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Molecular evaluation of sheep and goats isolates of *Pasteurella multocida* and their antibiotic resistance

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Abstract

Pasteurella multocida exists as a commensal in the upper respiratory tracts of livestock, and poultry, and causes a wide variety of diseases in humans and animals. This study aimed to investigate the incidence of *P. multocida* by bacteriological and molecular characterization in sheep and goats and screening the existence of capsule-specific genes and their antibiotic resistance pattern. Totally, 1650 nasopharyngeal swabs were collected from apparently healthy sheep and goats and 460 lung tissues were collected from slaughtered animals in Fars province, Iran. All samples were cultured and suspected colonies were examined by biochemical tests, antimicrobial assay and polymerase chain reaction (PCR). Among 165 *P. multocida* (104 sheep and 61 goats) isolates, the *capA*, *capD*, and *capB* genes were amplified in 98, 48, and 12 isolates, respectively. The occurrence of four virulence-associated genes of *P. multocida* isolates were determined by PCR. Most isolates harbored the *toxA* (79.40%) and *hgbB* genes (70.90%) and 59.40% of isolates had the *pfhA* gene. Almost half of the isolates (46.10%) contained the *tbpA* gene. According to the current study, *P. multocida* capsular type A had the most frequency followed by type D. In addition, the high frequency of *tbpA*, *pfhA*, *toxA*, and *hgbB* genes revealed that these genes are possibly important in the pathogenesis of *P. multocida*. Oxytetracycline, enrofloxacin, florfenicol, and tilmicosin were the most effective drugs.

Introduction

Pasteurella multocida is a small, Gram-negative, non-motile, non-spore-forming cocco-bacillus with bipolar staining features.[1] Under the light microscope, *P. multocida* cells could be seen single and sometime as pairs or short chains. *P. multocida* often exists as a commensal in the upper respiratory tracts of many livestock, poultry, and domestic pet species, especially cats and dogs. *Pasteurella* species are some of the most prevalent commensal bacteria present in domestic and wild animals worldwide. Also, as a human pathogen they can cause bite wounds, respiratory diseases and infection of central nervous system.[2] The virulence and etiology of *P. multocida* is strongly associated with its serotype. Isolates of *P. multocida* are classified according to the composition of capsular polysaccharide serotyping, which divides isolates into one of the five capsular serogroups A-F.[3-5] Then subtypes of isolates are identified based on their lipopolysaccharide (LPS) which separates isolates further into 16 serovars.[6] Isolate determination usually contain of a capsular serogroup letter followed by a somatic serovar number (e.g., A:1, A:2, A:3, B:2, etc.). Of the five capsular serotypes identified, serotype A is the most prevalent in sheep and goat infection. Serotype B and E are found commensally in the upper respiratory tract of healthy cattle, but can produce hemorrhagic septicemia in bovines, frequently causing high mortality rates that have devastated wild and domestic populations.[7] In a study conducted by Tahamtan *et al*. in Fars province all *P. multocida* isolated from sheep and goats belonged to capsular type A.[8]

It is known that multiple virulence factors play important roles in the pathogenesis of *P. multocida*.[9] The main structures associated with a virulence that has been identified in *P. multocida* strains are the capsule and LPS.[10] Furthermore *P. multocida* has some more important virulence factors.[9] These factors include genes encoding outer membrane proteins (ompH, oma87), genes related to iron metabolism (*hgbA*, *hgbB*, *exBD-tonB*), and those encoding fimbriae and adhesins (*ptfA*, *pfhA*, *tad*).[11] Previous studies found that the prevalence of some

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virulence-associated genes carried by *P. multocida*, including those detected for colonization factors (*ptfA*, *fimA*, *hsf2*), iron acquisition factors (*exbB*, *exbD*, *tonB*, *Fur*), superoxide dismutase (*sodA*, *sodC*), and outer membrane proteins (*ompA*, *ompH*, *oma87*, *plpB*), were broadly characteristic of the pig origin *P. multocida* isolates.[12-14]

Antimicrobial agents represent the most powerful tools to control *P. multocida* infections. However, acquisitions of resistance genes, as well as development of resistance-mediating mutations strongly reduce the efficacy of the antimicrobial agents.

The aims of the present study were epidemiological and molecular evaluation of *P. multocida* isolates according to presence of *pfhA*, *tbpA*, *hgbB*, and *toxA* genes in diseased or apparently healthy sheep and goats in Fars province as well as screening the existence of capsule-specific genes and evaluation of the most recent data on the antibiotic resistance status of such isolates.

Materials and Methods

Sample collection. From September 2017 to October 2018, nasopharyngeal swab samples were obtained aseptically from 1,650 apparently healthy sheep and goats from 75 husbandries in Fars province. Considering the expected prevalence of pasteurolosis in sheep and goats (based on previous studies) which was about 12.00%, with 3.00% accuracy and 95.00% confidence, the sample size selected by simple random sampling was 450. However, since the study was conducted in the province and sampling was necessarily multi-stage, taking into account the design effect of approximately equivalents 3, the final sample size was 1350. Considering that Fars province has 30 cities, in each city, depending on the livestock population, 2 to 3 herds were randomly selected and in each herd, depending on the number, between 15 to 20 animals were considered for sampling. From herds up to 50 animals, 15 samples, herds from 50 to 100 animals, 16 to 17 samples and larger herds from 18 to 20 samples were taken so that the final sample volume was provided. This sample size was used to evaluate the frequency of infection in sheep and goats. Sterile cotton-tipped swabs, moistened in sterile Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke, UK), then in aseptic condition and gentle rolling swabs were directed into the nasal cavities and returned in to the BHI broth containing test tubes and transported to the laboratory of bacteriology, Faculty of Veterinary Medicine, Shiraz University in cold conditions. Also, 460 pneumonic lungs of sheep and goats that contained hyperemia, rough surface, hepatization, and consolidation in cranio-ventral lobes from slaughtered animals were collected immediately after the slaughter. Each lung sample was separately placed in sterile zip-lock plastic bags, labeled and kept cool in the icebox, and transported to the laboratory of bacteriology.

Culture and isolation. As standard procedures, naso-pharyngeal swab samples were streaked on blood agar (Oxoid) containing 5.00% fresh sheep blood and MacConkey agar (Merck, Darmstadt, Germany) plates and incubated at 37.00 ˚C for 24 to 48 hr. Upon inspection, from the edge of the lung lesion, tissue samples were taken aseptically and prepared for inoculation into BHI medium. Before cutting and taking samples from inside the lung tissue, the surface of the tissue pieces was sterilized by hot metal blades and then sampling was done by sterile swabs. Then swabs inoculated onto blood agar and MacConkey agar plates and for 24 - 48 hr were placed in an incubator at 37.00 ˚C. After the incubation time, suspected colonies were inoculated onto the new blood agar plates and incubated as above. Finally typical colonies were selected and Gram stained and checks cell morphology. For final identification, mixed and Gram-negative bacteria were sub-cultured again onto the blood agar plates. According to the general appearance (morphology, color, shape, size, absence of hemolysis, and consistency) colonies on blood agar were characterized. For genus determination of isolated bacteria pure culture of single colonies further evaluated for oxidase, catalase and oxidation-fermentation reactions. Identification of bacterial species were done according to the results of primary traits, biochemical tests, sugar metabolism and indol production.[15] The isolates were confirmed as *P. multocida* via standard bacteriological methods and stored at – 80.00 ˚C for further use.

DNA extraction. Confirmed *P. multocida* isolates were cultured in the BHI broth and boiled for DNA extraction according to the method of Ewers *et al*.[16] The quantity and quality of the extracted DNA were evaluated by comparison of OD at 260 nm and 280 nm (Nanodrop; Thermo Scientific, Waltham, USA).

Master mix of polymerase chain reaction (PCR). *Pasteurella multocida* isolates, previously recognized via morphological and biochemical methods, finally were confirmed by PCR.[17] *Pasteurella multocida* isolates were investigated for capsule biosynthesis (*capA*, *B*, *D*, *E*, and *F*) and four virulence-associated genes (*pfhA*, *tbpA*, *hgbB*, and *toxA*) using PCR. The nucleotide sequences of the primers used in this study are shown in Table 1. For all the PCR tests, the PCR reaction mixture contained 12.50 µL master mix (Ampliqon, Odense, Denmark), 1.50 µL of each specific primer, 3.00 µL template DNA solutions and 6.50 µL deionized water were prepared. After initial denaturation (94.00 ˚C per 5 min), PCR amplifications were done for 35 cycles in a thermocycler (Analytic Jena, Jena, Germany), with a denaturation temperature of 94.00 ˚C lasting for 1 min, annealing temperature as shown in table 1 lasting for 1 min and extended for 1 more min at 72.00 ˚C, continuing for 7 min at 72.00 ˚C as the final extension. Then the thermocycler was set at 4.00 ˚C until the samples were collected. A negative control consisting of all components of the reaction mixture except the DNA template was

included in the PCR. The PCR products were evaluated by electrophoresis in agarose gel. After the completion of electrophoresis, to investigate amplified DNA fragments, agarose gel was transferred onto ultraviolet trans-illuminator and the existing bands were visualized (TM-26; Labnet International, Edison, USA) according to molecular weight and negative and positive controls in comparison to genomic DNA marker.

Antibiotic susceptibility assay. The isolates were subjected to an *in vitro* antibiotic susceptibility test by disc diffusion method. Isolated *P. multocida* were evaluated for antibiotic susceptibility against seven different generally using antibiotics for treatment of pasteurellosis such as penicillin, streptomycin, oxytetracycline, enrofloxacin, florfenicol, tylosin, and tilmicosin using the standard method proposed by Clinical and Laboratory Standards Institute (CLSI) and following the disc diffusion method.[18,19] From an overnight culture of each isolate on blood agar, bacterial colonies suspended in 0.85% saline with concentration equal to 0.50 of McFarland standard. Bacterial suspension was cultured onto the Muller-Hinton agar plates by sterile swabs. The cultured plates were allowed to dry for 10 min by putting them upside down in room temperature. Using sterile forceps, antibiotic discs (PadtanTeb Co., Tehran, Iran) were placed on the plate at an appropriate distance from each other and press gently to ensuring complete contact with the agar surface. Then the plates were incubated for 24 hr at 37.00 ˚C. The diameters of inhibition zones surrounding the antibiotic discs were measured by a ruler to the nearest mm. According the zone of growth inhibition; the isolates were assorted descriptively as susceptible, intermediate, and resistant. The results were analyzed using Chi-square tests in SPSS Software (version 20.0; IBM Corp., Armonk, USA) with a confidence level of 95.00%.

Results

Through the culture, 165 *P. multocida* isolates with small, glistening, non-hemolytic colonies on blood agar plates were obtained (from 1650 nasopharyngeal swabs and 460 lung samples of healthy and diseased animals), including 104 sheep and 61 goat's isolates. The isolates were rod shape in Gram staining, and their catalase, oxidase, and indol tests were positive. Finally, according to the results of other biochemical tests such as metabolism of sugars and inability to grow on MacConkey agar, it was confirmed as *P. multocida*. On lung tissues culture in this study other bacteria like *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Proteus*, *Pseudomonas*, *Bacillus*, and *Mannheimia* were also isolated. Biochemically verified isolates of *P. multocida* were approved by using the kmt1 species-specific primers and PCR assays.

Among 165 *P. multocida* isolates, the *capA gene* was obtained from 98 (59.39%), *capD* gene from 48 (29.09%), and *capB* genes from 12 isolates (7.27%). However, *capE* and *capF* genes were not detected in any of the isolates, and seven of the isolates (2.24%) lacked all capsular genes (Fig. 1A, Table 2). In the capsular typing, among the sheep and goats isolates, type A was the most common capsular type, followed by capsular type D. The existence of four virulence-associated genes in isolated *P. multocida* strains was determined by PCR (Table 2), and their association with capsular types and host origin were screened by the Chi-square test (Table 3). Most isolates harbored the *toxA* (79.39%) and *hgbB* genes (70.90%). The *pfhA* gene was detected in the 59.39% of sheep and goats isolates. Almost half of the isolates (46.06%) presented the *tbpA* gene (Fig. 1B). Based on statistical studies to investigate the

Table 1. Details of the primers and citations used for the detection of capsular type and virulence-associated genes in *P. multocida* isolates.

Primers	Sequence (5' to 3')	Target genes	Annealing temperature (˚C)	Amplicon size (bp)	References
KMT1T7 **KMT1S6**	F: ATCCGCTATTTACCCAGTGG R: GCTGTAAACGAACTCGCCAC	***kmt1***	56.00	460	17
capA	F: TGCCAAAATCGCAGTGAG R: TTGCCATCATTGTCAGTG	***hyaD-hyaC***	55.00	1,044	17
capB	F: CATTTATCCAAGCTCCACC R; GCCCGAGAGTTTCAATCC	***bcbD***	55.00	760	17
capD	F: TTACAAAAGAAAGACTAGGAGCCC R: CATCTACCCACTCAACCATATCAG	***dcbF***	55.00	657	17
capE	F: TCCGCAGAAAATTATTGACTC R: GCTTGCTGCTTGATTTTGTC	***ecbJ***	55.00	511	17
capF	F: AATCGGAGAACGCAGAAATCAG R: TTCCGCCGTCAATTACTCTG	***fcbD***	55.00	851	17
pfhA	F: AGCTGATCAAGTGGTGAAC R:TGGTACATTGGTGAATGCTG	***pfhA***	55.00	275	16, 20
toxA	F: CTTAGATGAGCGACAAGGTT R: GGAATGCCACACCTCTATA	***toxA***	55.00	865	16, 20
hgbB	F: ACCGCGTTGGAATTATGATTG R: CATTGAGTACGGCTTGACAT	***hgbB***	55.00	788	16, 20
tbpA	F: TTGGTTGGAAACGGTAAAGC R: TAACGTGTACGGAAAAGCCC	***tbpA***	57.00	728	16, 20

relationship between the presence of virulence genes and the sample taken from lung tissue or nasal swabs, it was found that there was a significant correlation between the presence of *pfhA* and *hgbB* genes, but no significant correlation was observed for *toxA* and *tbpA* genes ($p < 0.05$).

Also, *P. multocida* isolates were evaluated against seven antimicrobial agents. The majority of the isolates were susceptible to oxytetracycline, enrofloxacin, florfenicol, and tilmicosin followed by penicillin, tylosin, and streptomycin. There was no susceptibility or resistance of 100% to any antibiotic. A similar susceptibility pattern was obtained in different isolates (Table 4).

Discussion

Pasteurellosis is known as an important concern in the industry of livestock breeding. Therefore, evaluating and increasing our knowledge about the pathogenesis of *P. multocida* is of great importance. Determining the identity of pathogenesis factors can be effective in determining the pathogenesis mechanisms and developing regulative methods like producing effective vaccines.[20]

According to capsular typing, *P. multocida* isolates have been classified into five capsular types and each capsular type is generally associated with a specific disease in different hosts.[21,22] In present study, the prevalence rate

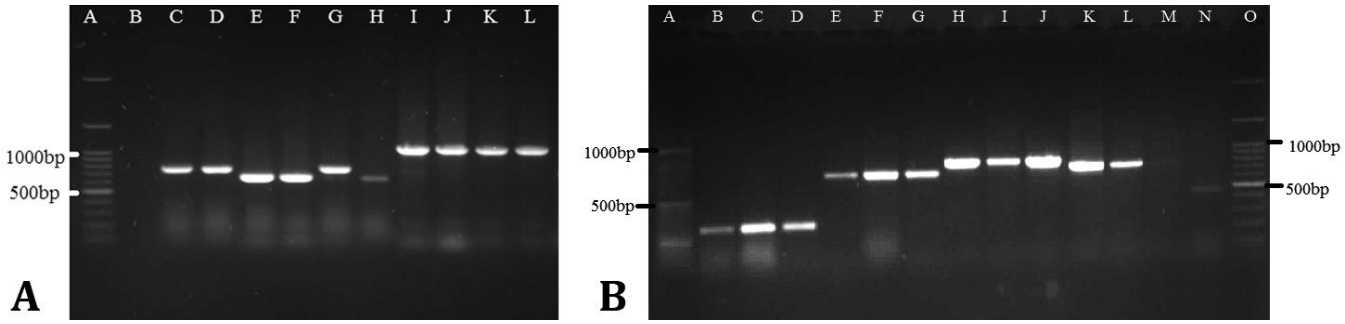

Fig. 1. A) The agarose gel electrophoresis of the polymerase chain reaction products of the capsule biosynthesis genes of *P. multocida* isolates. Lane A: 100 bp DNA marker; Lane: B negative control, Lanes C, D and G: *capB* (760 bp), Lanes E, F and H: *capD* (657 bp), Lanes I-L: *capA* (1044 bp). **B)** The agarose gel electrophoresis of the polymerase chain reaction products of the 4 putative virulence genes of *P. multocida* isolates. Lanes A and O: the 50 and 100 bp DNA marker; Lanes B-D: *pfhA* gene (275 bp), Lanes E-G: *tbpA* gene (725 bp), Lanes H-J: *toxA* gene (865 bp), Lanes K-M: *hgbB* gene (788 bp) and Lane N: kmt1 (460 bp).

Table 2. Prevalence of the capsular type and virulence-associated genes in *P. multocida* isolates.

Genes	Tissue sample (% of 91)			Swab sample (% of 74)			Total number (% of 165)
	Goat	Sheep	Total	Goat	Sheep	Total	
capA	21	25	46 (50.55)	17	35	52 (70.27)	98 (59.39)
capB	1	4	5 (5.49)	1	6	7 (9.46)	12 (7.27)
capD	19	17	36 (39.56)	3	9	12 (12.22)	48 (29.09)
Negative capsular type	1	3	4 (4.40)	0	3	3 (4.05)	7 (2.24)
pfhA	23	23	46 (50.55)	13	39	52 (70.27)	98 (59.39)
toxA	36	38	74 (81.320)	12	45	57 (77.03)	131 (79.39)
hgbB	22	30	52 (57.14)	15	50	65 (87.84)	117 (70.91)
tbpA	22	17	39 (42.86)	5	32	37 (50.00)	76 (46.06)

Table 3. Prevalence of the virulence-associated genes in *P. multocida* isolates in respect to the capsular type from swab and tissue samples.

Virulence factor genes	No. (%) of virulence factors within the following capsule serotypes				Total
	Capsular type A (n = 52)	Capsular type B (n = 7)	Capsular type D (n = 12)	Negative capsular type (n = 3)	
Swab sample (Total = 74)					
pfhA	35 (67.30)	6 (85.71)	9 (75.00)	2 (66.67)	52 (70.27)
toxA	37 (71.15)	7 (100.00)	11 (91.67)	2 (66.67)	57 (77.03)
hgbB	45 (86.54)	7 (100.00)	10 (83.33)	3 (100.00)	65 (87.84)
tbpA	20 (38.46)	3 (42.85)	12 (100.00)	2 (66.67)	37 (50.00)
Tissue sample (Total = 91)					
Virulence factor genes	**Capsular type A (n = 46)**	**Capsular type B (n = 5)**	**Capsular type D (n = 36)**	**Negative capsular type (n = 4)**	**Total**
pfhA	23 (50.00)	4 (80.00)	18 (50.00)	1 (25.00)	46 (50.55)
toxA	36 (78.26)	5 (100.00)	30 (83.33)	3 (75.00)	74 (81.32)
hgbB	33 (71.74)	4 (80.00)	13 (36.11)	2 (50.00)	52 (57.14)
tbpA	18 (39.13)	4 (80.00)	16 (44.44)	1 (25.00)	39 (4285)

Table 4. Antibiotics susceptibility assay of *P. multocida* isolates (74) from swab and (91) from tissue samples.

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
Swab sample			
Tilmicosin(15.00 µg)	56 (75.68)	11 (14.86)	7 (9.46)
Streptomycin (10.00 µg)	34 (45.95)	25 (33.78)	15 (20.27)
Tylosin (30.00 µg)	46 (62.20)	0 (0.00)	28 (37.84)
Oxytetracycline (30.00 µg)	53 (71.62)	3 (4.05)	18 (24.32)
Enrofloxacin (5.00 µg)	58 (78.38)	10 (13.51)	6 (8.11)
Florfenicol (30.00 µg)	61 (82.43)	8 (10.81)	5 (6.76)
Penicillin (10.00 IU)	46 (62.16)	12 (16.22)	16 (21.62)
Tissue sample			
Tilmicosin (15.00 µg)	70 (76.92)	7 (7.69)	14 (15.38)
Streptomycin (10.00 µg)	49 (53.85)	11 (12.09)	31 (34.07)
Tylosin (30.00 µg)	51 (56.04)	0 (0.00)	40 (43.96)
Oxytetracycline (30.00 µg)	73 (80.22)	10 (10.99)	8 (8.79)
Enrofloxacin (5.00 µg)	78 (85.71)	8 (8.79)	5 (5.49)
Florfenicol (30.00 µg)	75 (82.42)	5 (5.49)	11 (12.09)
Penicillin (10.00 IU)	60 (65.93)	14 (15.38)	17 (18.68)

of *capA* gene was higher than other micellar capsule genes. A higher number of isolates containing *capA* gene was observed in the studies of Ewers *et al*. in Germany (92.30% in bovine), Ferreira *et al*. in Brazil (75.60% in cats isolates), and Bethe *et al*. in Germany (53.40%in swine isolates).[16,23,24] Moreover, the results of the prevalence of capsule genes in this study were similar to the result of other studies carried out in Iran.[25-27] In the other hands, Tahamtan *et al*. reported that only the capsular type A was identified in all the isolates of *P. multocida* from sheep and goats in tropical and sub-tropical climate of Iran.[8]

It has been determined that *P. multocida* has some pathogenesis factors that play a significant role in the prevalence of increasing bacteria's survival .[1,16,26,28] Among these pathogenesis genes, siderophore genes (*exbBD-tonB*, *hgbA*, *hgbB*, *tbpA* and *fur*) can provide mechanisms for acquiring different sources of Heme iron in different situations.[29] Therefore, proteins codified by these genes may be required in different stages or they may be among factors that are important in *P. multocida* pathogenesis in a wide range of animals.[24] For example, hgbA and hgbB proteins can assist bacteria's growth through Hemin as a source of iron.[30]

Through PCR, 70.90% of isolates were positive for *hgbB* gene. Further, the amount of *hgbB* gene in the isolates obtained from nasopharyngeal swabs of goats was lower than isolates from sheep and this difference was statically significant ($p < 0.05$).

Based on different reports by Ewers *et al*., Bethe *et al*., and Sarangi *et al*.; most isolate harbor *hgbA* gene but presence of *hgbB* gene in the *P. multocida* isolated from different sources was different.[16,24,31] Contrary to the results of Shayegh *et al*. regarding the low prevalence of *hgbB* gene in sheep isolates (36.40%), the present study showed a high prevalence of this gene (70.90%) in sheep and goats isolates.[25]

Here, a low frequency of *tbpA* gene (46.10%) in the isolates obtained from sheep and goat was observed. In most previous studies, the presence of *tbpA* gene has a close relationship with pathogenesis of *P. multocida* and this can be an index of epidemiology among these animals.[16,32]

Hatfaludi *et al*. stated that outer membrane proteins act as selective barriers and control the entrance of harmful materials which is an important factor in protecting bacteria in different media. The gene related to connective factor (pfhA) that plays a critical role in the primary replacement of bacteria in the upper respiratory tract showed a high prevalence in the *P. multicide* isolates (59.40%).[29]

Other researchers stated that for induction of clinical signs of atrophic rhinitis in pigs and pneumonia in small ruminant's presence of *toxA* gene is very important.[1,23,25] This study, similarly, confirmed the frequency of this gene (79.40%) in small ruminants' isolates.

Statistical data indicated that some particular pathogenesis factors are significantly different in different capsular types, for example, *pfhA* and *tbpA* genes have higher frequencies in isolates with capsule type B and capsule type A, respectively. Some researchers reported that isolatas of capsuletype A as compared to type D have shown higher frequencies of *pfhA* gene.[24,33] Also, in the present study results was roughly alike and *pfhA* gene shown higher frequency in type A isolates (60.20%) in comparison with type D isolates (58.30%). However, it is not clear why this kind of gene acquisition occurs with higher frequency in type A strains.[24]

Based on our finding, isolates of capsule types A, B, and D contained *toxA* gene. Sarangi *et al*. reported that in type A isolates of small ruminants' origin *toxA* gene has high prevalence, while based on other studies capsular type D harbor *toxA* gene.[1,23,24,31] The findings of the present study are in agreement with report of Sarangi *et al*. that *P. multocida* type A strains harbore *toxA* gene in high frequency (74.50%).[31] For the determination of antibiotic resistance in *P. multocida*

isolates monitoring of anti-microbial susceptibility is very critical. An increase in resistance against antibiotics in both *P. multocida* and *Mannheimia haemolytica* isolates have been reported.[34] In present study, the majority of the isolates were susceptible to oxytetracycline, enrofloxacin, florfenicol, and tilmicosin, followed by penicillin, tylosin and streptomycin were lower susceptibilities. There was no sensitivity or resistance of 100% to any antibiotic. A similar susceptibility pattern was obtained in different isolates. According to the results of antibiogram obtained in a study carried out in Ethiopia, chloramphenicol (100%), sulfamethoxazole (89.10%), and tetracycline (84.40%) were the most active drugs, whereas ampicillin (53.10%)was the only intermediate drug.[35] Results of another study carried out in the Faculty of Veterinary Medicine at Shahid Chamran University of Ahvaz indicated that all *P. multocida* isolates were susceptible to nitrofurantoin, florfenicol, ciprofloxacin, enrofloxacin, trimethoprim-sulfamethoxazole, oxytetracycline, and ceftriaxone. The most common finding was resistance to tylosin (90.90%) followed by resistance to oxacillin (54.54%).[36]

In addition, according to the results of the study in Bangladesh, *P. multocida* isolates were susceptible to ciprofloxacin (95.00%) and azithromycin (80.00%), intermediate susceptibility to gentamycin (85.00%), tetracycline (75.00%), amoxicillin (75.00%), and erythromycin (70.00%), but resistant to penicillin G (100%).[37]

According to the current study, the type A isolates of *P. multocida* had the most frequency. In addition, the frequency of investigated *tbpA*, *pfhA*, *toxA*, and *hgbB* genes revealed that these genes may be important factors in evaluating the pathogenesis of *P. multocida*. Oxytetracycline, enrofloxacin, florfenicol, and tilmicosin were the most effective drugs. However, before using any drugs in practice an antibiotic susceptibility test should be done and the best drug for treating pasteurellosis must be prescribed accordingly. Studies with a larger number of specimens and high levels of accuracy in all stages (from sampling to the final stage), are critical.

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Conflict of interest

The authors state that there is no conflict of interest.

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