Research Article

Virulence Genotyping of Pasteurella multocida Isolated from Multiple Hosts from India

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In this study, 108 P. multocida isolates recovered from various host animals such as cattle, buffalo, swine, poultry (chicken, duck, and emu) and rabbits were screened for carriage of 8 virulence associated genes. The results revealed some unique information on the prevalence of virulence associated genes among Indian isolates. With the exception of toxA gene, all other virulence associated genes were found to be regularly distributed among host species. Association study between capsule type and virulence genes suggested that pfhA, nanB, and nanH genes were regularly distributed among all serotypes with the exception of CapD, whereas toxA gene was found to be positively associated with CapD and CapA. The frequency of hgbA and nanH genes among swine isolates of Indian origin was found to be less in comparison to its equivalents around the globe. Interestingly, very high prevalence of tbpA gene was observed among poultry, swine, and rabbit isolates. Likewise, very high prevalence of pfhA gene (95.3%) was observed among Indian isolates, irrespective of host species origin.

1. Introduction

Pasteurella multocida belonging to family Pasteurellaceae is a ubiquitous organism affecting multihost species, thus causing several diseases like haemorrhagic septicaemia in cattle and buffalo, enzootic bronchopneumonia in cattle, sheep, and goats, atrophic rhinitis in swine, fowl cholera in poultry and snuffles in rabbits [1, 2]. These diseases are known to cause severe financial loss to livestock industry, especially in tropical countries. Conventional vaccines have been used for several decades as a control strategy, but major limitation of these vaccines is their ineffectiveness in inducing long acting cross protective immunity [3, 4]. Therefore, several outer membrane proteins (OMPs) have been proposed as candidate antigen for subunit vaccine [3, 5].

The OMPs of Gram negative bacteria play an essential role in the disease process. They are involved in the process of nutrient uptake, transport of molecules in and out of the cell, colonization and invasion of the host, evasion of host immune response, injury to host tissue, and so forth, required for productive infection [6]. These proteins are subjected to different selection pressures, thereby exhibiting varying degree of interstrain heterogeneity. Therefore, these virulence associated genes can be used to assess intraspecies diversity and also to obtain epidemiological relationships [7]. In addition, these OMPs are good immunogens and can be used as vaccine components to provide protection [8–10]. Hence, virulence profiling can be used as a typing method for characterization of bacterial pathogens [11] and also for development of subunit vaccine in vaccine strain selection. For the first time, virulence profiling of P. multocida isolates was carried out by Ewers et al. [12], and subsequently it has been used by many workers to understand the diversity of the pathogen recovered from different host origin [13–19].
The previous study carried out in our laboratory on carriage of 19 virulence genes among *P. multocida* isolates, recovered from small ruminants, revealed some novel information on the frequency of virulence genes like very high prevalence of pfhA gene, 48.9% prevalence of toxA gene with the highest prevalence among serotype A followed by serotype D, and in one isolate each of capsular types B and F (Sarangi et al., submitted for publication). These findings from small ruminant isolates encouraged sampling of more isolates of Indian origin from various hosts to have a clear understanding on the heterogeneity of the bacteria. Therefore, this study was extended to *P. multocida* isolates recovered from multiple host species for 8 important virulence associated genes, encoding proteins involved in bacterial survival and pathogenesis. It included genes encoding transferrin binding protein (TbpA) and haemoglobin binding protein (HgbA, HgbB) associated with iron acquisition, filamentous haemagglutinin (PfhA), subunit of type IV fimbriae (PtfA), sialidases (NanB, NanH) involved in initial colonization and adhesion, and dermonecrotxin (ToxA).

### 2. Materials and Methods

#### 2.1. Bacterial Strains. In the present study, 108 *P. multocida* isolates recovered from large ruminants (buffalo, *n* = 23, cattle, *n* = 18), avians (chicken, *n* = 18, duck, *n* = 8, and emu, *n* = 4), swine (*n* = 34), and rabbit (*n* = 3), maintained at Division of Bacteriology & Mycology, Indian Veterinary Research Institute, Izatnagar, were used. Selection of isolates were carried out on the basis of host origin, year and place of isolation in order to incorporate isolates from all over India. The details of the isolates (isolate number, host origin, capsular type, year and place of isolation, and disease symptom (if available)) are given in Table 1.

#### 2.2. Confirmation of *P. multocida* Isolates. The isolates were revived in brain heart infusion broth by 18–24 h incubation at 37°C and plated subsequently onto blood agar to study cultural characteristics. The cultures were then tested for purity by biochemical tests as per standard techniques [20]. The genomic DNA of the isolates was extracted by CTAB method [21], and the isolates were reconfirmed as *P. multocida* by PM-PCR followed by determination of capsular type by multiplex PCR [22, 23].

#### 2.3. Detection of Virulence Associated Genes by PCR. The isolates were then subjected to screening of 8 virulence genes encoding iron binding proteins (TbpA, HgbA, HgbB), colonization and adhesion related protein (PfhA, PtfA), sialidases (NanB, NanH), and dermonecrotxin (ToxA) by individual PCR reactions, utilizing oligonucleotide primers described previously. The details of the virulence genes, sequences of the oligonucleotide primers, and citations used are listed in Table 2.

#### 2.4. Statistical Analysis. Statistical analysis of the data generated from the study was performed with SPSS 16.0 (SPSS Inc., Chicago). *P* values of <0.05 were considered as statistically significant.

### 3. Results and Discussion

*P. multocida* is an economically important veterinary pathogen, causing wide range of diseases in livestock and poultry. The bacteria have been classified into five capsular types (A, B, D, E, and F) based on capsular typing, with each capsule type being predominantly associated with a particular disease in a host species. But isolation of other capsular types from such hosts by cross species infection is not uncommon [2, 7]. Ability of the bacteria to infect and survive in several hosts as commensal exposes it to various selection pressures, resulting in emergence of divergent strains in field scenario. Molecular epidemiological study by employing REP-PCR, ERIC-PCR, MLST analysis, and so forth has confirmed the diversity of *P. multocida* circulating in India and also the possibility of transboundary spread of strains across evolutionary time [24, 25]. Therefore, a detailed study on the presence of virulence associated genes recovered from different host species in Indian subcontinent will be helpful to understand the disease process and to develop disease control measures in future.

In this study, *P. multocida* isolates recovered from various host species were screened for presence of 8 important virulence associated genes (tbpA, hgbA, hgbB, pfhA, ptfA, nanB, nanH, and toxA) involved in bacterial pathogenesis. The results confirmed that, with the exception of toxA gene, all other virulence associated genes are regularly distributed among the isolates of different host origin. The result of individual PCR reaction for each isolate is presented in Table 1. Among the genes encoding iron binding proteins, *tbpA* gene was present in 82.4% of isolates which range from 69.6% in buffalo to 100% in cattle, emu, and rabbits (Table 3). Similarly, *hgbB* gene was found to be regularly distributed among all isolates affecting different hosts with the lowest prevalence among swine isolates (73.5%). Gene *hgbB* has the lowest prevalence among the three iron binding proteins screened in this study and was found in 72.2% of the isolates. The percentage prevalence of this gene was found to be more among avian isolates (90%) in comparison to large ruminants (65.9%) and swine (67.6%) isolates. Of the two sialidases present in *P. multocida* isolates, the percentage prevalence of *nanB* gene was found to be more than *nanH* gene. Overall very high prevalence of *pfhA* gene (93.5%) was observed in this study with 100% prevalence among avian isolates. Dermonecrotxin gene (toxA) was found only in 17.6% of strains with majority of the isolates belonging to porcine origin. One buffalo and 3 duck isolates were also found to carry toxA gene (Table 3).

Iron acquisition and uptake are essential for bacterial survival, and many bacteria have developed different iron sequestering system for uptake of iron. The expression of iron acquisition proteins increases under iron limiting condition, as well as in vivo condition (reviewed in [5]). *P. multocida* utilizes various receptors for adapting to variations in supply of different haem iron sources [5]. Among these, TbpA protein is necessary for extraction of iron from transferrin and has been reported to be an important virulence factor and epidemiological marker in cattle [12, 19, 26]. Previous studies reported that *tbpA* gene is either absent or rarely
| Sample id | Species | Serotype | Place     | Year | Disease/symptom | tbpA | hgbA | hgbB | pfiA | pfa | toxA | nanB | nanH |
|-----------|---------|----------|-----------|------|-----------------|------|------|------|------|-----|------|------|------|
| 10        | Cattle  | F        | Pune      | 1992 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 11        | Cattle  | F        | Pune      | 1992 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 51        | Pig     | A        | UP        | 1995 | N.A.            | P    | P    | P    | P    | P   | P    | A    | A    |
| 53        | Buffalo | B        | Pune      | 1996 | N.A.            | A    | P    | P    | P    | P   | A    | P    | P    |
| 98        | Duck    | A        | Tripura   | 2001 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 117       | Cattle  | B        | Bhubaneswar | 2001 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 118       | Cattle  | B        | Bhubaneswar | 2001 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 120       | Cattle  | B        | Bhubaneswar | 2001 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 128       | Cattle  | B        | Bangalore | 2001 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 132       | Buffalo | A        | Palampur  | 2001 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 133       | Buffalo | A        | Palampur  | 2001 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 134       | Buffalo | A        | Palampur  | 2001 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 141       | Chicken | A        | Chennai   | 2001 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 202       | Chicken | A        | Chennai   | 2002 | N.A.            | A    | P    | P    | P    | P   | A    | P    | P    |
| 206       | Chicken | B        | Chennai   | 2002 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 222       | Buffalo | A        | Mathura   | 2002 | N.A.            | P    | P    | A    | P    | P   | A    | P    | P    |
| 258       | Chicken | A        | Nasik     | 2002 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 330       | Chicken | B        | Anand     | 2002 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 288       | Buffalo | B        | Bhubaneswar | 2003 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 291       | Pig     | B        | Guwahati  | 2003 | N.A.            | P    | P    | A    | P    | P   | A    | P    | P    |
| 292       | Pig     | B        | Guwahati  | 2003 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 366       | Cattle  | B        | Palampur  | 2004 | N.A.            | P    | P    | A    | P    | P   | A    | P    | P    |
| 390       | Buffalo | B        | Palampur  | 2005 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 400       | Buffalo | B        | Ludhiana  | 2005 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 407       | Chicken | B        | Ludhiana  | 2005 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 409       | Buffalo | B        | Jammu     | 2005 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 410       | Buffalo | B        | Jammu     | 2005 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 425       | Duck    | B        | Chennai   | 2005 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 448       | Rabbit  | B        | Palampur  | 2006 | N.A.            | P    | P    | A    | P    | P   | A    | P    | P    |
| 456       | Chicken | A        | Chennai   | 2006 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 460       | Chicken | A        | Chennai   | 2006 | N.A.            | A    | A    | A    | P    | P   | A    | P    | P    |
| 464       | Chicken | A        | Chennai   | 2006 | N.A.            | A    | P    | P    | P    | P   | A    | P    | P    |
| 569       | Chicken | A        | Chennai   | 2007 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 537       | Pig     | A        | Guwahati  | 2007 | N.A.            | A    | A    | A    | P    | P   | A    | P    | P    |
| 540       | Pig     | A        | Guwahati  | 2007 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 543       | Pig     | D        | Guwahati  | 2007 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 550       | Duck    | A        | Guwahati  | 2007 | N.A.            | P    | A    | P    | P    | P   | A    | P    | P    |
| 555       | Buffalo | B        | Palampur  | 2007 | N.A.            | P    | P    | A    | P    | P   | A    | P    | P    |
| 559       | Rabbit  | B        | Palampur  | 2007 | N.A.            | P    | P    | A    | P    | P   | A    | P    | P    |
| 563       | Cattle  | B        | Anand     | 2007 | N.A.            | P    | P    | A    | P    | P   | A    | P    | P    |
| 583       | Pig     | A        | Guwahati  | 2008 | N.A.            | P    | P    | A    | P    | P   | A    | P    | P    |
| 587       | Pig     | A        | Guwahati  | 2008 | N.A.            | P    | P    | A    | P    | P   | A    | P    | P    |
| 602       | Buffalo | B        | Palampur  | 2008 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 608       | Rabbit  | A        | Palampur  | 2008 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| 610       | Buffalo | B        | Ludhiana  | 2008 | N.A.            | A    | A    | A    | P    | P   | A    | P    | P    |
| 618       | Chicken | B        | Palampur  | 2008 | N.A.            | P    | P    | P    | P    | P   | A    | P    | P    |
| Sample id | Species | Serotype | Place         | Year | Disease/symptom                        | tbpA | hgbA | hgbB | pfhA | ptfA | toxA | nanB | nanH |
|-----------|---------|----------|---------------|------|---------------------------------------|------|------|------|------|------|------|------|------|
| 632       | Buffalo | B        | Anand         | 2008 | N.A.                                  | A    | A    | A    | P    | P    | A    | P    | P    |
| 633       | Chicken | B        | Bangalore     | 2008 | N.A.                                  | P    | A    | P    | P    | A    | P    | P    | P    |
| 655       | Buffalo | D        | Guwahati      | 2008 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 701       | Pig      | A        | Guwahati      | 2009 | N.A.                                  | P    | P    | A    | P    | P    | P    | P    | P    |
| 702       | Pig      | A        | Guwahati      | 2009 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 703       | Pig      | D        | Guwahati      | 2009 | N.A.                                  | P    | P    | P    | P    | P    | P    | A    | P    |
| 704       | Cattle   | B        | Guwahati      | 2009 | N.A.                                  | P    | P    | A    | A    | P    | A    | P    | P    |
| 720       | Pig      | B        | UP            | 2009 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 721       | Pig      | B        | UP            | 2009 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 722       | Pig      | B        | UP            | 2009 | N.A.                                  | P    | P    | A    | P    | P    | P    | P    | P    |
| 725       | Buffalo | A        | Ludhiana      | 2009 | N.A.                                  | P    | P    | P    | P    | P    | A    | A    | P    |
| 733       | Pig      | D        | Guwahati      | 2009 | N.A.                                  | P    | A    | P    | P    | P    | A    | P    | P    |
| 736       | Pig      | D        | Guwahati      | 2009 | N.A.                                  | P    | P    | P    | A    | A    | P    | A    | P    |
| 737       | Pig      | D        | Guwahati      | 2009 | N.A.                                  | P    | P    | A    | P    | A    | A    | P    | A    |
| 749       | Cattle   | A        | Palampur      | 2009 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 746       | Cattle   | A        | Palampur      | 2009 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 747       | Cattle   | A        | Palampur      | 2009 | N.A.                                  | P    | P    | P    | A    | A    | P    | A    | P    |
| 754       | Cattle   | A        | Palampur      | 2009 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 782       | Chicken | A        | Anand         | 2009 | N.A.                                  | A    | P    | A    | P    | P    | A    | P    | P    |
| 794       | Chicken | A        | Thrissur      | 2009 | Necrotic foci in liver and haemorrhage in heart | P    | P    | P    | P    | A    | P    | A    | P    |
| 652       | Buffalo | B        | Guwahati      | 2010 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 653       | Buffalo | B        | Guwahati      | 2010 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 784       | Chicken | A        | Anand         | 2010 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 803       | Chicken | A        | Chennai       | 2010 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 804       | Chicken | A        | Anand         | 2010 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 811       | Cattle   | A        | Palampur      | 2010 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 852       | Pig      | A        | Guwahati      | 2011 | Diseased                              | P    | P    | P    | P    | P    | P    | P    | P    |
| 860       | Pig      | B        | Guwahati      | 2011 | Diseased                              | P    | P    | P    | P    | A    | P    | P    | P    |
| 876       | Pig      | A        | Thrissur      | 2011 | Fever                                 | A    | A    | A    | P    | P    | A    | A    | P    |
| 877       | Pig      | A        | Thrissur      | 2011 | Fever                                 | A    | A    | P    | P    | A    | P    | P    | P    |
| 879       | Pig      | A        | Thrissur      | 2011 | Fever                                 | A    | P    | A    | P    | A    | A    | P    | P    |
| 890       | Emu      | A        | Chennai       | 2011 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| 2751      | Cattle   | B        | Palampur      | 2011 | Nasal discharge                       | P    | P    | A    | P    | A    | P    | P    | P    |
| 2766      | Cattle   | B        | Palampur      | 2011 | Nasal discharge                       | P    | P    | P    | P    | A    | P    | P    | P    |
| 3324      | Cattle   | B        | Palampur      | 2011 | Nasal discharge                       | P    | P    | A    | P    | A    | P    | P    | P    |
| 4312      | Cattle   | B        | Palampur      | 2011 | Nasal discharge                       | P    | P    | A    | P    | A    | P    | P    | P    |
| BP23      | Pig      | B        | Guwahati      | 2011 | N.A.                                  | P    | A    | A    | P    | A    | P    | P    | P    |
| BP28      | Pig      | A        | Guwahati      | 2011 | N.A.                                  | P    | A    | P    | P    | P    | P    | P    | P    |
| BP37      | Pig      | A        | Guwahati      | 2011 | N.A.                                  | P    | P    | A    | P    | P    | P    | P    | P    |
| EMU2      | Emu      | A        | Chennai       | 2011 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| JP18      | Pig      | A        | Guwahati      | 2011 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| NP23      | Pig      | B        | Guwahati      | 2011 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| NP37      | Pig      | B        | Guwahati      | 2011 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| PP1A      | Pig      | A        | Thrissur      | 2011 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
| PP2A      | Pig      | A        | Thrissur      | 2011 | N.A.                                  | P    | P    | P    | P    | P    | P    | P    | P    |
| PP4A      | Pig      | A        | Thrissur      | 2011 | N.A.                                  | P    | P    | P    | P    | A    | P    | P    | P    |
Table 1: Continued.

| Sample id | Species | Serotype | Place | Year | Disease/symptom | tbpA | hgbA | hgbB | pfhA | ptfA | toxA | nanB | nanH |
|-----------|---------|----------|-------|------|-----------------|------|------|------|------|------|------|------|------|
| 914       | Duck    | A        | Thrissur | 2012 | N.A.            | P    | P    | P    | P    | P    | A    | P    | P    |
| 920       | Emu     | A        | Anand   | 2012 | N.A.            | P    | P    | P    | P    | P    | A    | P    | P    |
| 922       | Emu     | A        | Anand   | 2012 | N.A.            | P    | P    | P    | P    | P    | A    | P    | P    |
| DP53      | Duck    | A        | Thrissur | 2013 | N.A.            | P    | P    | P    | P    | P    | A    | P    | A    |
| DP54      | Duck    | A        | Thrissur | 2013 | N.A.            | P    | P    | P    | P    | P    | A    | P    | P    |
| DP55      | Duck    | A        | Thrissur | 2013 | N.A.            | A    | P    | P    | P    | A    | P    | P    | P    |
| DP56      | Duck    | A        | Thrissur | 2013 | N.A.            | P    | P    | P    | P    | A    | P    | P    | P    |
| P14       | Pig     | D        | Guwahati | 2013 | N.A.            | A    | A    | A    | A    | P    | P    | A    | A    |
| P15       | Pig     | A        | Guwahati | 2013 | N.A.            | A    | A    | A    | A    | P    | A    | P    | A    |
| P16       | Pig     | D        | Guwahati | 2013 | N.A.            | A    | A    | A    | A    | P    | A    | P    | A    |
| PAB 78    | Buffalo | B        | Anand   | 2013 | N.A.            | P    | P    | A    | P    | P    | A    | P    | P    |
| PAB 80    | Buffalo | B        | Anand   | 2013 | N.A.            | P    | P    | A    | P    | A    | P    | P    | P    |
| PAB 86    | Buffalo | B        | Anand   | 2013 | N.A.            | A    | A    | A    | A    | P    | A    | P    | P    |
| PAP 88    | Chicken | A        | Anand   | 2013 | N.A.            | P    | P    | P    | P    | A    | P    | P    | P    |
| LDHB 106  | Buffalo | B        | Ludhiana | 2014 | N.A.            | A    | P    | A    | P    | P    | A    | P    | P    |
| MSRB 108  | Buffalo | B        | Ludhiana | 2014 | N.A.            | A    | P    | P    | P    | A    | P    | P    | P    |

(N.A. = not available; A = absence of virulence gene; P = presence of virulence gene as detected in PCR reaction).

Table 2: Details of primers and citations used for the detection of capsular type and virulence associated genes in strains of *Pasteurella multocida*.

| Gene          | Primer                  | Primer sequence (5’→3’) | Reference |
|---------------|-------------------------|-------------------------|-----------|
| PM-PCR and Capsular serotypes |                         |                         |           |
| KMT1          | PMPCR-F                 | ATCCGCTATTTACCCCGTGG    | [22]      |
|               | PMPCR-R                 | GCCTGAACAGAACCCTGAC     |           |
| hyaD-hyaC     | capA F                  | GATGCCAAAATCGCAATGAG    | [23]      |
|               | capA R                  | TGTTGCCATTGTGTCAATG     |           |
| bcbD          | capB F                  | CATTTATCCAAGCTCCACC     | [23]      |
|               | capB R                  | GCACCGAGATTTCAATTCC     |           |
| dcbF          | capD F                  | TTACAAAAAGAAGACATGAGAGC| [23]      |
|               | capD R                  | CATCTACCACCTCAACATCATC |           |
| ecbF          | capE F                  | TCAGAGAAAAATTTGACTC     | [23]      |
|               | capE R                  | GCTAGCTGTCGTTTTGTC      |           |
| fcbD          | capF F                  | AATCGGAGAACCCGAGAATCCG | [23]      |
|               | capF R                  | TCCGCCCCGTTAAPACTCTG    |           |
| Iron acquisition genes |                         |                         |           |
| tbpA          | tbpA F                  | GGACAGTGCATATAACTGTGG   | [32]      |
|               | tbpA R                  | GGACAGTGCATATAACTGTGG   |           |
| hgbA          | hgbA F                  | CATATCAGATCATTTTGGAAGA  | In-house  |
|               | hgbA R                  | GAATGTCGAGATCTGATATTCTG | [33]      |
| hgbB          | HgbB F                  | ACCGCATGTTGATATGATG     | [12]      |
|               | HgbB R                  | CATTGACTGTCGTTTACATG    |           |
| Adhesins      | ptfA F                  | AAGTGCATGAAAACGAGACATGT | In-house  |
|               | ptfA R                  | GAGACGCTTATACCCGACG     |           |
| pflA          | pflA F                  | TAAGCCCTATCGGTCCAGT     | [12]      |
|               | pflA R                  | GTAATAACAGGATCCGATAT    |           |
| Sialidases    | NanB F                  | GTCCTATAAAGTGACGCCG     | [12]      |
|               | NanB R                  | AAGACGAAAGAGAGCCTGTCC   |           |
|               | nanH F                  | CACTGCTTATAGCCCGATTCT   | [12]      |
|               | nanH R                  | AGCAGCTGTTACCAGAACC     |           |
| Dermonecrotoxin | ToxA F                  | TCTTAGATGAGCGACAAGG     | [34]      |
|               | ToxA R                  | GAATGCCACCTCTCTATAG     |           |
Table 3: Prevalence of virulence associated genes among *Pasteurella multocida* isolates recovered from various host species of India.

| Host origin/capsular type | No. of strains | *tbpA* (%) | *hgbA* (%) | *hgbB* (%) | *pfhA* (%) | *ptfA* (%) | *nanB* (%) | *nanH* (%) | *toxA* (%) |
|--------------------------|----------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Buffalo                  | 23             | 69.6       | 82.6       | 65.2       | 95.7       | 95.7       | 100        | 78.3       | 4.3        |
| Cap type A               | 5              | 80.0       | 80.0       | 80.0       | 100        | 100        | 100        | 60.0       | 20.0       |
| Cap type B               | 17             | 64.7       | 82.3       | 58.8       | 94.1       | 94.1       | 100        | 82.3       | 0.0        |
| Cap type D               | 1              | 100        | 100        | 100        | 100        | 100        | 100        | 100        | 0.0        |
| Cap type F               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Cattle                   | 18             | 100        | 94.4       | 66.7       | 94.4       | 94.4       | 100        | 100        | 0.0        |
| Cap type A               | 5              | 100        | 100        | 100        | 100        | 100        | 100        | 100        | 0.0        |
| Cap type B               | 11             | 100        | 90.9       | 45.4       | 90.9       | 100        | 100        | 100        | 0.0        |
| Cap type D               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Cap type F               | 2              | 100        | 100        | 100        | 100        | 100        | 100        | 100        | 0.0        |
| Large ruminants (cattle + buffalo) | 41             | 82.9       | 87.8       | 65.9       | 95.1       | 95.1       | 100        | 87.8       | 2.4        |
| Cap type A               | 10             | 90.0       | 90.0       | 90.0       | 100        | 90.0       | 100        | 80.0       | 10.0       |
| Cap type B               | 28             | 78.5       | 85.7       | 53.5       | 92.8       | 96.4       | 100        | 89.2       | 0.0        |
| Cap type D               | 1              | 100        | 100        | 100        | 100        | 100        | 100        | 100        | 0.0        |
| Cap type F               | 2              | 100        | 100        | 100        | 100        | 100        | 100        | 100        | 0.0        |
| Chicken                  | 18             | 77.8       | 83.3       | 83.3       | 100        | 100        | 100        | 100        | 61.1       | 0.0        |
| Cap type A               | 13             | 69.2       | 84.6       | 76.9       | 100        | 100        | 100        | 100        | 53.8       | 0.0        |
| Cap type B               | 5              | 100        | 80.0       | 100        | 100        | 100        | 100        | 100        | 0.0        |
| Cap type D               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Cap type F               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Duck                     | 8              | 87.5       | 87.8       | 100        | 100        | 100        | 100        | 100        | 50.0       | 37.5       |
| Cap type A               | 7              | 85.7       | 85.7       | 100        | 100        | 100        | 100        | 100        | 42.8       | 28.5       |
| Cap type B               | 1              | 100        | 100        | 100        | 100        | 100        | 100        | 100        | 42.8       | 28.5       |
| Cap type D               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Cap type F               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Emu                      | 4              | 100        | 100        | 100        | 100        | 100        | 100        | 100        | 0.0        |
| Cap type A               | 4              | 100        | 100        | 100        | 100        | 100        | 100        | 100        | 0.0        |
| Cap type B               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Cap type D               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Cap type F               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Avian (chicken + duck + emu) | 30             | 83.3       | 86.7       | 90.0       | 100        | 100        | 100        | 100        | 63.3       | 10.0       |
| Cap type A               | 24             | 79.1       | 87.5       | 87.5       | 100        | 100        | 100        | 100        | 58.3       | 8.3        |
| Cap type B               | 6              | 100        | 83.3       | 100        | 100        | 100        | 100        | 100        | 83.3       | 16.6       |
| Cap type D               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Cap type F               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Rabbit                   | 3              | 100        | 100        | 33.3       | 100        | 100        | 100        | 100        | 0.0        |
| Cap type A               | 1              | 100        | 100        | 100        | 100        | 100        | 100        | 100        | 0.0        |
| Cap type B               | 2              | 100        | 100        | 0.0        | 100        | 100        | 100        | 100        | 0.0        |
| Cap type D               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Cap type F               | 0              | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       | N.A.       |
| Pig                      | 34             | 79.4       | 73.5       | 67.6       | 85.3       | 100        | 88.2       | 67.6       | 44.1       |
present in poultry, swine, and rabbit isolates (Table 4) [12–16]. In contrast to these findings, we observed a very high occurrence of \( tbpA \) gene among poultry (83.3%), swine (79.4%), and rabbit (100%) isolates (Tables 3 and 4). Among the host species, the prevalence of this gene was found to be lowest (69.6%) in buffalo (Table 3). The difference in prevalence of this gene among isolates of cattle and buffalo origin was found to be statistically significant \((P < 0.05)\), which is quite unexpected. Therefore more number of isolates from both host origins should be carried out before reaching any conclusion. In this study, \( tbpA \) gene was found to be frequently distributed among four capsule types, including CapF (Table 3). This is in contrast to Ewers et al. [12], who observed \( tbpA \) gene in 70% of CapB strains, followed by 37% of CapA, 9.5% of CapD strains and nil in CapF strains. Similarly, Katsuda et al. [18] reported positive association of CapA strain with \( tbpA \) gene. 

*P. multocida* utilizes two proteins (HgbA and HgbB) for acquiring iron directly from haem component. Morton et al. [27] reported that the presence of both proteins might provide increased uptake of iron and protection against negative effects of mutation in one of the encoding genes. Between these two proteins, \( hgbA \) gene was found to be regularly distributed (>95% prevalence) among isolates [12–15, 17, 18]. In the present study, 73.5% of porcine isolates were found to carry this gene, which is lower in comparison to previous findings, that is, nearly 100% prevalence (Table 4) [12–15]. The frequency of \( hgbB \) gene varies among strains of different host origin and also with disease status of the animal [12, 15, 16, 18, 19]. In this study, 72.2% of the isolates were found to carry this gene with highest frequency observed among avian strains (90%), which is in agreement with previous reports (Table 4) [12, 17].

Among the genes encoding proteins involved in bacterial colonization and adhesion, \( ptfA \) gene has the highest (98.1%) prevalence (Table 3). This gene encodes type 4 fimbria subunit and has been associated with bovine diseases [19]. Worldwide, this gene is regularly distributed with more than 85% prevalence among *P. multocida* isolates, irrespective of host origin and capsule type (Table 4).

\( pfhA \) 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 [12, 13, 18, 19, 28]. Almost all previous studies reported low prevalence of this gene with varying frequencies in between 46–52%, 45–60%, and 15–40.5% among isolates of cattle, poultry, and pig origin, respectively (Table 4) [12–15]. But interestingly, very high prevalence, 85.3% (pig) to 100% (avian), of this gene was observed among Indian isolates (Table 3). 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 Indian strains/clones.

Sialidases play an important role in colonization to epithelial surface. They enhance bacterial virulence by unmasking key host receptor and by reducing the effectiveness of mucin [5, 29]. Of the two sialidases (NanB and NanH) present in *P. multocida* isolates, the *nanB* gene was found in almost all isolates, whereas the prevalence of *nanH* varied according to host origin and geographical location. In this study, the frequency of *nanH* gene among porcine isolates was found to be low (63.3%), which is in contrast to the report of Furian et al. [17] (Table 4). Similarly, the carriage of *nanH* gene among isolates of pig origin from India was also found to be lower (67.6%) in comparison to isolates from other parts of the globe, which reported higher (>97%) frequency (Table 4) [12–15].

Dermonecrotoxin (sometimes called *P. multocida* toxin) is encoded by \( toxA \) gene. This gene was initially detected in serotype D isolates and was found to be associated with atrophic rhinitis in pigs. Later on, it was detected in strains of serotype A from pigs and other hosts [30]. In this study, \( toxA \) gene was detected in 44.1% of pig isolates. Further, one buffalo and three duck isolates were also found positive for \( toxA \) gene (Table 3). Two serotype B isolates were found to carry this gene which is in agreement with our previous findings (Sarangi et al., submitted for publication). A lysogenic bacteriophage infection of *P. multocida* resulting in horizontal gene transfer could be the reason [31].

The association of virulence associated genes with particular capsular type and host origin was assessed by the Chi-square and Fisher’s exact test. Out of the 8 virulence associated genes studied \( toxA, pfhA, nanB, \) and \( nanH \) were found to be associated (positive or negative) with capsular type. \( pfhA, nanB, \) and \( nanH \) genes were found to be regularly distributed among all serotypes with the exception of serotype D. Negative association of \( pfhA \) gene with CapD strains has been reported previously [12, 14, 18]. Dermonecrotoxin encoded by \( toxA \) gene was found to be positively associated with capD and CapA. Ewers et al. [12] observed clear association of \( toxA \) gene with CapD strains which was later supported by similar reports from other workers [13, 14]. Among cattle

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**Table 3: Continued.**

| Host origin/capsular type | No. of strains | \( tbpA \) (%) | \( hgbB \) (%) | \( hgbA \) (%) | \( ptfA \) (%) | \( pfhA \) (%) | \( nanB \) (%) | \( nanH \) (%) | \( toxA \) (%) |
|--------------------------|----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Total (all isolates)     | 108            | 82.4        | 83.3        | 72.2        | 93.5        | 98.1        | 96.3        | 75.0        | 17.6        |
| Cap type A               | 53             | 79.2        | 83.0        | 79.2        | 98.0        | 98.0        | 96.2        | 71.7        | 24.5        |
| Cap type B               | 45             | 86.7        | 86.7        | 60.0        | 95.6        | 97.8        | 100         | 88.9        | 4.4         |
| Cap type D               | 8              | 75.0        | 62.5        | 87.5        | 50.0        | 100         | 100         | 75.0        | 12.5        |
| Cap type F               | 2              | 100         | 100         | 100         | 100         | 100         | 100         | 100         | 0.0         |
| Gene/host | Cattle (reference) | Buffalo (reference) | Poultry (reference) | Pig (reference) | Rabbit (reference) |
|-----------|-------------------|---------------------|---------------------|----------------|-------------------|
| tbpA      | India (this study) | 18                  | 23                  | 30             | 34                |
|           | Germany [12]      | 104                 | 07                  | 20             | 52                |
|           | Japan [18]        | 378                 | 97.2                | 20             | 83.3              |
|           | India [19]        | 23                  | 100                 | 20             | 79.4              |
|           | Country (reference) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) |
|           |                   |                     |                     |                |                   |                     |                     |                |                   |
| hgbA      | India (this study) | 18                  | 94.4                | 23             | 86.7              |
|           | Germany [12]      | 104                 | 95.2                | 07             | 90.0              |
|           | Japan [18]        | 378                 | 95.5                | 07             | 85.0              |
|           | India [19]        | 23                  | 100                 | 25             | 100               |
|           | Country (reference) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) |
|           |                   |                     |                     |                |                   |                     |                     |                |                   |
| hgbB      | India (this study) | 18                  | 66.7                | 23             | 95.5              |
|           | Germany [12]      | 104                 | 57.7                | 07             | 85.0              |
|           | Japan [18]        | 378                 | 61.4                | 07             | 90.0              |
|           | India [19]        | 23                  | 86.9                | 25             | 100               |
|           | Country (reference) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) |
|           |                   |                     |                     |                |                   |                     |                     |                |                   |
| ptfA      | India (this study) | 18                  | 94.4                | 23             | 95.7              |
|           | Germany [12]      | 104                 | 99.0                | 07             | 90.0              |
|           | Japan [18]        | 378                 | 94.7                | 07             | 85.0              |
|           | India [19]        | 23                  | 86.9                | 25             | 100               |
|           | Country (reference) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) |
|           |                   |                     |                     |                |                   |                     |                     |                |                   |
| pfhA      | India (this study) | 18                  | 94.4                | 23             | 95.7              |
|           | Germany [12]      | 104                 | 46.2                | 07             | 45.0              |
|           | Japan [18]        | 378                 | 52.4                | 07             | 60.0              |
|           | India [19]        | 23                  | 100                 | 25             | 100               |
|           | Country (reference) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) |
|           |                   |                     |                     |                |                   |                     |                     |                |                   |
| nanB      | India (this study) | 18                  | 100                 | 23             | 100               |
|           | Germany [12]      | 104                 | 100                 | 07             | 100               |
|           | Japan [18]        | 378                 | 100                 | 07             | 100               |
|           | India [19]        | 23                  | 0.0                 | 25             | 100               |
|           | Country (reference) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) |
|           |                   |                     |                     |                |                   |                     |                     |                |                   |
| nanH      | India (this study) | 18                  | 100                 | 23             | 78.3              |
|           | Germany [12]      | 104                 | 88.5                | 07             | 65.0              |
|           | Japan [18]        | 378                 | 88.4                | 07             | 96.0              |
|           | India [19]        | 23                  | 100                 | 25             | 100               |
|           | Country (reference) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) |
|           |                   |                     |                     |                |                   |                     |                     |                |                   |
| toxA      | India (this study) | 18                  | 0.0                 | 23             | 4.3               |
|           | Germany [12]      | 104                 | 5.8                 | 07             | 5.0               |
|           | India [19]        | 23                  | 0.0                 | 25             | 0.0               |
|           | Country (reference) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) | No. of strains tested | Prevalence (%) |
|           |                   |                     |                     |                |                   |                     |                     |                |                   |
isolates, a significant difference ($P = 0.021$) was observed in the distribution of $hgbB$ gene among serotypes (Table 3). Similarly, for pig isolates the frequency of $pfhA$ and $nanH$ gene among serotypes was found to be statistically significant (Table 3). However, as the number of strains tested under each serotype was less, more number of samples should be tested before reaching any definite conclusion. In order to ascertain any trend in the distribution of virulence genes over time period, the strains used in the study were divided into two groups, contemporary (2009–2014) and archived (1992–2008), and statistical analysis was carried out. But no statistically significant difference was observed between the two groups with respect to virulence gene distribution (Table 1).

The prevalence of virulence associated genes was found to vary among $P. multocida$ isolates recovered from various host species. Significant association between $toxA$ and $nanH$ genes with host origin was also observed. Dermonecrototoxin gene was found to be positively associated with porcine isolates, whereas $nanH$ gene was found to be positively associated with large ruminant isolates, more specifically with cattle isolates, which agrees well with the findings of Ewers et al. [12].

The combination of genes among $P. multocida$ isolates was assessed by the Chi-square and Fisher’s exact test. Significant association was observed between $tbpA$-$hgbA$, $tbpA$-$hgbB$, $tbpA$-$pfhA$, $tbpA$-$nanB$, $hgbA$-$hgbB$, $hgbA$-$pfhA$, $hgbA$-$nanB$, $hgbA$-$nanH$, $pfhA$-$nanB$, and $pfhA$-$nanH$. Similar association among iron acquisition genes, as well as between various virulence associated genes, has been reported previously by Ewers et al. [12].

To sum up, the present study revealed some unique epidemiological information on the prevalence of virulence associated genes among Indian strains in comparison to its equivalents in other parts of the globe. The result shows that with the exception of $toxA$ gene the virulence associated genes are regularly distributed among $P. multocida$ isolates. The occurrence of $pfhA$, $hgbA$, and $nanH$ genes among swine isolates of Indian origin was found to be less in comparison to other countries. Gene encoding dermonecrototoxin was observed in 17.6% of the total isolates studied. This gene is present mostly among swine isolates, with few occurrences in buffalo and duck isolates. Interestingly, very high prevalence of $tbpA$ gene was observed among poultry, swine, and rabbit isolates. Likewise, very high prevalence of $pfhA$ gene was observed among Indian isolates, irrespective of host species origin. As proper history of majority of the isolates with respect to its disease status was not available, it was not possible to perform association study between virulence gene and disease status of the animal, which could have enhanced the significance of this study. Therefore, more number of isolates with proper history on disease status of the host should be carried out in future, which will be helpful to make a more definite conclusion, to provide insight into mechanism of pathogenesis, association of genes with outcome of the disease, and in future vaccine strategies.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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**References**

[1] M. Harper, J. D. Boyce, and B. Adler, “$P. multocida$ pathogenesis: 125 years after pasteur,” *FEBS Microbiology Letters*, vol. 265, no. 1, pp. 1–10, 2006.

[2] F. Dziva, A. P. Muhairwa, M. Bisgaard, and H. Christensen, “Diagnostic and typing options for investigating diseases associated with $P. multocida$,” *Veterinary Microbiology*, vol. 128, no. 1–2, pp. 1–22, 2008.

[3] S. B. Shivachandra, K. N. Viswas, and A. A. Kumar, “A review of hemorrhagic septicemia in cattle and buffalo,” *Animal Health Research Reviews*, vol. 12, no. 1, pp. 67–82, 2011.

[4] R. Verma and T. N. Jaiswal, “Haemorrhagic septicaemia vaccines,” *Vaccine*, vol. 16, no. 11-12, pp. 1184–1192, 1998.

[5] T. Hatfuludi, K. Al-Hasani, J. D. Boyce, and B. Adler, “Outer membrane proteins of $P. multocida$,” *Veterinary Microbiology*, vol. 144, no. 1–2, pp. 1–17, 2010.

[6] J. Lin, S. Huang, and Q. Zhang, “Outer membrane proteins: key players for bacterial adaptation in host niches,” *Microbes and Infection*, vol. 4, no. 3, pp. 325–331, 2002.

[7] R. L. Davies, R. MacCorquodale, and S. Reilly, “Characterisation of bovine strains of $P. multocida$ and comparison with isolates of avian, ovine and porcine origin,” *Veterinary Microbiology*, vol. 99, no. 2, pp. 145–158, 2004.

[8] A. W. Confer, “Immunogens of $P. multocida$,” *Veterinary Microbiology*, vol. 37, no. 3–4, pp. 353–368, 1993.

[9] A. W. Confer, S. H. Nutt, S. M. Dabo, R. J. Panciera, and G. L. Murphy, “Antibody responses of cattle to outer membrane proteins of $P. multocida$ A3,” *The American Journal of Veterinary Research*, vol. 57, no. 10, pp. 1453–1457, 1996.

[10] S. M. Dabo, A. W. Confer, and G. L. Murphy, “Outer membrane proteins of bovine $P. multocida$ serogroup A isolates,” *Veterinary Microbiology*, vol. 54, no. 2, pp. 167–183, 1997.

[11] V. Blume, I. Luque, A. I. Vela et al., “Genetic and virulence-phenotype characterization of serotypes 2 and 9 of $S. suis$ swine isolates,” *International Microbiology*, vol. 12, no. 3, pp. 161–166, 2009.

[12] C. Ewers, A. Lübbe-Becker, A. Bethe, S. Kiebling, M. Filter, and L. H. Wieler, “Virulence genotype of $P. multocida$ strains isolated from different hosts with various disease status,” *Veterinary Microbiology*, vol. 114, no. 3–4, pp. 304–317, 2006.
[13] A. Bethe, L. H. Wieler, H.-J. Selbitz, and C. Ewers, "Genetic diversity of porcine Pasteurella multocida strains from the respiratory tract of healthy and diseased swine," *Veterinary Microbiology*, vol. 139, no. 1-2, pp. 97–105, 2009.

[14] X. Tang, Z. Zhao, J. Hu et al., "Isolation, antimicrobial resistance, and virulence genes of Pasteurella multocida strains from swine in China," *Journal of Clinical Microbiology*, vol. 47, no. 4, pp. 951–958, 2009.

[15] N. García, J. F. Fernández-Garayzábal, J. Goyache, L. Domínguez, and A. I. Vela, "Associations between biovar and virulence factor genes in Pasteurella multocida isolates from pigs in Spain," *Veterinary Record*, vol. 169, no. 14, p. 362, 2011.

[16] T. S. P. Ferreira, M. R. Felizardo, D. D. Sena de Gobbi et al., "Virulence genes and antimicrobial resistance profiles of Pasteurella multocida strains isolated from rabbits in Brazil," *The Scientific World Journal*, vol. 2012, Article ID 685028, 6 pages, 2012.

[17] T. Q. Furian, K. A. Borges, S. L. S. Rocha et al., "Detection of virulence-associated genes of Pasteurella multocida isolated from cases of fowl cholera by multiplex-PCR," *Pesquisa Veterinária Brasileira*, vol. 33, no. 2, pp. 177–182, 2013.

[18] K. Katsuda, K. Hoshinoo, Y. Ueno, M. Kohmoto, and O. Mikami, "Virulence genes and antimicrobial susceptibility in Pasteurella multocida isolates from calves," *Veterinary Microbiology*, vol. 167, no. 3–4, pp. 737–741, 2013.

[19] S. Verma, M. Sharma, S. Katoch et al., "Profiling of virulence associated genes of Pasteurella multocida isolated from cattle," *Veterinary Research Communications*, vol. 37, no. 1, pp. 83–89, 2013.

[20] S. T. Cowan and K. J. Steel, *Manual for the Identification of Medical Bacteria*, Cambridge University Press, Cambridge, UK, 1970.

[21] K. Wilson, "Preparation of genomic DNA from bacteria," in *Current Protocols in Molecular Biology*, F. M. Ausubel, R. Brent, R. E. Kingston et al., Eds., vol. 1, pp. 2.4.1–2.4.5, Wiley-Interscience, Brooklyn, NY, USA, 1987.

[22] K. M. Townsend, A. J. Frost, C. W. Lee, J. M. Papadimitriou, and H. J. S. Dawkins, "Development of PCR assays for species- and type-specific identification of Pasteurella multocida isolates," *Journal of Clinical Microbiology*, vol. 36, no. 4, pp. 1096–1100, 1998.

[23] K. M. Townsend, J. D. Boyce, J. Y. Chung, A. J. Frost, and B. Adler, "Genetic organization of Pasteurella multocida cap loci and development of a multiplex capsular PCR typing system," *Journal of Clinical Microbiology*, vol. 39, no. 3, pp. 924–929, 2001.

[24] S. B. Shivachandra, A. A. Kumar, R. Gautam et al., "Characterization of avian strains of Pasteurella multocida by restriction endonuclease and amplified fragment length polymorphism," *Research in Veterinary Science*, vol. 81, no. 1, pp. 8–18, 2006.

[25] L. N. Sarangi, P. Thomas, S. K. Gupta, S. Kumar, K. N. Viswas, and V. P. Singh, "Molecular epidemiology of Pasteurella multocida circulating in India by Multilocus sequence typing," *Transboundary and Emerging Diseases*, 2014.

[26] A. J. Cox, M. L. Hunt, J. D. Boyce, and B. Adler, "Functional characterization of HgbB, a new hemoglobin binding protein of Pasteurella multocida," *Microbial Pathogenesis*, vol. 34, no. 6, pp. 287–296, 2003.

[27] D. J. Morton, P. W. Whitby, H. Jin, Z. Ren, and T. L. Stull, "Effect of multiple mutations in the hemoglobin- and hemoglobin-haptoglobin-binding proteins, HgpA, HgpB, and HgpC, of Haemophilus influenzae type b," *Infection and Immunity*, vol. 67, no. 6, pp. 2729–2739, 1999.