Occurrence of virulence factors and antimicrobial resistance in Pasteurella multocida strains isolated from slaughter cattle in Iran

Faham Khamesipour¹, Hassan Momtaz²* and Morteza Azhdary Mamoreh³

¹ Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
² Department of Microbiology, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
³ Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

INTRODUCTION

Cattle rearing is one of the important sources of income in Iran, involving both dairy and beef breeds. The sector faces a number of constraints ranging from limited feed resources to diseases. Of the diseases, those caused by infectious agents are of great importance which include bacteria and viruses affecting the respiratory system (Hemmatzadeh et al., 2001; Haji Hajikolaei and Seyfi Abad Shapouri, 2007; Sakhaee et al., 2009). The most important bacteria that play a role in pneumonia include: Mannheimia haemolytica, Pasteurella multocida, and Haemophilus somnus pneumonia, which presence of these bacteria in pneumonia lesions of slaughtered cattle around Iran have been also reported (Haji Hajikolaei et al., 2010).

A total of 30 Pasteurella multocida strains isolated from 333 pneumatic and apparently healthy cattle were examined for capsule biosynthesis genes and 23 virulence-associated genes by polymerase chain reaction (PCR). The disc diffusion technique was used to determine antimicrobial resistance profiles among the isolates. Of the isolates, 23 belonged to capsular type A, 5 to capsular type D and two isolates were untypeable. The distribution of the capsular types in pneumatic lungs and in apparently healthy lungs was statistically similar. All virulence genes tested were detected among the isolates derived from pneumatic lungs; whereas isolates derived from apparently healthy lungs carried 16 of the 23 genes. The frequently detected genes among isolates from pneumatic lungs were exbD, hgbA, hgbB, ompA, ompH, oma87, and sodC; whereas tadD, toxA, and pmHAS genes occurred less frequently. Most of the adhesins and superoxide dismutases; and all of the iron acquisition and protectin proteins occurred at significantly (p ≤ 0.05) higher frequencies in isolates from pneumatic lungs. Isolates from apparently healthy lungs didn’t carry the following genes; hsf-1, hsf-2, tadD, toxA, nanB, nanH, and pmHAS. One adhesion (hsf-1) and two iron acquisition (exbD and tonB) genes occurred at significantly (p ≤ 0.05) higher frequencies among capA isolates. All the P. multocida isolates were susceptible to ciprofloxacin, co-trimoxazole, doxycycline, enrofloxacin, nitrofurantoin, and tetracyclines. Different proportions of the isolates were however resistant to ampicillin, amoxicillin, erythromycin, lincomycin, penicillin, rifampin, streptomycin, and florfenicol. Our results reveal presence of virulence factors (VFs) in P. multocida strains isolated from symptomatic and asymptomatic bovids. A higher frequency of the factors among isolates from symptomatic study animals may suggest their role in pathogenesis of P. multocida-associated bovine respiratory disease (BRD). The results further reveal occurrence of antimicrobial resistance among some isolates. Control strategies for this pathogen, which could include development of an effective vaccine, are warranted so as to mitigate the social and economic consequences attributable to natural infections with this bacterium.

Keywords: Pasteurella multocida, virulence factors, antimicrobial resistance, cattle, Iran

Bovine respiratory disease (BRD) is a significant cause of morbidity and mortality among beef cattle in the world (Dagleish et al., 2010; Hotchkiss et al., 2010; Portis et al., 2012). Among others, Pasteurella multocida has been identified as a major bacterial etiologic agent for this disease (Confer, 2009; Griffin et al., 2010). It is a zoonotic Gram negative bacterium responsible for a range of infections in domestic animals causing substantial economic losses (Steen et al., 2010). The organism causes fowl cholera in domestic and wild birds, bronchopneumonia and hemorrhagic septicemia in bovids, atrophic rhinitis in porcines and snuffles in rabbits (Mannheim, 1984; Hunt et al., 2000). Most human infections with P. multocida result from dog and cat bites, but infections through
the respiratory tract may also occur (Hubbert and Rosen, 1970).

Several host and pathogen-specific attributes do determine the outcome of infections caused by *P. multocida* (Verma et al., 2013). Of the pathogen factors important ones include the capsular and virulence-associated genes (Katsuda et al., 2013). These virulence factors (VFs) and outer membrane proteins are important for pathogenesis, functionality, protective immunity and vaccine development against *P. multocida* infections (Harper et al., 2006; Hatfalahdi et al., 2010). Based on capsular antigens, *P. multocida* strains are differentiated into five serogroups i.e., type A causing fowl cholera pathogen and bovine shipping fever, type B causing hemorrhagic fever in ungulates, type D causing atrophic rhinitis in swine, type E, an African serotype, infecting cattle and buffalo; and type F also causing fowl cholera (Carter, 1955, 1961, 1967; Rimler and Rhoades, 1987). Virulence associated genes described for *P. multocida* isolates and their examples include adherence and colonization factors (*ptfA, fimA, hsf-1, hsf-2, pfhA, and tadD*), iron-regulated and acquisition proteins (*exbB, exbD, tonB, hgbA, hgbB, and Fur*), extracellular enzymes such as neuraminidase (*nanB and nanH*), hyaluronidase (*pmHAS*) and superoxide dismutases (*soda, sodC, and tlpA*), toxins (*toxA*), lipopolysaccharides (LPS), capsule and a variety of outer membrane proteins such as protectins (*ompA, omph, oma87*, and *plpB*) (Katoch et al., 2014).

Increased use of antibiotics in modern animal production has been associated with emergence of antimicrobial resistant bacteria with potential for transfer of resistance from animals to humans (Witte, 1998). As a result, antimicrobial resistance among bacterial pathogens has of recent become a big problem in both the veterinary and human medicine fields (Levy, 1998; Caprioli et al., 2000; Kehrenberg et al., 2001; White et al., 2002; Shea, 2003). The implication of the problem is increased treatment cost, prolonged illness due to treatment failure and sometimes death (Kelly et al., 2004).

The present study was conducted with the aim to detect the occurrence of VFs in *P. multocida* isolated from pneumatic and apparently healthy lungs in cattle. It was also to determine the occurrence of antimicrobial resistance among the isolates.

**MATERIALS AND METHODS**

**SAMPLE COLLECTION**

A total of 333 samples, from both pneumatic (219) and apparently healthy (114) lungs, were collected randomly from slaughter cattle in an industrial abattoir in Shahrekord province during the period of September 2013 to March 2014. The abattoir receives cattle from different herds within and outside the province. For the purpose of this study pneumatic lungs referred to those lungs with gross lesions such as consolidation, fibrin deposition on the pleura, pleurisy, and/or adhesion; and apparently healthy lungs was used to describe those lungs without gross lesions. A simple random procedure was used to select pre-identified pneumatic and apparently health lungs. Random numbers were generated in Microsoft excel®. Specimens were obtained aseptically using a sterile scalpel while taking precautions to prevent surface contamination. Following collection the samples were conveyed to the microbiology laboratory in special ice-filled containers within 6 h of sampling.

**P. MULTOCIDA SCREENING**

Isolation of *P. multocida* was done using techniques described previously by other authors (Soner and Post, 2005). Briefly, swabs were obtained from the collected samples and were plated on tryptic soy agar (Difco, Detroit, MI) containing 10 μg/ml NAD (Sigma, St. Louis, MO) and 5% bovine serum, MacConkey agar, and blood agar (5% fresh sheep blood). All plates were incubated at 37°C in air for a minimum of 48 h.

**IDENTIFICATION OF ISOLATES**

Preliminary identification of *P. multocida* isolates was carried out according to standard biochemical tests as described earlier (Soner and Post, 2005). The isolates were gram-negative cocccobacilli and were indole, catalase and oxidase-positive. But, citrate, Methyl red (MR), Voges–Proskauer (VP), and gelatin liquefaction negative. They don’t grow on MacConkey agar and do not show hemolysis on blood agar. Confirmation of the isolates was done by polymerase chain reaction (PCR) assay with primers specific for the amplification of the KMT1 gene, adopting the methodology previously described by Townsend et al. (1998). All confirmed isolates of *P. multocida* were subsequently characterized by capsular serotyping using PCR. Primers for amplification of *hyaD-hyaC* and *DcbF* genes were used for detection of capsular type A and capsular type D, respectively (*Table 1*). *P. multocida* isolates which didn’t yield bands on PCR when the two primers were used were classified as untyped. Following confirmation and characterization all isolates were freeze-dried and kept at −20°C.

**DETECTION OF VIRULENCE GENES**

The virulence genes of *P. multocida* isolates were detected by PCR. They included adhesins (*ptfA, fimA, hsf-1, hsf-2, pfhA, and tadD*),

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**Table 1 | Primers used for the detection of serogroups in strains of *P. multocida*.**

| Serogroup | Gene | Primer name | Primer sequence (5′–3′) | Amplic size (bp) | Anneal. Temp (°C) | Reference |
|-----------|------|-------------|-------------------------|-----------------|--------------------|-----------|
| All       | KMT1 | KMT17T      | ATCCGCTATTTACCAGTCAGGG  | 460             | 55                 | Townsend et al., 1998 |
|           |      | KMT1SP6     | GCTGTAACGAAATCGCCAC    |                 |                    |           |
| Capsular type A | hyaD-hyaC | CAPA-F | CATTATCCAAGCTCCACC | 760             | 55                 |           |
|           |      | CAPA-R      | GCGCGAGGTTTCATCC       |                 |                    |           |
| Capsular type D | DcbF | CAPD-F      | TTAAAGAAGAAGACTAGAGGCC | 657             | 55                 |           |
|           |      | CAPD-R      | CATCTACCCACTCAACCATCAC |                  |                    |           |
toxin (toxA), iron acquisition (exbB, exbD, tonB, hgbA, hgbB, and Fur), sialidases (nanB and nanH), hyaluronidase (pmHAS), protectins (ompA, ompH, oma87, and plpB) and superoxide dismutases (soda, sodC, and tbpA) (Table 2). The base sequences and the predicted sizes of the amplified products for the specific oligonucleotide primers used in detection of the genes in this study are shown in Table 3. The bacterial lysates used as templates for the PCR were prepared as follows. A loopful of bacteria from a fresh overnight culture on a tryptic soy agar plate was resuspended homogeneously in 200 μl of sterile water, and the mixture was boiled at 100°C for 5 min to release the DNA and centrifuged. A 4 μl volume of the supernatant was used as a template for each 25 μl PCR mixture. The amplified products were analyzed in 1% agarose gels by electrophoresis, and the results were recorded with a gel documentation system. All tests were repeated three times in parallel with the relevant positive (P. multocida strains ATCC 15742, ATCC 12945, and ATCC 12946) and negative (distilled water) controls. Discrepant results for each VF were investigated further, and samples were sequenced for gene verification.

**ANTIMICROBIAL RESISTANCE TEST**

Antimicrobial resistance profiles of the isolates to 20 antimicrobial agents were determined by the disc diffusion method on Muller Hinton agar with 5% blood (Carter and Subronto, 1978). The plates were inoculated with a cotton swab dipped into a 0.5 McFarland standard suspension of each isolates, according to the procedures outline in NCCLS (NCCLS, 2008). Then, the plates were incubated at 37°C for 24 h. The inhibition zones around each disc were measured and interpretation of results made according to the guidelines provided by manufacturers (Pattan-Teb, Tehran, Iran) and those provided by NCCLS (2008). The results were interpreted as resistant (R), intermediate (I), and susceptible (S).

**STATISTICAL DATA ANALYSIS**

Data analysis was performed in SPSS software version 12.0 (SPSS Inc., Chicago, IL). Descriptive statistics were computed to determine the proportions of the different VFs among the isolates; and proportions of isolates resistant to different antimicrobial agents. Chi square test adopted for determination of statistical significance of differences between the proportions.

**RESULTS**

**PREVALENCE OF P. MULTOCIDA IN COLLECTED SAMPLES**

The prevalence of *P. multocida* in collected lung samples is indicated in Table 4. Overall 9.0% (30/333) of the sampled cattle were infected with the organism. The frequency of infection with the organism was higher in pneumatic lungs than in apparently health lungs and the difference was statistically significant at *p* ≤ 0.05.

**DISTRIBUTION OF VFs ACCORDING TO CAPSULAR SEROTYPES**

Two capsular types (A and D) were detected among 28 of the 30 isolates obtained as seen in Tables 5, 6. The majority (76.7%) of the isolates were of capsular type A. The distribution of the capsular types in pneumatic lungs and in apparently health lungs (Table 5) didn't show any statistically significant difference. The distribution of capsular serotypes for each individual isolate is displayed in Table 6.

**DISTRIBUTION OF VFs ACCORDING TO ASSOCIATED VF GENES**

All isolates from pneumatic lungs harbored at least one virulence gene as displayed in Table 7. Table 8 shows the distribution of virulence genes by capsular serotypes. The detected virulence genes for each isolate obtained in this study is presented in Table 6. Most of the adhesins and superoxide dismutases; and all of the iron acquisition and protectin proteins occurred at significantly (*p* ≤ 0.05) higher frequencies in isolates from pneumatic lungs. One adhesion (hhs1-1) and two iron acquisition (exbD and tonB) genes occurred at significantly (*p* ≤ 0.05) higher frequencies among capA isolates.

| Table 2 | Tested virulence-associated genes in strains of *P. multocida*. |
|---------|----------------------------------------------------------------|
| **Gene function and gene** | **Description** |
| **ADHESINS** | |
| ptfA | Type 4 fimbriae |
| fimA | Fimbriae (from Pm70) |
| hsf-1 | Autotransporter adhesion (from Pm70) |
| hsf-2 | Autotransporter adhesion (from Pm70) |
| pfhA | Filamentous hemagglutinin |
| tadD | Putative non-specific tight adherence protein D |
| toxA | Dermonecrotic toxin |
| exbB | Accessory protein Ton-dependent transport of iron compounds |
| exbD | Accessory protein Ton-dependent transport of iron complex |
| tonB | Iron transporters, transport ferric-siderophore complexes |
| hgbA | A hemoglobin-binding protein |
| hgbB | B hemoglobin-ion uptake |
| Fur | Ferric uptake regulation protein |
| **SIALIDASES nanB** | |
| nanB | Outer membrane-associated proteins, an autotransporter protein |
| nanH | Outer membrane-associated proteins, small sialidases |
| **HYALURONIDASE** | |
| pmHAS | Hyaluronan synthase |
| **SUPEROXIDE DISMUTASE** | |
| soda | Superoxide dismutase |
| sodC | Superoxide dismutase |
| tbpA | Superoxide dismutase |
| **PROTECTINS** | |
| ompA | Outer membrane protein A |
| ompH | Outer membrane protein H |
| oma87 | Outer membrane protein 87 |
| plpB | Lipoprotein B |
| Gene function and gene | Primer sequence (5′–3′) | Amplicon size (bp) | Annealing temp (°C) | References |
|------------------------|--------------------------|--------------------|---------------------|------------|
| ADHESINS               |                          |                    |                     |            |
| ptfA                   | TGTGAATTCAGCATTTAGTGTC   | 488                | 55                  | Townsend et al., 1998 |
| fimA                   | CCATCGGATCTAAACGACCTA    | 866                | 55                  |            |
| hsf-1                  | TTAGTGCCGCTGAGTCTTCG     | 654                | 54                  |            |
| hsf-2                  | ACCGCAACCATGCTCTTAC      | 433                | 54                  |            |
| pfhA                   | TCCAGGGGATCAAATCTTCG     | 286                | 55                  |            |
| tadD                   | TGTACATTTCAGGAAGCAGC     | 416                | 55                  |            |
| TOXINS                 |                          |                    |                     |            |
| toxA                   | CTTAGATGAGCCGCAAAGG      | 864                | 55                  | Townsend et al., 1998 |
| SUPEROXIDE DISMUTASE   |                          |                    |                     |            |
| sodA                   | TACCAAGATTAGGCTACGC      | 361                | 55                  | Ewers et al., 2006 |
| tbpA                   | TTGGTGGAACGGGAAAAGC      | 728                | 54                  |            |
| sodC                   | AGTATAGCGGGGATTGGCA      | 235                | 55                  | Lainson et al., 1996 |
| SIALIDASES nanB        |                          |                    |                     |            |
| nanB                   | CATTGCACTACATACACCCT     | 555                | 55                  | Townsend et al., 1998 |
| nanH                   | GTGGGAACCGGAATTGTA       | 287                | 55                  |            |
| PROTECTINS             |                          |                    |                     |            |
| ompA                   | CGCATAGCACTCACGTTTCTCC   | 201                | 55                  | Townsend et al., 1998 |
| ompH                   | CGCGTATAGGGTTTAGGT       | 438                | 55                  |            |
| oma87                  | GGCCAGCGGCAACAGATAACGC   | 838                | 55                  |            |
| plpB                   | TTTGGTGGTGCGTAGTCTCTTCT  | 282                | 55                  |            |
| HYALURONIDASE          |                          |                    |                     |            |
| pmHAS                  | TCAATGTGGCGATAGTCCCTAGA  | 430                | 54                  | Townsend et al., 1998 |
| IRON ACQUISITION       |                          |                    |                     |            |
| exbB                   | TTGGCCTTGTGATGCTAAGC     | 283                | 55                  | Townsend et al., 1998 |
| exbD                   | CGTTCTGATTACGCTTCTT      | 247                | 55                  |            |
| tonB                   | CGACGCTAGAACTCGACCC     | 261                | 55                  |            |
| hgbA                   | TCAACGGGATGAAATACGCGG    | 267                | 55                  |            |
| Fur                    | GTTTACGTGATATGACCA       | 244                | 55                  | Ewers et al., 2006 |
| hgbB                   | ACCGCGTGGAAATTGTGGTGATAG| 788                | 55                  |            |
in this study are displayed in thromycin, and florfenicol was observed at different frequencies. Resistance to ampicillin, lin-
tible to ciprofloxacin, co-trimoxazole, doxycycline, enrofloxacin,

Table 4 | Prevalence of *P. multocida* in collected cattle lung samples.

| Lung samples       | Number of samples | Number of positive samples |
|--------------------|-------------------|---------------------------|
| Pneumonic lungs    | 219               | 25 (11.4%)                |
| Healthy lungs      | 114               | 5 (4.4%)                  |
| Total              | 333               | 30 (9.0%)                 |

Table 5 | Distribution of capsular serotypes among the isolates.

| Capsular types | Overall prevalence (n = 30) | Pneumonic lung isolates (n = 25) | Apparently healthy lung isolates (n = 5) |
|----------------|----------------------------|---------------------------------|----------------------------------------|
| Type A         | 23 (76.7%)                 | 18 (72.0%)                      | 5 (100.0%)                             |
| Type D         | 5 (16.7%)                  | 5 (20.0%)                       | –                                      |
| Untyped        | 2 (6.7%)                   | 2 (8.0%)                        | –                                      |

**ANTIMICROBIAL RESISTANCE AMONG THE ISOLATES**

Antimicrobial resistance profiles of *P. multocida* isolates obtained in this study are displayed in Table 9. All the isolates were susceptible to ciprofloxacin, co-trimoxazole, doxycycline, enrofloxacin, nitrofurantoin, and tetracyclines. Resistance to ampicillin, lin-
comycin, penicillin, rifampin, streptomycin, amoxicillin, ery-
thermycin, and florfenicol was observed at different frequencies.

**DISCUSSION**

VFs play a key role in disease production by bacterial pathogens (Nanduri et al., 2009). Among others, their functions include competence, adherence, synthesis, and export of capsules; and evasion of host immune responses (Nanduri et al., 2009). In the present study the factors have been detected in *P. multocida* isolated from the lungs of slaughter cattle. The higher frequency of the factors among isolates from pneumonic lungs suggests the role of these factors in disease occurrence. It was pointed out that virulence gene occurrence in *P. multocida* has a strong positive association with the outcome of infection with the organism in cattle (Katsuda et al., 2013). On the other hand occurrence of the factors in apparently healthy lungs could possibly indicate early infection or contained infection which couldn’t lead to disease. It was previously reported that this facultative anaerobic bacterium is commonly found in clinically healthy calves (Lainson et al., 2013).

In this study capsular types A and D were detected using PCR among the obtained *P. multocida* isolates. A small proportion (6.7%; 2/30) of *P. multocida* strains were untypeable, a similar observation to what was reported by Arumugam et al. (2011). Capsular type A was predominant among the strains accounting for 76.6%. Our observation is similar to a finding by Katsuda et al. (2013) who also detected capsular types A and D among cattle derived *P. multocida* isolates; with type A occurring at higher frequency. A higher frequency of capsular type A among cattle derived *P. multocida* isolates has also been reported in a study conducted earlier by Davies et al. (2004) who found that 99.3% of bovine *P. multocida* strains (n = 153) were of this capsular type. *P. multocida* isolates of serotype A are common in bovids occurring as normal flora in the nasopharynx; or as causes of disease including BRD and hemorrhagric septicemia (Ewers et al., 2004; Dabo et al., 2007). The capsular type A is also most frequently described for rabbits (Ewers et al., 2006) and pigs (Garcia et al., 2011).

Of the protectins; *ompA* and *oma* were the most frequently detected genes particularly in the isolates from pneumonic lungs. Slightly higher frequencies of the two genes were noted for isolates of the capA serogroup than those belonging to the capD serogroup. The *ompA* gene has a significant role in stabilizing the cell envelope structure by providing physical linkage between the outer membrane and peptidoglycan (Katoch et al., 2014). It mediates *P. multocida* host cells interaction through heparin and/or fibronectin binding and thus acts as an important invasive molecule which could determine the outcome of infection with the organism (Katoch et al., 2014).

The type 4 fimbria (ptfA gene) was described in 92.0% of the isolates tested in the current study. The gene plays a key role of fixing bacterial pathogens on the surface of the epithelial cells of hosts, a phenomenon which is more common in rabbits (Ewers et al., 2006). Consequently a study conducted on rabbits described a high prevalence of the ptfA gene (93.4%; 43/46).

Presence of adhesins on the bacterial surface is usually linked to virulence as these proteins are known to play a crucial role in facilitating host invasion and colonization (Kline et al., 2009). Studies by Ewers et al. (2000) and Tang et al. (2009) have demonstrated that, of the adhesins; fimA, hsf-2, and ptfA are of frequent occurrence among pathogenic isolates of *P. multocida*. In the current study the three adhesins were demonstrated at higher frequencies than others in both capA and capD serogroup isolates. On the other hand, gene *tadD* was the least frequently detected adhesin among *P. multocida*, occurring only in 48.0% of the isolates (n = 30). In these organisms the gene is known to be a putative non-specific tight adherence protein D (May et al., 2001). A more or less similar low frequency (43.3%; 100/233) of tadD was described in a field study involving pigs (Tang et al., 2009). A work on rabbits, however, observed a higher frequency (91.3%; 42/46) of this gene among *P. multocida* strains.

It is noteworthy that the dermonecrotxin encoding *toxA* was the least frequently detected gene among the isolates; demonstrated only in those of capsular type A obtained from pneumonic lungs. Some other researchers indicated that this particular gene is more frequently expressed by strains of serogroup D and is responsible for the clinical symptoms associated with atrophic rhinitis in porcines (Harper et al., 2006; Ferreira et al., 2012). The observation in the current study could be attributed to the small sample size of capsular type D isolates. In a study conducted earlier the gene was detected in *P. multocida* isolates from avians, swine, sheats and cattle; but was only associated with disease in pigs (Ewers et al., 2006). Pullinger et al. (2004) points out that the toxA gene is not inserted into the bacterial chromosome but in a lysogenic bacteriophage that infects the agent.

The *tbpA* encoding gene is known to be of common occurrence among ruminant *P. multocida* strains (Ewers et al., 2006; Atashpaz et al., 2009). Its prevalence was however relatively low when compared to other superoxide dismutases (sodA and sodC) tested in this study. Ferreira et al. (2012) found a low frequency (8.6%;
### Table 6 | Capsular types and virulence genes detected among *P. multocida* isolates obtained from cattle lungs in Iran.

| Strain ID | Capsule type | Virulence genes |
|-----------|--------------|-----------------|
| 1         | Type A       | ptfA, fimA, hsf-1, tonB, hgbA, hgbB, Fur, nanB, nanH, pmHAS, ompA, oomph, plpB, soda, sodC |
| 2         | Type A       | ptfA, fimA, hsf-1, exbD, tonB, hgbA, hgbB, Fur, nanB, nanH, pmHAS, ompA, oomph, oma87, plpB, soda, sodC, tbpA |
| 3         | Type A       | ptfA, fimA, hsf-1, tonB, hgbA, hgbB, Fur, nanB, nanH, pmHAS, ompA, oomph, oma87, plpB, soda, sodC, tbpA |
| 4         | Type D       | ptfA, hsf-2, pfhA, exbB, exbD, hgbA, hgbB, Fur, nanB, nanH, ompA, oomph, oma87, plpB, soda, sodC, tbpA |
| 5         | Type D       | ptfA, fimA, hsf-2, pfhA, tadD, exbB, hgbB, Fur, nanB, nanH, ompA, oomph, oma87, plpB, soda, sodC, tbpA |
| 6-25      | Untyped     | pfhA, Fur, nanB, nanH, ompA, oomph, oma87, plpB, tbpA |
| 26-30      | Type D       | ptfA, fimA, hsf-1, hsf-2, pfhA, tadD, exbB, hgbA, hgbB, Fur, nanB, nanH, pmHAS, ompA, oomph, oma87, plpB, soda, sodC, tbpA |

(Continued)
4/46) of this gene in a study conducted on rabbits. Variable frequencies of the genes encoding proteins with different functions, such as adhesins (fimA, hsf-1, hsf-2, and plpB), iron acquisition (exbB, exbD, tonB, hgbA, hgbB, and Fur), sialidases (nanB and nanH), hyaluronidase (pmHAS), and protecins (ompA and plpB) were found in the isolates. This finding is similar to what was reported in previous works which involved ruminants, porcine, poultry, and rabbits (Ewers et al., 2006; Tang et al., 2009; Ferreira et al., 2012).

**Infections with *P. multocida*** are commonly managed by broad-spectrum antimicrobials (Kehrenberg et al., 2001; Lion et al., 2006; Brogden et al., 2007). Studies have however reported occurrence of resistance to a large number of antimicrobial agents among *P. multocida* isolates (Hunt et al., 2001; Davies et al., 2004; Arashima and Kumasaka, 2005). In the current study all the *P. multocida* isolates were susceptible to ciprofloxacin, co-trimoxazole, doxycycline, enrofloxacin, nitrofurantoin, and tetracyclines. Similar observations for ciprofloxacin and co-trimoxazole (Mohamed et al., 2012); and for enrofloxacin, tetracycline, and doxycycline (Ferreira et al., 2012) have also been made earlier. These antibiotics can therefore be used for prevention and treatment of bovine *P. multocida* infections in the study area. Unlike other authors who reported poor (Gutiérrez Martin and Rodríguez Ferri, 1993; Yoshimura et al., 2001) and moderate (Mohamed et al., 2012) activity of aminoglycoside antibiotics against *P. multocida*, our current study kanamycin, gentamicin, amikacin, and streptomycin exhibited high activity against the tested isolates. The frequencies of resistant isolates to other antibiotics varied greatly as reported by other researchers (Salmon et al., 1995; Kehrenberg et al., 2001; Welsh et al., 2004).

The major limitation in the discussion of the findings of the current study was large differences in the sample sizes of comparison groups as seen for isolates between isolates from pneumatic lungs and those from apparently healthy lungs; and isolates of different capsular types. This made it difficult to infer on the observed variations as they could be attributed to chance.

In summary, our results reveal presence of VFs in *P. multocida* strains isolated from the lungs of symptomatic and asymptomatic...

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**Table 7 | Distribution of VFs according to associated VF genes.**

| Virulence genes | Overall prevalence (n = 30) | Pneumonic lung isolates (n = 25) | Apparently healthy lung isolates (n = 5) |
|-----------------|-----------------------------|---------------------------------|--------------------------------------|
| **ADHESINS**    |                             |                                 |                                      |
| ptfA            | 24 (80.0%)                  | 23 (92.0%)                      | 1 (20.0%)                            |
| fimA            | 24 (80.0%)                  | 23 (92.0%)                      | 1 (20.0%)                            |
| hsf-1           | 18 (60.0%)                  | 18 (72.0%)                      | –                                    |
| hsf-2           | 23 (76.7%)                  | 23 (92.0%)                      | –                                    |
| plpB            | 25 (83.3%)                  | 25 (100%)                       | –                                    |
| **TOXINS**      |                             |                                 |                                      |
| toxA            | 3 (10.0%)                   | 3 (12.0%)                       | –                                    |
| **IRON ACQUISITION** |                         |                                 |                                      |
| exbB            | 25 (83.3%)                  | 24 (96.0%)                      | 1 (20.0%)                            |
| exbD            | 26 (86.7%)                  | 25 (100%)                       | 1 (20.0%)                            |
| tonB            | 25 (83.3%)                  | 24 (96.0%)                      | 1 (20.0%)                            |
| hgbA            | 26 (86.7%)                  | 25 (100%)                       | 1 (20.0%)                            |
| hgbB            | 28 (93.3%)                  | 25 (100%)                       | 3 (60.0%)                            |
| Fur             | 25 (83.3%)                  | 24 (96.0%)                      | 1 (20.0%)                            |
| **SIALIDASES nanB** |                         |                                 |                                      |
| nanB            | 25 (83.3%)                  | 25 (100%)                       | –                                    |
| nanH            | 24 (80.0%)                  | 24 (96.0%)                      | –                                    |
| **HYALURONIDASE** |                         |                                 |                                      |
| pmHAS           | 10 (33.3%)                  | 10 (40.0%)                      | –                                    |
| **PROTECTINS**  |                             |                                 |                                      |
| ompA            | 27 (90.0%)                  | 25 (100%)                       | 2 (40.0%)                            |
| ompH            | 26 (86.7%)                  | 25 (100%)                       | 1 (20.0%)                            |
| oma87           | 27 (90.0%)                  | 25 (100%)                       | 2 (40.0%)                            |
| plpB            | 25 (83.3%)                  | 24 (96.0%)                      | 1 (20.0%)                            |
| **SUPEROXIDE DISMUTASE** |                   |                                 |                                      |
| sodA            | 25 (83.3%)                  | 24 (96.0%)                      | 1 (20.0%)                            |
| sodC            | 26 (86.7%)                  | 25 (100%)                       | 1 (20.0%)                            |
| tbpA            | 20 (66.7%)                  | 18 (72.0%)                      | 2 (40.0%)                            |

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**Table 8 | Distribution of VFs according to capsule serotypes among 30 bovine isolates of *P. multocida*.**

| Virulence genes | Overall prevalence (n = 30) | capA (n = 23) | capD (n = 5) | Untyped (n = 2) |
|-----------------|-----------------------------|---------------|--------------|----------------|
| **ADHESINS**    |                             |               |              |                |
| ptfA            | 24 (80.0%)                  | 19 (82.6%)    | 5 (100.0%)   | –              |
| fimA            | 24 (80.0%)                  | 20 (87.0%)    | 3 (60.0%)    | 1 (50.0%)      |
| hsf-1           | 18 (60.0%)                  | 18 (78.3%)    | –            | –              |
| hsf-2           | 23 (76.7%)                  | 18 (78.3%)    | 5 (100.0%)   | –              |
| plpB            | 18 (60.0%)                  | 13 (56.5%)    | 4 (80.0%)    | 1 (50.0%)      |
| **TOXINS**      |                             |               |              |                |
| toxA            | 3 (10.0%)                   | 3 (13.0%)     | –            | –              |
| **IRON ACQUISITION** |                         |               |              |                |
| exbB            | 25 (83.3%)                  | 19 (82.6%)    | 5 (100.0%)   | 1 (50.0%)      |
| exbD            | 26 (86.7%)                  | 23 (100.0%)   | 3 (60.0%)    | –              |
| tonB            | 25 (83.3%)                  | 23 (100.0%)   | –            | 2 (100.0%)     |
| hgbA            | 26 (86.7%)                  | 20 (87.0%)    | 5 (100.0%)   | 1 (50.0%)      |
| hgbB            | 28 (93.3%)                  | 23 (100.0%)   | 5 (100.0%)   | –              |
| Fur             | 25 (83.3%)                  | 19 (82.6%)    | 5 (100.0%)   | 1 (50.0%)      |
| **SIALIDASES nanB** |                         |               |              |                |
| nanB            | 25 (83.3%)                  | 19 (82.6%)    | 5 (100.0%)   | 1 (50.0%)      |
| nanH            | 24 (80.0%)                  | 18 (78.3%)    | 4 (80.0%)    | 2 (100.0%)     |
| **HYALURONIDASE** |                         |               |              |                |
| pmHAS           | 10 (33.3%)                  | 8 (34.8%)     | 2 (40.0%)    | –              |
| **PROTECTINS**  |                             |               |              |                |
| ompA            | 27 (90.0%)                  | 20 (87.0%)    | 5 (100.0%)   | 2 (100.0%)     |
| ompH            | 26 (86.7%)                  | 21 (91.3%)    | 3 (60.0%)    | 2 (100.0%)     |
| oma87           | 27 (90.0%)                  | 21 (91.3%)    | 5 (100.0%)   | 1 (50.0%)      |
| plpB            | 25 (83.3%)                  | 18 (78.3%)    | 5 (100.0%)   | 2 (100.0%)     |
| **SUPEROXIDE DISMUTASE** |                   |               |              |                |
| sodA            | 25 (83.3%)                  | 23 (100.0%)   | 2 (40.0%)    | –              |
| sodC            | 26 (86.7%)                  | 23 (100.0%)   | 3 (60.0%)    | –              |
| tbpA            | 20 (66.7%)                  | 18 (78.3%)    | 1 (20.0%)    | 1 (50.0%)      |
slaughter cattle. Frequent detection of the factors among isolates from symptomatic study animals may suggest their role in pathogenesis of BRD caused by these organisms. Occurrence of antimicrobial resistance among some isolates is of great concern. Control strategies for this pathogen, which could include development of an effective vaccine, are warranted so as to mitigate the social and economic consequences attributable to natural infections with this bacterium. Further, the use of antimicrobial agents in modern livestock farming need to be controlled so as to minimize the emergence and eventually spread of resistance not only in target microbes but also in other important zoonotic pathogens.

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