

Virulence of *Pasteurella Multocida* Isolated During Pasteurellosis Outbreak among Saigas in Kazakhstan



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Abstract

For the past few years mass deaths of saiga antelopes was recorded in the Republic of Kazakhstan. The outbreaks of *Pasteurella* infection caused drastic reduction of saiga population. The isolates of *P.multocida* belong to multocida subspecies. Dedicated from the dead saigas in 2010-2011 gg. *Pasteurella multocida* isolates contained virulent genes. The study was performed with use of 25 (2010) and 8 (2011) *Pmultocida* field isolates from different organs of dead saigas.

Fifteen virulence-associated genes (ptfA, ompA, ompH, oma87, plpB, fimA, hsf-2, pfhA, exbB, tonB, hgbA, fur, nanB, nanH and pmHAS) were detected by multiplex PCR. In isolates of *Pmultocida* from saigas (year 2010) the following genes were detected: tonB (100%), ompA (92%), nanH (92%), fimA (68%), ptfA (68%), exbB (68%), oma87 (48%), plpB (44%), ompH (16%) and pfhA (12%). The genomes of *Pmultocida* isolated from saigas in 2011 contained the following genes: hgbA (100%), ompA (100%), tonB (100%) and nanH (100%); fimA (87.5%), ptfA (87.5%) and exbB (87.5%); pfhA (62.5%), hsf2 (62.5%), fur (62.5%) and none of plpB, oma87, ompH, nanB and pmHAS genes was found in them. All assayed isolates showed high content of virulent hgbA, ompA, tonB and nanH genes. Field isolates of *Pmultocida* (years 2010 and 2011) from saiga antelopes differ in virulence gene profiles.

Keywords: *Pasteurella multocida*; virulence factors; PCR; saiga; Kazakhstan

Introduction

Saiga antelope (*Saiga tatarica*) is entered in the International Red Book because of drastic decline of its population. Mass death of saigas was observed on the territory of the Republic of Kazakhstan in 2010-2011. About 12000 in 2010 and over 500 animals in 2011 died [1] Taking into account the current low saiga numbers emergence of epizooty can be a real threat to the life story of this animal species. Assay of samples from dead saigas (Borsy village, West Kazakhstan oblast, Republic of Kazakhstan) at the RIBSP in 2010-2011 resulted in isolation of pathogenic organisms that were differentiated as *Pasteurella* [2]. is a Gram negative bacterium belonging to Pasteurellaceae family. Subject to the specific type of capsular antigens *P.multocida* has serologic differences and subdivides into capsular serogroups (A, B, D, E, F) [3,4]. The currently known virulence factor genes include genes encoding the outer membrane proteins (ompA, ompH, oma87, plpB), adhesins (hsf1, hsf2), iron transport genes (fimA, ptfA, exbB, tadd, fur), enzymes (nanH, nanB, pmHAS). It should be also noted that there are genes responsible for high virulence of *P.multocida*; they are pfhA gene encoding filamentous hemagglutinin, hgbB gene that is responsible for hemoglobin control in a body [5-7].

The work was conducted towards determining subspecies and serotyping of *Pmultocida* bacteria isolated from saiga antelopes in 2010 and 2011 in the Republic of Kazakhstan. Pathogenicity of *P.multocida* strains and isolates is assessed by presence (%) of virulent genes [8,9]. It is clear that high mortality rate among saiga antelopes in the Republic of Kazakhstan is directly related to the virulence factors of *P.multocida*. On the basis of the above stated the objective of the study was to detect the virulent genes in the genomes of *Pmultocida* isolated from saiga antelopes in 2010 and 2011 in the Republic of Kazakhstan.

Materials and Methods

All ethics, field and laboratory studies were reviewed and approved by the appropriate committees of the Research Institute for Biological Safety Problems (RIBSP), Ministry of Education & Science in Gvardeiskiy, Zhambylskaya oblast, Republic of Kazakhstan.

Clinical examination, sampling and specimen delivery

Twenty five samples taken from dead saigas in 2010 and 8 samples of year 2011 were delivered to the RIBSP by veterinary specialists from West Kazakhstan oblast within the network of

epidemiological surveillance to determine the cause of saigas illness and death. Suspected pasteurellosis clinical cases were reported, and biological samples were taken from dead saigas for diagnosis. The samples taken from each animal were placed separately in indelibly marked plastic bags with the species

name, animal i.d. and tissue name. These were packed in iced refrigerator bags and delivered frozen to the RIBSP. Detailed information on clinical signs and samples taken is shown in Table 1.

Table 1: Description of samples used in the study.

No	Outbreak Locus	Clinical Signs	Date of Delivery of Biological Samples	Samples Delivered
1	Borsy village, West Kazakhstan oblast (Republic of Kazakhstan)	Dead animals had abdominal swelling, salivary discharge and nasal bleeding. Post portem examination showed pneumonia.	05.21.2010	lungs, liver, kidneys, intestine, spleen of 25 dead saigas
2	Borsy village, West Kazakhstan oblast (Republic of Kazakhstan)	Dead animals had abdominal swelling, foaming at mouth and nose. Post portem examination showed pneumonia.	05.27.2011	lungs, liver, kidneys, intestine, spleen of 8 dead saigas

DNA Extraction

DNA was extracted with use of “PrepMan Ultra Sample Preparation Reagent” kit (Applied Biosystems) following the manufacturer’s guidelines.

Pasteurella multocida species specific PCR

The species specific primers designed by Townsend et al. [10] KMTIT7 and KMTISP6 were used to amplify the gene sequences in *P.multocida*. The PCR reaction mixture and the thermal cycle protocol were as follows: initial denaturation–94

°C for 5 min, followed by 30 cycles, each cycle consisting of 3 steps; denaturation at 95°C for 1 min; annealing at 55 °C for 1 min; extension at 72 °C for 1 min; final extension at 72 °C for 9 min

Detection of virulence associated genes by multiplex PCR

AccuPrime Taq DNA Polymerase High Fidelity kit manufactured by Invitrogen (USA) was used to produce PCR products of *P.multocida* virulent genes. Multiplex polymerase chain reaction (PCR) with 15 primer pairs was used for amplification (Table 2) [4,5,11,12].

Table 2: Characteristics of *P.multocida* virulence factor gene primers.

No	Primers	Primer’s sequence (5’-3’)	Primer length	PCR product size (bp)
1	ptfAs	TGTGGAATTCAGCATTTAGTGTGTC	26	488
	ptfAa	TCATGAATTCCTTATGCGCAAAATCCTGCTGG	31	
2	ompAs	CGCATAGCACTCAAGTTTCTCC	22	201
	ompAa	CATAAACAGATTGACCGAAACG	22	
3	ompHs	CGCGTATGAAGTTTAGGT	19	438
	ompHa	TTTAGATTGTGCGTAGTCAAC	21	
4	oma87s	GGCAGCGAGCAACAGATAACG	21	838
	oma87a	TGTTTCGTCAAATGTCGGGTGA	21	
5	plpBs	TTTGGTGGTGCATGTCTTCT	22	282
	plpBa	AGTCACTTTAGATTGTGCGTAG	22	
6	fimAs	CCATCGGATCTAAACGACCTA	21	866
	fimAa	AGTATTAGTTCCTGCGGGTG	20	
7	hsf-2s	ACCGCAACCATGCTCTTAC	19	433
	hsf-2a	TGACTGACATCGGCGGTAC	19	
8	pfhAs	TTCAGAGGGATCAATCTTCG	20	286
	pfhAa	AACTCCAGTTGGTTTGTCG	19	
9	exbBs	TTGGCTTGTGATTGAACGC	19	283
	exbBa	TGCAGGAATGGCGACTAAA	19	
10	tonBs	CGACGGTGAAACCTGAGCCA	20	261
	tonBa	CCGAGCGATAAGCATTGACT	20	
11	hgbAs	TCAACGGCAGATAATCAGGG	20	267
	hgbAa	GCGGAATGCTGAAGATAAG	20	
12	fur-s	GTTTACCGTGATTAGACCA	20	244
	fur-a	CATTACTACATTTGCCATAC	20	

13	nanBs	CATTGCACCTAACACCTCT	19	555
	nanBa	GGACACTGATTGCCCTGAA	19	
14	nanHs	GTGGGAACGGGAATTGTGA	19	287
	nanHa	ACATGCCAAGTTGCCCTA	19	
15	pmHASs	TCAATGTTTGGGATAGTCCGTTAG	24	430
	pmHASa	TGGCGAATGATCGGTGATAGA	21	

Table 3: Component formulae of the reaction mixtures for amplification of virulence factor genes.

Multiplex PCR	Amplification Mixtures
Multiplex - 1	10x buffer-2.5µl; forward and reverse primers (10 pmol)-0.5 µl each; 0.08U Taq DNA Polymerase; DNA samples-4µl; water -up to 25µl.
Multiplex - 2	10x buffer-2.5µl; forward and reverse primers (10 pmol)-0.8µl each; 0.06U Taq DNA Polymerase; DNA samples-5µl; water -up to 25µl.
Multiplex - 3	10x buffer-2.5µl; forward and reverse primers (10 pmol)-1µl each; 0.08U Taq DNA Polymerase; DNA samples-5µl; water -up to 25µl.
Multiplex - 4	10x buffer-2.5µl; forward and reverse primers (10 pmol)-1 µl each; 0.1U Taq DNA Polymerase; DNA samples-4µl; water -up to 25µl.

The parameters of the multiplex PCR that enable to detect several virulence gene profiles at a time have been chosen. While performing multiplex PCR optimal component structures of PCR mixtures and modes for amplification of virulence factor genes were selected. The parameters of the multiplex PCR are shown in Table 3 & 4.

Table 4: Temperature-time modes for amplification of virulence factor genes.

Multiplex PCR	Virulence Gene	Amplification Modes			
		Denaturation	Annealing	Replication	Cycle Number
Multiplex - 1	<i>pfhA, hgbA</i>	94°C-30 s	55 °C-30 s	68 °C-3 min	35
Multiplex - 2	<i>ompA, ompH, oma87, plpB, hsf-2</i>	94°C-30 s	55 °C-30 s	72 °C-1 min	35
Multiplex - 3	<i>ptfA, fimA, exbB, tonB, fur</i>	94 °C-30 s	54 °C-1 min	72°C-1 min 20 s	30
Multiplex - 4	<i>pmHAS, nanH, nanB</i>	94°C-30 s	57 °C-30 s	68 °C-1 min	30

DNA electrophoresis in 2% agarose gel was performed with TBE buffer containing 1 µl/ml ethidium bromide in the unit for electrophoresis of nucleic acids GNA-100, Pharmacia (Sweden).

Statistical Analysis

The resulted statistical data were processed with use of SPSS software for Windows 12.0. The diagrams were drawn with the help of Microsoft Excel 2010. Statistical analysis was performed using Fisher’s exact test (two tailed) in the GraphPadPrism 6.0 software.

Results

All 25 samples taken from dead saigas in 2010 and 8 samples of year 2011 were identified as *Pmultocida*. It has been

shown that KMTI gene is specific for reference strains and all *Pmultocida* isolates [10]. Size of the specific product obtained with use of primers KMTI77 and KMTISP6 is 460 bp. The results of the assay are shown on Figure 1.

As Figure 1 shows PCR fragments of KMTI gene sized 460 bp have been found in DNA of *Pmultocida* isolates under study. So, the assayed isolates are of *Pasteurella* genus, *multocida* species. The mechanisms that take place in virulence factor genes of *Pmultocida* provide a molecular basis for studying the degree of its pathogenicity. Therefore our task was to detect the presence of virulent genes in the genomes of *Pmultocida* isolated from saiga antelopes. The results of the multiplex PCR for detection of virulent genes in *Pmultocida* isolated from saiga antelopes in Kazakhstan are shown on Figure 2 & 3.

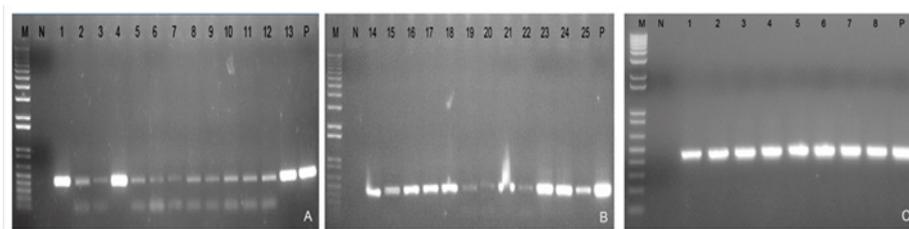


Figure 1: PCR fragments of KMTI gene in *Pmultocida* isolated from saigas in the Republic of Kazakhstan. M – 1 kb marker Fermentas, N – negative control (DNase- and RNase-free water), P – positive control *Pasteurella multocida* ATCC 10544, A and B (1-25) - isolates of *Pmultocida* that circulated in 2010 in saiga antelope populations and C (1-8) – isolates of *Pmultocida* that circulated in 2011 in saiga antelope populations.

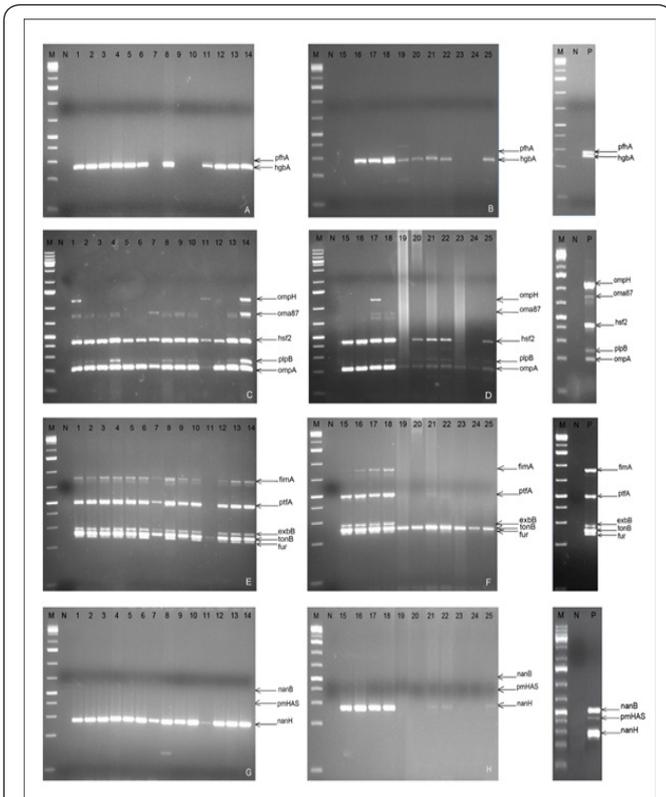


Figure 2: PCR fragments of virulent genes from *P. multocida* isolated in 2010. M – 1 kb marker, Fermentas, N – negative control (DNase- and RNase-free water), P – positive control *Pasteurella multocida* ATCC 10544, 125 – *P. multocida* that circulated in 2010 in saiga antelope populations. A, B – genes responsible for high virulence (pflA-286 bp, hgbA-262 bp); C, D – genes encoding outer membrane proteins (ompA-201bp, ompH-1200 bp, oma87-838 bp, plpB-282 bp) and adhesin (hsf2-433 bp); E, F – adhesins (fimA-866 bp, pflA-488 bp), genes associated with iron acquisition related factors (exbB-283 bp, tonB-261 bp, fur-244 bp); G, H – genes encoding sialidase proteins (nanB-555 bp, pmHAS-430 bp, nanH-287 bp).

Figure 2 shows the PCR fragments of virulent genes encoding the outer membrane proteins, adhesins, iron acquisition related factors and sialidase proteins. HgbA-267 bp gene responsible for hemoglobin regulation in a body was not found in genomes of isolates Nos. 7, 9, 10, 15, 23, 24. Region of pflA-286 bp gene encoding filamentous hemagglutinin was detected only in genomes of isolates Nos. 8, 11, 18, 21.

Figures 2C & 2D show PCR fragments of virulent genes encoding outer membrane proteins and adhesins of *Pasteurella* bacteria isolated from saiga antelopes in 2010. Region of OmpH gene encoding outer membrane protein H 1200 bp long was found only in genomes of 4 assayed isolates: 1, 11, 14 and 17. Fragment of the virulent Oma87 gene was detected in genomes of 13 isolates under study (1, 2, 3, 4, 7, 8, 9, 10, 13, 14, 16, 17, 18). Fragment of hsf2 gene sized 433 bp was present in genomes of all isolates except Nos. 19, 23 and 24. PlpB gene was detected in genomes of 11 isolates (1, 2, 3, 4, 7, 8, 9, 10, 13, 14, 18) and it was absent in 14 isolates (5, 6, 11, 12, 15, 16, 17, 19, 20, 21, 22, 23, 24 and 25). Fragment of OmpA gene encoding outer membrane

protein A 201 bp long was detected in all isolates except Nos. 19 and 24.

Figures 2E & 2F show PCR fragments of virulent genes associated with iron acquisition related factors: exbB-283 bp, tonB-261 bp, fur-244 bp and adhesins: fimA-866 bp, pflA-488 bp. As one can see the PCR fragments of virulent fimA, pflA and exbB genes are present in genomes of 17 isolates except Nos. 11, 19, 20, 21, 22, 23, 24 and 25. PCR fragment of tonB gene sized 261 bp was detected in genomes of all isolates. PCR fragment of fur gene 244 bp long was found in genomes of 18 isolates and not detected in genomes of isolates 7, 11, 19, 20, 21, 23 and 24.

On Figures 2G & 2H PCR fragments of virulent genes encoding sialidase proteins (nanB-555 bp; pmHAS-430 bp; nanH-287 bp) are shown. We failed to find virulent nanB and pmHAS genes in genomes of all assayed isolates. Site of virulent nanH gene 287 bp long were present in all isolates except Nos. 11, 19, 20, 23 and 24.

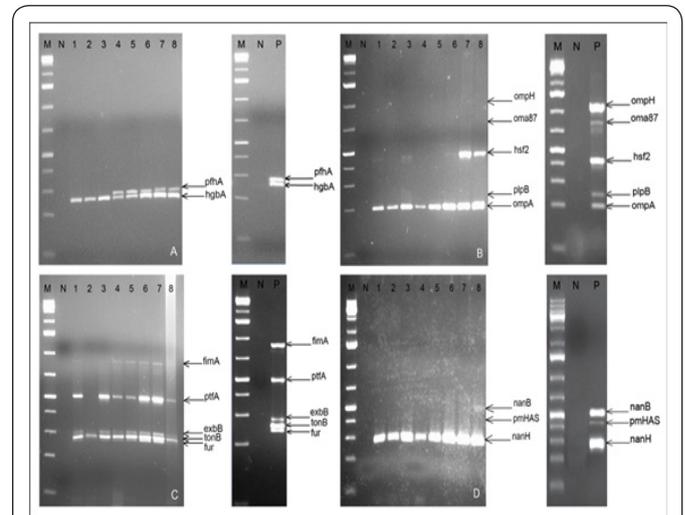


Figure 3: PCR fragments of virulent genes from *P. multocida* that circulated among saiga antelopes in 2011. M – 1 kb marker, Fermentas, N – negative control (DNase- and RNase-free water), P – positive control *Pasteurella multocida* ATCC 10544, 1-8 – *P. multocida* that circulated in 2011 in saiga antelope populations. A – genes responsible for high virulence (pflA-286 bp, hgbA-262 bp); B – genes encoding outer membrane proteins (ompA-201bp, ompH-1200 bp, oma87-838 bp, plpB-282 bp) and adhesin (hsf2-433 bp); C – adhesins (fimA-866 bp, pflA-488 bp), genes associated with iron acquisition related factors (exbB-283 bp, tonB-261 bp, fur-244 bp); D – genes encoding sialidase proteins (nanB-555 bp, pmHAS-430 bp, nanH-287 bp).

Figure 3A shows that PCR fragment of hgbA gene sized 267 bp has been found in the genomes of the assayed isolates. Product of pflA gene 286 bp long was detected in genomes of isolates Nos. 4, 5, 6, 7, 8. The results of PCR for presence of ompH, oma87, plpB, hsf2, plpB and ompA genes (Figure 3B) show that ompH, oma87 and plpB genes are absent in *P. multocida* isolates. The region of hsf2 gene sized 433 bp was detected in genomes of isolates Nos. 1, 3, 6, 7, 8. OmpA gene encoding the outer membrane protein A was present in genomes of all isolates.

Figure 3C shows PCR products of *fimA*, *ptfA*, *exbB*, *tonB*, *fur* genes. *TonB* and *exbB* genes associated with iron acquisition related factors were present in genomes of all isolates whereas

the *fimA*, *ptfA* and *fur* genes were not detected in the genome of isolate No. 2.

Table 5: Genetic profiles of virulent genes from *P.multocida* isolated from saigas.

№	Genes	Isolates of Year 2010		Isolates of Year 2011		P Value
		Total (n=25)	Total (%)	Total (n=8)	Total (%)	
1	<i>pfhA</i>	3/25	12	5/8	62.5	0.0099
2	<i>hgbA</i>	19/25	76	8/8	100	0.2964
3	<i>ompA</i>	23/25	92	8/8	100	1.0000
4	<i>ompH</i>	4/25	16	0/8	0	0.5503
5	<i>oma87</i>	12/25	48	0/8	0	0.0299
6	<i>plpB</i>	11/25	44	0/8	0	0.0313
7	<i>hsf2</i>	22/25	88	5/8	62.5	0.1365
8	<i>fimA</i>	17/25	68	7/8	87.5	0.3939
9	<i>ptfA</i>	17/25	68	7/8	87.5	0.3939
10	<i>exbB</i>	17/25	68	7/8	87.5	0.3939
11	<i>tonB</i>	25/25	100	8/8	100	1.0000
12	<i>fur</i>	18/25	72	5/8	62.5	0.6728
13	<i>nanH</i>	20/25	80	8/8	100	0.3023
14	<i>nanB</i>	0/25	0	0/8	0	0.0000
15	<i>pmHAS</i>	0/25	0	0/8	0	0.0000

Only one gene (*nanH*) sized 287bp, of all genes encoding sialidase proteins was found in the assayed isolates (Figure 3D). In genomes of *P.multocida* isolated from dead saigas in 2011 *nanB* and *pmHAS* genes were absent. Table 5 shows quantitative content (%) of virulent genes in genomes of *P.multocida* isolated from saiga antelopes in years 2010 and 2011.

plotted (Figure 4) to reflect the background of virulence gene profiles of *P.multocida* isolated from dead saigas in the Republic of Kazakhstan during years 2010-2011.

Judging on the results of the comparative analysis of data plotted on diagram (Figure 4) the background of virulence gene profiles of *P.multocida* isolated from saiga antelopes in 2010 include 13 genes while in *P.multocida* isolated from saigas in 2011 only 10 genes were detected. In 2011 sharp drop in mortality rate among saigas (only about 500 animals) versus year 2010 (≈12000 animals) was recorded maybe owing to reduction of the virulence background due to the influence of some biological, physical and chemical factors on the causative agent.

Percentage of virulent genes in the genomes of isolates under study appeared to be different, e.g., content of *tonB* gene was 100%, of *fur* gene-72%, of *hgbB* gene-76%, *pfhA*-12%, *ompA*-92%, *oma87*-48%, *plpB*-44%, *ompH*-16%, *nanH*-80 %, and percentage of *fimA*, *ptfA* and *exbB* genes was 68%. Statistical analysis of data has been performed using Fisher's exact test and the resulted p values are shown in Table 4 and Figure 4. Differences in content of virulent *pfhA*, *oma87* and *plpB* genes in *P.multocida* isolated from saiga antelopes in years 2010 and 2011 were considerable ($p < 0.05$). As it concerns virulent *hgbA*, *ompA*, *ompH*, *hsf2*, *fimA*, *ptfA*, *exbB*, *tonB*, *fur* and *nanH* genes the differences were insignificant ($p > 0.1$).

Determination of the virulent gene profiles of *P.multocida* isolated from saigas in 2011 showed 100% content of *hgbA*, *ompA*, *tonB* and *nanH* genes. Percentage of *fimA*, *ptfA* and *exbB*

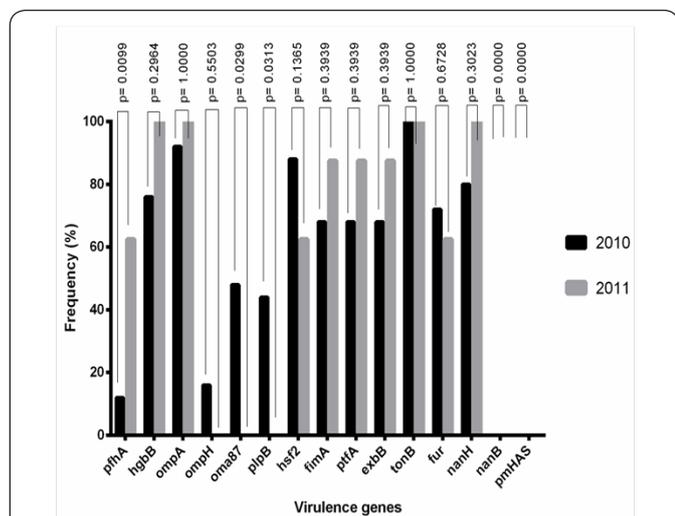


Figure 4: Background of virulence gene profiles of *P.multocida* isolates, 2010-2011 years.

The study towards determination of virulent gene profiles of *P.multocida* isolated from dead saiga antelopes in 2010 Table 5 has shown that *hgbA*, *pfhA*, *ompA*, *ompH*, *oma87*, *plpB*, *hsf2*, *fimA*, *ptfA*, *exbB*, *tonB*, *fur*, *nanH* genes are present in the genomes of the isolates while *nanB* and *pmHAS* genes are absent. On the basis of the obtained data (Figures 2,3 & 5) the diagram has been

genes in genomes of *P.multocida* isolates was 87.5%, of genes *pfhA*, *hsf2* and *fur*-62.5%. *PlpB*, *oma87*, *ompH*, *nanB* and *pmHAS* genes were absent.

Discussion

So far the principles of pathogenicity mechanisms and immune processes in *P.multocida* are not known. The putative determinants of virulence such as genes encoding the outer membrane proteins, genes participating in iron transport, enzymes, toxins and adhesins play the certain role in these processes and their presence in *P.multocida* genomes is associated with virulence.

Some authors recommend to use *hgbA* gene as an epidemiological marker for characterization of bacterial strains and isolates of *P.multocida* [4,13]. Content of the virulent *hgbB* gene in genomes of Kazakhstanean isolates from saigas in 2010 was 76% whereas in isolates of year 2011 it was 100%. A number of foreign scientists have shown 100% presence of the virulent *hgbA* gene in genomes of *P.multocida* isolated from different animal species [7].

Filamentous hemagglutinin *pfhA* gene is used as an epidemiological marker in *P.multocida* isolated from cattle. In our study only 12% of the assayed isolates of year 2010 contained the virulent *pfhA* gene whereas its content in the isolates of year 2011 was 62.5% ($p<0.05$). Adler et al. [14] paid special attention to detection of surface antigens participating in pathogenesis of *P. multocida*. They extracted the protective protein of outer membrane *oma87* that participates in export of other virulence factors from a bacterial cell. Our studies show 48% content of *oma87* gene in genomes of Kazakhstanean isolates of *P.multocida* from saiga antelopes in 2010 and its absence in isolates of year 2011 ($p<0.05$).

Also the assayed Kazakhstanean isolates of *P.multocida* showed low content of *ompH* gene, e.g. only 16% of *P.multocida* isolated in 2010 contained this gene, and in isolates of year 2011 this gene was not detected. One more gene encoding the outer membrane protein is *ompA* gene. As Table 3 shows content of this gene in the genomes of Kazakhstanean isolates of *P.multocida* is high. In isolates of year 2010 the percentage of this gene is 92%, and in isolates of year 2011 it is 100%.

According to Lu YS et al. [15] the protein encoded by *ompA* gene as well as the protein encoded by *ompH* gene is protective, that is why the results of molecular and genetic analysis are significant in studying pathogenesis of *P.multocida*.

Content of *plpB* gene encoding outer membrane lipoprotein in the bacteria isolated in 2010 was 44%, whereas in *P.multocida* isolates of year 2011 it was absent ($p<0.05$). Adhesins are essential to colonization and invasion of the host by *P.multocida* and as a rule their presence on bacterial surface is associated with virulence [3]. They include *fimA* (fimbrial), *hsf-1* and *hsf-2* (autotransporter adhesins), *pfhA* (filamentous hemagglutinins),

ptfA (subunits of type IV pili) genes. Out of all isolates of year 2010 68% contained virulent *fimA* gene and it was detected in 87.5% of isolates of year 2011. Occurrence of virulent *ptfA* gene in genomes of *P.multocida* isolated in Kazakhstan in years 2010 and 2011 was respectively 68% and 87.5%. Content of the virulent *hsf-2* gene in genomes of Kazakhstanean isolates of years 2010 and 2011 was respectively 88% and 62.5%. These data are close to the results of other authors [6].

The virulent *tonB* gene was detected in all assayed Kazakhstanean isolates of *P.multocida*. Content of the virulent *exbB* gene in isolates of year 2010 was 68%, and in isolates of year 2011 it was 87.5%. Virulent *fur* gene was present respectively in 72% and 62.5% of *P.multocida* isolated in years 2010 and 2011.

Sialidase proteins encoded by *nanH* and *nanB* genes can localize on the surface of host cells and destroy the receptors of the immune system [16,17]. In our study virulent *nanH* gene was detected respectively in 80% and 100% of bacterial genomes of *P.multocida* isolated in years 2010 and 2011. Virulent *nanB* gene was absent in genomes of Kazakhstanean isolates. Virulent *pmHAS* and *nanB* genes were not found in genomes of *P.multocida* isolated from saiga antelopes in Kazakhstan in years 2010 and 2011.

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