agree with those mentioned by **Ramdani et al., [31] and Varte et al., [11]. Khamesipouret al., [15]** reported that 30 *Pasteurella multocida* strains isolated from pneumonic and apparently health slaughter cattle.

Also this research was carried out to determine capsular types of *Pasteurellamultocida* isolated from healthy and diseased buffaloes. The results of capsular typing of *P. multocida* strains are present in **Table (3) Fig (1).**

PCR showed that capsular type A and D *Pasteurellamultocida* isolates exhibited two different banding patterns ranging between 657-1000 bp **(Fig1).**

In the present study, it was also observed that PCR pattern of *P. multocida capsular type A* was different from that of capsular type D but no discriminatory patterns were recorded within the same capsular type. This result of this study is also in accordance with previously published reports by various research groups. **Dutta et al., [32]** and **Ranjan et al., [18]**characterized by *P. multocida* strains of different serotypes by PCR and observed a unique banding pattern for individual serotypes.

Two genotypes (A and D) were found among isolated strains of *P. multocida*, 30(69.76%), 13(30.23%) isolates were capsular type A and D respectively. These results agree with

Varte et al.,[11]isolated *P. multocida* from nasal swabs 15 swabs (3.75%) which grouped under capsular type A by multiplex PCR. and all isolates were found to be negative for PCR indicating that the isolates is non-toxigenic. **Shayegh et al., [24]**isolated *P. multocida* from cattle and buffaloes were under capsular serogroups A, B, and D were found among both *P. multocida* and septica subspecies, type A (46.15%) as type B while type D (26.92%).

On the other hand **Naz et al., [26]** isolates *P.multocida* from (HS) of buffaloes and serologically all isolates were associated with carter's serogroups B. **Khamesipouret al., [15]** reported that 30 *Pasteurella multocida* strains isolated from pneumonic and apparently health slaughter cattle. Of the isolates, 23 belonged to capsular type A, 5 to capsular type D and two isolates were untypeable.

Antibiogram pattern upon forty three pure isolates of *P. multocida* were tested for their sensitivity against (13) different antibiotics available for the treatment of bacterial infections in animals as shown in table (4). All of the 43 isolates were found highly sensitive to ciprofloxacin, Gentamicin and Ceftriaxone, while moderate sensitive with Norfloxacin, Enrofloxacin, Ceftazidime ,Cefotaxime.(*P. multocida* type A was sensitive). These result agree to those reported by many author, **Kumar et al., [27] and Shayegh et al., [33]** whosementioned that 100% sensitivity of the isolated *P. multocida* to ciprofloxacin and enrofloxacin, also **Varte et al., [11]**mentioned that Majority of the isolates of *P. multocida* were sensitive to ciprofloxacin, enrofloxacin, gentamicin. Also **Carty et al., [34]**reported thatamong aminoglycosides, gentamicin was found highly effective (87.5%) against field isolates. In contrast to our finding **Carty et al., [34]** observed the acquired resistance of *P. multocida* isolates to enrofloxacin **Yoshimura et al., [35]** also found aminoglycosides as less effective, also they study all tested strains of *P. multocida* were resistant to flumequine, cloxacillin, cefpodoxine, cephlothin and ampicillin and variable results with the remaining used of antibiotics.

Similar observations were also recorded by **Wattset al., [36]** who frequently encountered resistance of *P. multocida* of bovine origin to sulfamethazine.

Varte et al., [11]reported that the lower sensitivity of *P. multocida* to amikacin could be due to the gradual development of resistance towards these antimicrobial drug. Also**Badr, [37]** recorded that *P. multocida* was increase resistance to ampicillin.

In the last two decades, shift in antibiotic sensitivity spectrum of *Pasteurella* is well evident from observed clinical resistance against conventional antibiotics, which lead to problems in treating the animals suffering from Pasteurellosis. This warrants the need for pre-testing of antibiotics sensitivity against *Pasteurella*, so as to find out an effective antimicrobial agent to be used by the veterinarian. **Varte et al., [11].**

None of the isolates were untypeable. These results agree with those reported by many authors , **Choi et al., [38]**and **Shayegh et al., [33]** isolated *P. multocida* from cattle and buffaloes in Iran and carried out capsular typing of 17 isolates were under three capsular types (A , B and D) . In this study, a higher prevalence of type A of *P.multocida* and lower prevalence of type D. These results is also in accordance with previously published reports by various research groups **Kalorey et al., [19].**

This research was carried out to determine capsular types, and some virulence factor (*toxA* and *tbpA*), the high prevalence of capsular type A than type D among the isolated strains of *P. multocida*, furthermore PCR for some virulence gene detection showed important role of *toxA* genewith two types of isolated *P. multocida* in buffaloes Amplification of DNA bands with about 864bp, while all isolates were negative for (*tbpA*). This virulence factor showed their association with diseased status. On the other hand there is no incidence detections of *tbpA* in two types of isolated *P. multocida*. These results were opinion with those recorded by

Ranjan et al.,[18] also described that *toxA* gene based PCR can be used for direct analysis of toxigenic *P.multocida* without additional hybridization.

Shayegh et al.,[24]in detection of virulence genes using multiplex PCR in presence of specific primers Amplification of DNA bands with about 265bp ,728bp and 846bp size were addressed to the presence of *pfhA* and *toxA* from cattle and buffaloes. Also they reported the prevalence of *toxA* is bovine isolates in this study. Strain with *toxA* gene was previously reported in cattle **Jaglic et al.,** [39] and **Ewers et al.,** [16].

Shayegh et al., [24]found high prevalence of *Pasteurella* Filamintous haemagglutinin A(*pfhA*) and transferrin binding protein encoding gene(*tbpA* gene) from apparently healthy animals, that seem to be associated with disease status in cattle. *P.multocida pfhA*(+),*tbp*(+) strains are commensal in healthy hosts similar to *P.multocidapfhA*(-),*tbpA*(-) strains. But they can induce disease in susceptible animals under predisposing condition.

Conclusion,

All the field isolates were similar in cultural, morphological, biochemical and two capsular typing can be used for development of HS vaccine to control the disease. The local isolates of *Pasteurella multocida* showed increasing level of resistance to the antibiotics that are extensively used in field for treatment of the disease. It is therefore recommended to monitor the antibiotic sensitivity of *Pasteurella multocida* from time in future to design the effective regimen for treatment of the disease.

This investigation revealed that infections with *P.multocida* were the causes of many diseased conditions in buffalo in Egypt and were usually present concomitantly with different bacterial agents. Poor environmental conditions probably served as predisposing stress factors that may result in outbreaks among buffalos.

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