International Journal of Molecular and Clinical Microbiology 5(2) (2015) 556-563

International Journal of
Molecular and Clinical Microbiology

Distribution of virulence adhesin associated genes and antimicrobial susceptibility in *Pasteurella multocida* from ovine pasteurellosis in Iran

Ahmad Reza Jabbari[1]*, Elahe Rezaei[2], Majid Esmaelizad[3]

1. Pasteurella National Reasearch Laboratory, Razi Vaccine and Serum Research Institute, Agriculture Research, Education and Extention Organisation (AREEO), Karaj, Iran.
2. Faculty of Basic Siences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.
3. Central Laboratory, Razi Vaccine and Serum Research Institute, Agriculture Research, Education and Extention Organisation (AREEO), Karaj, Iran.

ARTICLE INFO

Article history:
Received 28 August 2015
Accepted 23 September 2015
Available online 1 December 2015

Keywords:
Pasteurella multocida;
Sheep;
Virulence;
Adhesins

ABSTRACT

Pasteurella multocida is an opportunistic pathogen responsible for fowl cholera in poultry, haemorrhagic septicaemia of cattle and buffalo, pneumonia of lambs and goats, respiratory atrophic rhinitis of swine and purulent rhinitis of rabbit. Although the molecular basis of the pathogenicity and host specificity of *P. multocida* is not well understood, several studies have reported that a number of proteins are correlated with the pathogenic mechanisms. Adhesins have a crucial role in mediating colonization and invasion of the host. Thus, their presence on the bacterial surfaces is usually correlated with virulence. The aim of this study was to identify the adhesin associated genes and antimicrobial susceptibility among ovine *P. multocida* isolates from Iran. Of the thirty tested strains, 3.4% were susceptible to all the tested antibiotics, while 83.4% (25/30) were resistant to at least one drug. The resistance was more frequent to streptomycin (83.3%), followed by tylosin and penicillin (46.6%). The results of PCR analysis for the frequency of virulence-associated genes indicated that the genes encoding adhesins (ptfA, fimA, hsf-1 and ompH), were each found in more than 93.0% of the isolates. However, the frequency of pfhA and tadD genes in the isolates was 36% only. All the tested isolates were positive for fimA and ompH (100%). Five different virulence profiles (P1 - P5) were obtained from the 30 isolates of ovine origin, among which profile P2, harboring all adhesin genes except *pfhA and ptfA*, had the highest frequency. The results of this investigation provides useful information for understanding the antimicrobial resistance patterns, capsular types and prevalence of adhesin genes in *P.multocida* strains isolated from sheep in Iran.

1. Introduction

P. multocida can be a primary or secondary agent involved in pneumonia in cattle (predominantly caused by serotype A:1), pigs and sheep (Odugbo et al., 2006). Sheep pasteurellosis, caused by *P. multocida* serotype A, is one of the common infectious diseases occurring in temperate and sub-tropical areas (Chandrasekaran, 1991; Ragavandhar et al., 2015).

The molecular basis of pathogenesis and protective immunity against *P. multocida* infections is not fully understood. Putative virulence determinants such as outer membrane proteins, proteins involved in iron uptake and acquisition, sialidases, toxins, and various

*Corresponding author: Dr. Ahmad Reza Jabbari
Tel: +982634502899
E-mail address: a.jabbari@rvsri.ac.ir

adhesins may play a part in these mechanisms (Ragavandhar et al., 2015).

Adhesins have a crucial role in mediating colonization and invasion of the host. Thus, their presence on the bacterial surfaces is usually correlated with virulence (Kline et al., 2009). Adhesins have been targeted in recent virulence genotyping studies, (Ewers et al., 2006; Tong et al., 2009) which demonstrated that among fimA (fimbriae), hsf-1, 2 (autotransporter adhesins), pfhA (filamentous hemagglutinin), tad (nonspecific tight adherence protein), and ptfA (subunit of type 4 fimbriae), only fimA, hsf-2, and ptfA are present in all or virtually all pathogenic isolates of *P. multocida* (Sellyei et al., 2010).

Although the molecular basis of the pathogenicity and host specificity of *P. multocida* is not well understood, several studies have reported that a number of VFs are correlated with the pathogenic mechanisms (Harper et al., 2006; Hunt et al., 2000).

These VFs facilitate the colonization and invasion of the host, the avoidance or disruption of host defense mechanisms, injury to host tissues, and/or stimulation of a noxious host inflammatory response (Harper et al., 2006; Hunt et al., 2000). Thus, the informed selection of the VFs to be targeted for the prevention of *P. multocida* infections requires knowledge of which VFs are prevalent in specific clinical syndromes, as may be revealed by epidemiological studies (Tang et al., 2009).

There are only a few studies that demonstrated the frequency of virulence gene patterns in this species (Ewers et al., 2006; Atashpaz et al., 2009; Bethe et al., 2009; Shayegh et al., 2009; Tang et al., 2009). There are even fewer data available examining strains of ovine origin. The data on the antibiotic resistance profiles and virulence factors of *P. multocida* from sheep differing in disease status may yield a better understanding of pasteurellosis in this important farm animal. Therefore, we aimed to determine the adhesin associate genes and antibiotic resistance pattern in ovine *P. multocida* strains which were isolated from sheep in Iran.

2. Materials and Methods

2.1. Bacterial isolates and culture conditions

A total of 30 nasal and tonsil swab samples were collected from ailing sheep (exhibiting pneumonic symptoms, respiratory distress, profuse nasal discharge, and sneezing), from endemic areas in Fars (10), Isfahan (3), Ghom (11), Kerman (3) and Tehran (3) provinces, Iran during 2008 and 2011. All animal procedures were carried out in accordance with the Animals (Scientific Procedure) Act, 1986. The swab samples were transported to the laboratory on dry ice.

The reference strains of *P. multocida* used in this study were PMI31 (capsular serogroupA) and PMI32 (capsular serogroup B) obtained from Aerobic Bacterial Vaccines Department, Razi Institute, Iran. The strains were stored in 1 ml of Brain Heart Infusion (BHI) broth supplemented with 20% (v/v) glycerol at -80^{o}C.

2.2. Bacterial Identification

The isolates were identified as *P. multocida* using standard bacteriological and biochemical tests. A PM-PCR for species-specific amplification of the *kmt1* gene, as described by Townsend et al. (1998) was performed and the capsular type determined by Cap-PCR as described previously (Townsend et al., 2001).

2.3. Antimicrobial Susceptibility Test

The antibiotic susceptibility profile of the isolates was determined by disc diffusion test according to the standard protocol of the M31-A3 Document, issued by the Clinical and Laboratory Standards Institute (CLSI). The antimicrobial agents tested included penicillin, nitrofurantoein, streptomycin, sulphamethoxazole - trimethoprim, florfenicol, lincospectin, tylosin, oxytetracyclin, flumequin, enrofloxacin, chloramphenicol, nalidixic Acid (Padtan teb). Reference strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as quality control organisms in all antimicrobial susceptibility tests (Ferreira et al., 2012).

2.4. DNA Extraction

Overnight culture of each sample in BHI was used for the extraction of DNA by heat treatment. One ml aliquot of each culture was centrifuged (13000 x g 15 min) and the resultant pellets were washed twice and re-suspended in 200 µl of HPLC-grade water. After boiling for

20 min and centrifugation (13000 x g 5 min) 3 µl of supernatant was used as DNA template for PCR assay. The concentration of the DNA template was determined using a spectrophotometer at OD260/280 nm (Eppendorf, Germany).

2.5. Detection of adhesin Associated Genes by PCR

All isolates were analyzed with PCR for the presence of the adhesin-associated genes. PCR analysis of adhesin-associated genes (ptfA, fimA, hsf-1, pfhA, tadD and ompH) was conducted as described previously (Ewers et al., 2006; Tang et al., 2009; Townsend et al., 1998).

The details of the adhesin genes, sequences of the oligonucleotide primers, and the expected size of each amplicon are described in Table 1.

The reactions were performed in a final volume of 25µl at the following reagent concentrations: 10mM Tris-Hcl, pH 8.3, 50mM Kcl, 200 µM dNTP, 0.5 µM of each primer, 1.5 mM Mgcl2, 2.5 U Taq polymerase enzyme, and 1µl of template DNA. Amplification were performed at 35 cycles: 94^{o}C for 1 min. 68^{o}C for 1 min,72^{o}C for 1 min., and final extension at 72^{o}C for 7 min. PCR products were resolved on 1.5% agarose gel (Invitrogen UltrapureTM Agarose®- Carlsbad, USA), stained with ethidium bromide and visualized under UV light. Molecular sizes were determined based on a 100 bp ladder molecular weight marker.

2.6. Sequencing

PCR products were purified by PCR product purification kit (Roche, Germany) and submitted for sequencing to Invitrogen Laboratory, South Korea. Alignments of the gene sequences from the Iranian field isolates and reference strains were conducted by using the GenBank database and the BLAST algorithm. (http://www.ncbi.nlm.nih.gov/BLAST/).

3. Results

Biochemical characterization and PM-PCR confirmed all of the isolates as *P. multocida*. Cap-PCR showed that all of the *P. multocida* isolates belonged to capsular type A. None of the isolates harbored the capB, capE and capF genes.

The results of antibiogram for 12 antimicrobial agents of the *P. multocida* isolates are shown in Table 1. Of the thirty tested strains, 3.4% (1/30) were susceptible to all tested drugs, and 83.4% (25/30) were resistant to at least one drug tested. The resistance was more frequent to streptomycin (83.3%), followed by tylosin and penicillin (46.6%).

The results of PCR analysis for the frequency of virulence-associated genes (ptfA, fimA, hsf-1, pfhA, tadD and ompH) are shown in Tables 3, 4 and 5.

Genes encoding adhesins (ptfA, fimA, hsf-1 and ompH), were each found in more than 93.0% of the isolates. In contrast, the frequency of 2 genes (pfhA and tadD) were 36%. All the tested isolates were positive for fimA and ompH (100%).

Five different virulence profiles (P1 - P5) were obtained among the 30 isolates of ovine origin, and profile P2, harboring all adhesin genes except *pfhA* and *ptfA*, had the highest frequency (Table 5).

The sequence alignments of the fragments were compared with the previous sequences in Gen Bank, showed 96-99% similarity.

4. Discussion

The basic requirement for bacterial infection is its attachment to host cell and therefore, adhesins that mediate such adherence are considered to be potential virulence factors. In the present study *P. multocida* isolates recovered from sheep pneumonia in Iran were tested for adhesin genes carriage and antibiotic susceptibility patterns.

In this study, *P. multocida* isolates recovered from pneumonia in sheep were screened for presence of five important adhesin associated genes (hsf1, pfhA, ptfA, ompH, and tad D) involved in bacterial pathogenesis.

Virulence profiling has been used as a typing method for characterization of bacterial pathogens including *P. multocida* (Ewers et al., 2006; Tang et al., 2009; Atashpaz et al., 2009; Ferreira et al., 2012).

Cats are often described as carriers of *P.multocida*. Recent study in Brazil showed that 10.4% (20/191) of tested cats were carrier of *P. multcida*. The feline isolates were positive for

presence of adhesin genes fimA, hsf1, ptfA and tad D with frequency range of 12.1- 63.4%. However, pfhA gene was not detected in feline *P. multocida* isolates (Ferreira et al., 2015).

The high frequency of genes encoding for ompH and fimA (100%) is relevant to the invasion potential of the tested strains. According to reports, ompH gene plays different roles in bacteria, such as nutrient absorption, importation and exportation of molecules and a close inter-action with host tissue (Ferreira et al., 2012, Hatfaludi et al., 2010).

The gene, which encodes fimbriae type IV protein (*ptfA)*, was found in 28/30 (93.3%) of ovine *P. multocida* isolates in present study. Similar results have been observed in other studies (Ewers et al., 2006; Bethe et al., 2009; Tang et al., 2009). Sarangi et al (2015) showed high prevalence of ptfA gene (95.45%) among *P. multocida* isolates of small ruminant origin in India. According to their report the frequency of ptfA and hsf1 genes were 95.5% and 49.2 % respectively. Similar findings were also reported by Verma and his colleagues (2013), who identified ptfA gene among all (100%) capsular types A and B of cattle and buffalo origin from India. They found that the presence of ptfA gene revealed a positive association with the disease outcome in cattle and could be an important epidemiological marker gene for characterizing *P. multocida* isolates.

pfhA gene encoding filamentous haemagglutinin is an important epidemiological marker and the presence of this gene has been correlated with occurrence of disease in cattle, swine and sheep (Ewers et al., 2006; Bethe et al., 2009; Katsuda, 2013; Verma et al., 2013; Shayegh et al., 2008). In this study, the hemagglutinin pfhA gene was identified in 11/30 ovine isolates (36.6%). This lower frequency was also found in other studies. Furrian et al., (2013) detected *pfhA* in 60% (15/25) of avian *P. multocida* isolates. Ewers et al. (2006) identified a variation in frequency between 7 and 100% according to the species studied and correlated the presence of the gene to the occurrence of pasteurellosis in cattle. However, Shayegh et al., (2008) reported a low percentage of *pfhA* in sheep and observed a correlation between the presence of the gene and disease in this species. Bethe et al., (2009) found a correlation between the presence of *pfhA* and the occurrence of respiratory disease in swine.

Interestingly, very high prevalence, 85.3% (pig) to 100% (avian), of this gene was observed among Indian isolates (Sarangi et al., 2014). This suggests *pfhA* gene might be providing survival advantage to the bacterium in the host and the occurrence of horizontal gene transfer has led to such high prevalence among the isolates.

The Type IV fimbriae are formed by repeated subunits and present a highly conserved N-terminal nucleotide sequence (Hatfaludi et al., 2010). In a recent study, Sellyei et al., (2010) identified only two major alleles of *ptfA* among 31 strains of different avian serotypes. For these reasons, despite the variations in immunogenicity among strains of *P. multocida* observed by Doughty et al., (2000), this gene is a candidate for heterologous vaccine development in poultry (Ewers et al., 2006; Furrian et al., 2013).

Previous study showed that, certain VFs varied significantly among the different capsular serogroups. For example, *hsf-1*, which has been described to be an autotransporter adhesin was more frequently seen in serogroup D, whereas *tadD*, described as putative nonspecific tight adherence protein D was concentrated significantly in serogroup A (Tang et al., 2009; May et al., 2001).

Monitoring the antimicrobial susceptibility trends of *P. multocida* is an important aid to veterinarians in selecting the most efficacious therapeutic agents. Tetracycline, penicillin and streptomycin are the main antimicrobial agents used for farm animals in Iran. The results of the present study indicate that fluorquinolones and florfenicol are the most efficient drugs to be used against *P. multocida*. Resistance to antimicrobial agents, including penicillin, streptomycin, tetracyclines and lincospectin, was observed in *P. multocaida* isolates collected from sheep. Similar results have been previously described in Brazil, France, North America and Japan (Rigobelo et al., 2013; Kehrenberg et al., 2001; Yoshimura et al., 2001).

OTC showed poor activity against *P. multocida* compared with other classes of antimicrobial agents; this is not unexpected. These findings agree well with results presented in previous reports (Portis et al., 2012). Therefore, continuous monitoring of antimicrobial susceptibility is necessary to determine the current susceptibility status of *P. multocida* isolates.

Presently, a number of key virulence factors in *P. multocida* are being identified. Further work is thus required to elucidate the mechanisms of pathogenesis and to determine unequivocally the role of these factors in immunity to pasteurellosis. Hence, a detailed investigations of the virulence associated genes recovered from different host species will be helpful to understand the disease process and to develop disease control measures in future.

Acknowledgments

We thank all the staff of the Pasteurella National Research Laboratory, Razi Vaccine and Research Institute, Karaj, Iran. This study was supported by a research project at Razi Institute and Agricultural Research, Education, Extension and organization (AREEO) with the grant No: 2-18-18-94104.

Conflict of interest

The authors declare that they have no conflict of interest.

Table 1. Details of primers and citations used for the detection of adhesin associated genes in ovine *P. multocida* isolates.

Gene	Description	Primer Name	Nucleotide sequence	Amplicon size	References
PM (kmt1)	Pasteurella multocida (all)	Kmt1 Sp6	5'-ATCCGCTATTTACCCAGTGG-3' 5'-GCTGTAAACGAACTCGCCAC-3'	460	Townsend et al., 1998
hyaD	Capsule type A	hyaD-F hyaD-R	5'-TGCCAAAATCGCAGTCAG-3' 5'-TTGCCATCATTGTCAGTG-3'	1044	Townsend et al., 2001
ompH	Outer Membrane Protein H	ompH-F ompH-R	5'-ACTATGAAAAAGACAATGGTAG-3' 5'-GATCCATTCCTTGCAACATATT-3'	1100	Luo et al., 1997
fimA	Fimbriae	fimA-F fimA-R	5'-CCATCGGATCTAAACGACCTA-3' 5'-AGTATTAGTTCCTGCGGGTG-3'	866	Tang et al., 2009
ptfA	Type 4 fimbriae	ptfA-F ptf-R	5'-TGTGGAATTCAGCATTTTAGTGTGTC-3' 5'-TCATGAATTCTTATGCGCAAAATCCTGCTGG-3'	488	Ewers et al., 2006
hsf1	Autotransporter adhesin	haf1-F hsf1-R	5'- TTGAGTCGGCTGTAGAGTTCG-3' 5'- ACTCTTTAGCAGTGGGGACAACCTC-3'	654	Tang et al., 2009
pfhA	Filamentous hemagglutinin	pfhA-F pfhA-R	5'- TTCAGAGGGATCAATCTTCG -3' 5'- AACTCCAGT TGGTTTGTCG -3'	286	Tang et al., 2009
tadD	Putative nonspecific tight adherence protein D	tadD-F tadD-R	5'- TCTACCCATTCTCAGCAAGGC-3' 5'- ATCATTTCGGGCATTCACC-3'	4416	Tang et al., 2009

Table 2. Frequency of antimicrobial susceptibility in *P. multocida* isolates from sheep in Iran.

Antibiotic	Sensitive No (%)	Intermediate No (%)	Resistance No (%)
Penicillin	16 (53.4)	-	14 (46.6)
Nitrofurantoein	19 (63.4)	6 (20)	5 (16.6)
Streptomycin	1 (3.4)	4 (13.3)	25 (83.3)
Sulphamethoxazole-trimethoprim	21 (70)	6 (20)	3 (10)
Florfenicol	28 (93.4)	-	2 (6.6)
Lincospectin	21 (70)	-	9 (30)
Tylosin	13 (43.4)	3 (10)	14 (46.6)
Oxytetracyclin	25(83.4)	2(6.6)	3(10)
Flumequin	30 (100)	-	-
Enrofloxacin	28 (93.4)	2 (6.6)	-
Chloramphenicol	23 (76.7)	-	7 (23.3)
Nalidixic Acid	30 (100)	-	-

Table 3. Isolates code, capsule type and presence (+) or absence (-) of adhesin associated genes of *P. multocida* isolates from sheep in Iran

Isolate code	capA	hsf-1	pfhA	ptfA	tadD	ompH	fimA
PM01	+	+	+	+	+	+	+
PM02	+	+	+	+	+	+	+
PM03	+	+	+	+	+	+	+
PM04	+	+	+	+	+	+	+
PM05	+	+	+	+	+	+	+
PM06	+	+	+	+	+	+	+
PM07	+	+	–	+	–	+	+
PM08	+	+	–	+	–	+	+
PM09	+	+	–	+	+	+	+
PM10	+	+	+	+	+	+	+
PM11	+	+	–	+	–	+	+
PM12	+	+	+	+	+	+	+
PM13	+	+	–	+	–	+	+
PM14	+	-	–	–	–	+	+
PM15	+	-	–	–	–	+	+
PM16	+	+	+	+	–	+	+
PM17	+	+	+	+	+	+	+
PM18	+	+	–	+	–	+	+
PM19	+	+	–	+	–	+	+
PM20	+	+	–	+	–	+	+
PM21	+	+	–	+	–	+	+
PM22	+	+	–	+	–	+	+
PM23	+	+	–	+	–	+	+
PM24	+	+	–	+	–	+	+
PM25	+	+	+	+	+	+	+
PM26	+	+	–	+	–	+	+
PM27	+	+	–	+	–	+	+
PM28	+	+	–	+	–	+	+
PM29	+	+	–	+	–	+	+
PM30	+	+	–	+	–	+	+

Table 4: Frequency of adhesin coding genes among *P. multocida* isolates from sheep

Gene	Adhesin factor	No of positives (%)
capA	Capsule type A	30 (100)
ompH	Outer Membrane Protein H	30 (100)
hsf1	Autotransporter adhesin	28 (93.3)
ptfA	Type 4 fimbriae	28 (93.3)
pfhA	Filamentous hemagglutini	11 (36.6)
tadD	Putative nonspecific tight adherence protein D	11 (36.6)
fimA	Fimbriae A	30 (100)

Table 5. Distribution of virulence adhesin patterns (P1-P5) among thirty ovine *P. multocida* isolates from Iran.

Virulence pattern	Isolates	No	percent
P1: ompH+,hsf1+, pfhA+, ptfA+, tadD+	PM01, PM02, PM03, PM04, PM05, PM06, PM10, PM12, PM17, PM25	10	33.3
P2: ompH+,hsf1+, pfhA-, ptfA+, tadD-	PM07, PM08, PM11, PM13, PM18, PM19, PM20, PM21, PM22, PM23, PM24, PM26, PM27, PM28, PM29, PM30	16	53.3
P3: ompH+,hsf1+, pfhA-, ptfA+, tadD+	PM09	1	3.3
P4: ompH+,hsf1-, pfhA-, ptfA-, tadD-	PM14, PM15	2	6.6
P5: ompH+,hsf1+, pfhA+, ptfA+, tadD-	PM16	1	3.3

Refereces

Atashpaz, S.I., Shayegh, J., Hejazi, M.S. 2009. Rapid virulence typing of Pasteurella multocida by multiplex PCR. Res Vet Sci. 87(3):355-7.

Bethe, A., Wieler, L.H., Selbitz, H.J., and Ewers, C. 2009. Genetic diversity of porcine Pasteurella multocida strains from the respiratory tract of healthy and diseased swine. Vet Microbiol. 139 (1-2): 97–105.

Chandrasekaran, S. 1991. Evaluation of combined Pasteurella vaccines in control of sheep pneumonia. Brit. Vet. J. 147(5): 437-443.

Doughty, S.W., Ruffolo, C.G. and Adler, B. 2000. The type 4 fimbrial subunit gene of Pasteurella multocida. Vet. Microbiol. 72:79-90.

Ewers, C., Lu¨bke-Becker, A., Bethe, A., Kiebling, S., Filter, M., and Wieler, L.H. 2006. Virulence genotype of Pasteurella multocida strains isolated from different hosts with various disease status. Vet. Microbiol. 114:304–317.

Ferreira, T.S.P.M.R., Felizardo, D.D., Sena de Gobbi., Gomes, C.R., Lima, P.H., Moreno, M., Paixao, R., Pereira, J.J. and Moreno, A.M. 2012. Virulence genes and antimicrobial resistance profiles of Pasteurella multocida strains isolated from rabbits in Brazil. The Sci World J. Article ID 685028, 6 pages.

Furian, T.Q., Borges, K.A., Rocha, S.L.S., Rodrigues, E.R., Nascimento, V.P., Moraes, C.T.P. 2013. Detection of virulence-associated genes of Pasteurella multocida isolated from cases of fowl cholera by multiplex-PCR. Pesquisa Veterin ´aria Brasileira. 33 (2): 177–182.

Gyles, C.L., Prescott, J.F., Songer, J., Thoen, C.O., 2004. Pasteurella and Mannheimia, In: Pathogenesis of bacterial infection in animals. 3rd edition. lowa: Wiley- Blackwell Publishing co. PP: 273-285.

Harper, M., Boyce, J.D., and Adler, B. 2006. Pasteurella multocida pathogenesis: 125 years after Pasteur. FEMS Microbiol. Lett. 265:1–10.

Hatfaludi, T., Al-Hasani, K., Boyce, J.D., and Adler, B. 2010. Outer membrane proteins of Pasteurella multocida. Vet. Microbiol. 144:1-17.

Hunt, M.L., Adler, B., and Townsend, K.M. 2000. The molecular biology of Pasteurella multocida. Vet. Microbiol. 72:3–25.

Katsuda, K.K., Hoshinoo, Y., Ueno, M., Kohmoto, M., and Mikami, O. 2013. Virulence genes and antimicrobial susceptibility in Pasteurella multocida isolates from calves. Veterinary Microbiology. 167(3-4): 737–741.

Kehrenberg, C., Schwarz, S. 2001. Occurrence and linkage of genes coding for resistance to sulfonamides, streptomycin and chloramphenicol in bacteria of the genera Pasteurella and Mannheimia. FEMS Microbiol. Lett. 205, 283–290.

Kline, K.A., Falker, S., Dahlberg, S., Normark, S., Henriques-Normark, B. 2009. Bacterial adhesins in host-microbe interaction. Cell Host Microbe. 5:580–592.

Luo, Y., Glisson, J.R., Jackwood, M.W., Hancock, R., Bains, M., Cheng, I.H. and Wang, C. 1997. Cloning and characterization of the major outer membrane protein gene (ompH) of Pasteurella multocida X-73. J. Bacteriol. 179: 7856-64.

May B.J., Zhang Q., Li L.L., Paustian M.L., Whittam, T.S. and Kapur, V. 2001. Complete genomic sequence of Pasteurella multocida, Pm70. Proc. Natl Acad. Sci. USA 98:3460-3465.

Odugbo, O.M., Odama, L.E., Umoh, L., Lamorde, A.G. 2006. Pasteurella multocida pneumonic infection in sheep: Prevalence, clinical and pathological studies. Small Ruminant Research. 66 (1-3): 273-277.

Portis, E., Lindeman, C., Johansen, L., Stoltman, G. 2012. A ten-year (2000-2009) study of antimicrobial susceptibility of bacteria that cause bovine respiratory disease complex-Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni-in the United States and Canada. J Vet Diagn Invest. 24:932-944.

Ragavendhar, K., Prabhakar, P., Thangavelu, A., Kirubaharan, J.J., Chandran, N.D.J. 2015.

Sequence analysis, 3-dimensional protein modelling and epitope prediction of Oma87 gene originating from Pasteurella multocida isolate of sheep. J. Inf. Mol. Biol. 3(1): 1-12.

Rigobelo, E.C., Blackall, P.J., Maluta, R.P., Avila, F. R. 2013. Identification and antimicrobial susceptibility patterns of Pasteurella multocida isolated from chickens and japanese quails in Brazil. Braz J Microbiol. 44:161-164.

Salomon, S.A., Watts, C.A., Case, J. 1995. Comparison of MICs of ceftiofur and other antimicrobial agents against bacterial pathogens of swine from United States, Canada and Denmark. J Clin Microbiol 33:2435-2444.

Sarangi, L.N., Priyadarshini, A., Kumar, S., Prasad Thomas, P., Gupta, S. K., Nagaleekar, V. K., and Vijendra Pal Singh, V.P., 2014. Virulence genotyping of Pasteurella multocida isolated from multiple hosts from India. Sci World J. Volume 2014, Article ID 814109, 10 pages http://dx.doi.org/10.1155/2014/814109.

Sellyei, B., Banyai, K., Magyar, T. 2010. Characterization of the ptfA gene of avian Pasteurella multocida strains by allele-specific polymerase chain reaction. J Vet Diagn Invest 22:607–610.

Shayegh, J., Atashpaz, S., and Hejazi, M.S. 2008. Virulence genes profile and typing of ovine Pasteurella multocida. Asian J of Animal and Vet Advances. 3 (4): 206–213.

Tang, X., Zhao, Z., Hu, J., Wu, B., Cai, X., He, Q., Chen, H. 2009. Isolation, antimicrobial resistance, and virulence genes of Pasteurella multocida strains from swine in China. J. Clin Microbiol. 47 (4): 951–958, 2009.

Townsend, K.M., Boyce, J.D., Chung, J.Y., Frost, A. J., and Adler, B. 2001. Genetic organization of Pasteurella multocida cap loci and development of a multiplex capsular PCR typing system. J. Clin. Microbiol. 39:924–929.

Townsend, K.M., O'Boyle, D., Phan, T.T., Hanh, X., Wijewardana, T.G., Wilkie, I., Trung, N.T., and Frost, A. 1998. Acute septicaemic pasteurellosis in Vietnamese pigs. Vet. Microbiol. 63:205–215.

Verma, S. M. Sharma, S. Katoch., Verma, L., Kumar, S., Dogra, V., Chahota, R., Dhar, P., Singh, G. (2013). Profiling of virulence associated genes of Pasteurella multocida isolated from cattle. Vet Res Commun. 37(1): 83–89. 2013.

Yoshimura, H.M., Ishimura, Y.S.E., Kojima, A. 2001. Antimicrobial susceptibility of Pasteurella multocida isolated from cattle and pigs. J Vet Med B. 48:555-560.