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ORIGINAL ARTICLE

Pathology, isolation and characterisation of virulent and diverse *Mannheimia haemolytica* and *Pasteurella multocida* associated with fatal pneumonia in sheep, Rajasthan, India

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Abstract
Pneumonia, a major sheep disease, causes high mortality among affected animals predominantly the lambs. It is caused by a number of microbes including *Mannheimia haemolytica*/*Pasteurella multocida*. It was aimed to explore the pathology in the lungs due to pneumonia, and to isolate and characterise the virulent *M. haemolytica*/*P. multocida* strains. The lung tissues and heart blood specimens were collected by necropsy of sheep ($n = 96$). These samples were used for isolation and characterisation of *M. haemolytica*/*P. multocida*. Moreover, the fresh and square-shaped lung pieces were also taken to perform the histopathological analysis. Grossly, the petechial and ecchymotic haemorrhages, congestion and consolidation in lung lobes were observed at necropsy. Pneumonia was diagnosed as acute fibrinous-bronchopneumonia (36), suppurative chronic bronchopneumonia (10), acute interstitial pneumonia (32) and acute pulmonary congestion (18) based on histological lesions. Bacterial isolation recovered *M. haemolytica* alone or along with *P. multocida* and/or *Bibersteinia trehalosi* identified by phenotypic characters and sequencing of 16S rRNA. Haemolytic colonies were documented to carry the *PHSSA*, *plpE* and *Rpt2* genes. *P. multocida* identification at species level was carried out by *KMT1* gene detection. These isolates were noted to carry the capsule types A (*hyaD-hyaC* gene) and D (*dcbF* gene) and outer membrane protein genes *ompH* and *omp87*. Sequencing revealed nucleotide variability in *Rpt2*, *ompH* and *omp87* genes.We highlighted the occurrence of fatal ovine pneumonic pasteurellosis/mannheimiosis caused and/or complicated by virulent *M. haemolytica*/*P. multocida* strains.

Keywords Bacterial infections · *Mannheimia haemolytica* · *Pasteurella multocida* · Pathology · Pneumonia · Virulence

Introduction

Among ovine diseases, pneumonia is very common in occurrence. Due to high mortality among affected lambs or adult animals, it poses huge economic implications for sheep enterprise (Marru et al. 2013). Onset and progression of pathological alterations in ovine pneumonia could not be tracked by the antemortem screening of the sick animals (Goodwin et al. 2004). Pneumonia lesions like focal to diffused consolidation, different types of haemorrhages, congestion and exudation in lung parenchyma were seen by necropsy. Pathological lesion development was not affected by the age or sex of animals.

Various organisms in different environmental conditions interact to evade the defence system of animal hosts and develop pneumonia (Valadan et al. 2014). *Mannheimia haemolytica* is considered as a predominant cause of ovine pneumonic pasteurellosis. *Pasteurella multocida* exhibits bronchopneumonia among adult animals and the septicaemia in nursing lambs (Watson and Davies 2002). These microbes are commensal to the healthy lungs without causing any pathology. However, their interaction with the body defence mechanism of the host animal is affected by certain factors which induce them to develop overt pneumonia. The infected mothers act as a common source of these microbes for their nursing lambs.

The various virulence elements such as lipopolysaccharide capsule, adhesin, outer membrane proteins (OMPs), and the proteases produced by *M. haemolytica*/*P. multocida*, help them to invade the host respiratory system and establish the

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infection (Mohamed and Abdelsalam 2008; Sarangi et al. 2015). These bacterial isolates could be typed and characterised by their virulence genes (Mohamed and Abdelsalam 2008; Kumar et al. 2015; Sarangi et al. 2015). Contrary to *M. haemolytica*, the reports on *P. multocida* related to ovine pneumonic pasteurellosis are lesser despite its proven linkages with the occurrence of lamb pneumonia (Odugbo et al. 2006). Phenotypic characters of the causative isolates may be useful for their presumptive identification. However, molecular tests are required for their confirmation and epidemiological relationships (Hassan et al. 2016). Therefore, we planned to perform the pathology-, isolation- and virulence-based characterisation of pasteurellae organisms with the occurrence of ovine fatal pneumonia.

Materials and methods

Geography of the area and disease overview

The study area includes six sheep farms at Avikanagar of Tonk district in the state of Rajasthan which comes under the semiarid eastern plain. It is located at 26.28° N 75.38° E with an average elevation of 132 m. The climate of the area is dry. The summer starts from March and extends up to June. The main source of rain is the south-west monsoon which starts from the month of June to the middle of September. The temperature declines after the onset of the monsoon but the weather becomes a little stressful due to the increase in relative humidity. The winters fall between December and February. Due to the semiarid climate of the area, the pasture land is dry and dusty with scanty fodder growth in fields during winters and summers followed by blooming pastures during the monsoon season. The animals graze in the pasture land during the daytime. In the evening, the animals come back to the shed where they are provided with dry and green fodder and the formulated concentrates. Major sheep breeds include Malpura, Avikalin, Patanwadi, Garole and their crosses.

Pneumonia was the common disease condition leading to the high deaths of the sheep at the farms. There was no any correlation between the deaths of the sheep with their age and breeds. Clinically, the sheep exhibited coughing and or/sneezing, nasal discharge or laboured breathing. The sick animals appeared dull, depressed and anorectic with pneumonic grunts and the course of the advanced disease was acute and short, leading to the unanticipated death of lambs (Dixit et al. 2010). However, its course was chronic and suppurative in adult or aged sheep. The survived animals became chronically ill with reduced productivity.

Sampling and sample processing

The individual lung pieces ($n = 96$) were collected in sterile plastic vials by performing the necropsy of fresh dead sheep from December 2014 to March 2016. Simultaneously, the small volume of heart blood from the same sheep was also collected directly in the syringes through heart puncture with the sole aim to isolate the *M. haemolytica/P. multocida*. At the same time, the lung tissue pieces were stored in 10% formal saline for histopathological evaluation. All the samples were shifted immediately to the microbiological laboratory for further investigation. Before bacterial culture, the lung pieces were aseptically processed in a bio-safety cabinet near the flame to make 20% *w*/*v* tissue homogenate in sterile phosphate-buffered saline.

Histopathology

The lung pieces after collection were processed by following the standard histological technique of Luna (1968). The sections with the thickness of about 5 μm were incised and applied for haematoxylin and eosin (H & E) staining for their histopathological analysis.

Bacteria culture

A methodology for *P. multocida* or *M. haemolytica* isolation was optimised in which the lung tissue triturates (20% *w*/*v*) were directly streaked on the blood agar base (HiMedia, Mumbai, India) supplemented with 7% defibrinated sheep blood or on the brain heart infusion (BHI) agar (HiMedia, Mumbai, India) and incubated up to 24–48 h at 37 °C (Odugbo et al. 2006). The individually grown colony was streaked on Mac-Conkey agar/deoxycholate citrate agar. Each colony representing a distinct isolate was examined by Gram's staining for their morphological features. The suspected colonies were further identified by testing them for catalase, indole and oxidase production. The identified colonies or isolates were streaked on the BHI agar for their growth at 37 °C for 48 h to isolate their genomic DNA. Moreover, the individual isolates were maintained in BHI agar slants for further use.

Antibacterial susceptibility test

All the isolates were tested for their susceptibility to amoxicillin (30 μg), amoxicillin-clavulanic acid (20/10 μg), cefotaxime (30 μg), amikacin (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg), enrofloxacin (5 μg), ofloxacin (5 μg), norfloxacin (10 μg), tetracycline (30 μg) and chlortetracycline (30 μg) discs (HiMedia, Mumbai, India) as per CLSI guidelines (CLSI 2008). *Escherichia coli* ATCC 25922 was used as a quality control strain. The interpretation of results was based on the CLSI breakpoints (CLSI 2010).

Isolation of DNA from bacterial colonies

About 10 healthy and growing colonies of each isolate from 48-h-old growth on BHI agar plates were transferred into 1.5-mL Eppendorf tubes. Colonies were washed twice in nuclease-free water by centrifugation at 10,000*g* for 3 min. The genomic DNA from the bacteria was extracted using the DNeasy Blood and Tissue Kit (Qiagen, USA).

Molecular identification

PCR amplification reactions were carried out in peqSTAR 96 universal gradient thermal cycler (Peqlab Biotechnology, Erlangen, Germany) using specific primers (Sigma-Aldrich, USA) to detect the different genes (Table 1). The reaction mixtures and amplification conditions were optimised for all the genes. Bacterial isolates were specifically identified by amplifying 16S rRNA using universal primers. Further, the *M. haemolytica* isolates were specified by amplification of the *PHSSA* gene, and the individual isolates suspected to *P. multocida* were confirmed by *KMT1* gene amplification. Individual isolates representing *P. multocida* serovars were differentiated by capsule typing and tested for the presence of *hyaD-hyaC* (encoding hyaluronan synthase or *capA*), *bcbD* (*capB*) and *dcbF* (*capD*) genes. All bacterial isolates were also screened for the *plpE* gene (encoding outer membrane lipoprotein) specific to *M. haemolytica*, *omp87* gene (encoding 87-kDa outer membrane protein) and *ompH* gene (encoding outer membrane protein H) peculiar to *P. multocida*. Moreover, the distinct *M. haemolytica* isolates were also tested for the *Rpt2* gene (encoding methyltransferase). For PCR amplification, about 50 ng of DNA was added to the 20-μL reaction mixture having 200 μM of dNTPs, 0.2 μM of each primer, 1.875 mM of $MgCl_2$ and 1 U of Taq DNA polymerase (Sigma-Aldrich, USA) in 1× PCR buffer.

Nucleotide sequencing

The specific genes were further amplified using Pfu polymerase. The gene products (50 μL) were purified using the QIAquick gel extraction kit (Qiagen, USA) as per the manufacturer's instructions. The purified PCR products were quantified (~ 100 ng/μL concentration) and subjected to nucleotide sequencing using bi-directional Sanger's method.

Results

All the moribund sheep were examined by necropsy for specific lesions of pneumonia. Grossly, the infected lungs were seen with various stages of consolidation, petechial or ecchymotic haemorrhages, congestion and exudation in the lobes (Fig. 1a, b). Apical, cardiac and the diaphragmatic lobes (particularly the anteroventral parts) of lungs were affected

Table 1 Primer sequences of different genes

Gene target	Primer sequence	Amplicon (bp)	Annealing temp (°C)	Reference
16S rRNA	27F 5'AGAGTTTGATCMTGGCTCAG3′ 1492R 5'CGGTTACCTTGTTACGACTT3′	~ 1466	52	Lane (1991)
PHSSA	F 5'TTCACATCTTCATCCTC3′ R 5'TTTTCATCCTCTTCGTC3′	327	48	Hawari et al. (2008)
Rpt2	F 5'GTTTGTAAGATATCCCATTT3′ R 5'CGTTTTCCACTTGCGTGA3′	~ 1022	48	Deressa et al. (2010)
plpE	F 5'AGGGCTAATCTACTACAGCC3′ R 5'GGTTTGATCTTCTACAGATG3′	~ 1300	49	Ayalew et al. (2006)
KMT1	F 5'ATCCGCTATTTACCCAGTGG3′ R 5'GCTGTAAACGAACTCGCCAC3′	457	56	Townsend et al. (1998)
hyaD-hyaC (*capA*)	F1 5'TGCCAAAATCGCAGTCAG3′ R1 5'TTGCCATCATTGTCAGTG3 F2 5'AATGTTTGCGATAGTCCGTTAGA3′ R2 5'ATTTGGCGCCATATCACAGTC3′	1046 564	55 55	Townsend et al. (2001) Gautam et al. (2004)
bcbD (*capB*)	F 5'CATTTATCCAAGCTCCACC3′ R 5'GCCCGAGAGTTTCAATCC3′	760	55	Townsend et al. (2001)
dcbF (*capD*)	F 5'TTACAAAAGAAAGACTAGGAGCCC3′ R 5'CATCTACCCACTCAACCATATCAG3′	648	55	Townsend et al. (2001)
ompH	F 5'ACTATGAAAAAGACAATCGTAG3′ R 5'GATCCATTCCTTGCAACATATT3′	~ 1210	55	Singh et al. (2011)
omp87	F1 5'ACCTCGGTTTTATGGCATTG3′ R1 5'CTTATTAGAACGTCCCACCA3′ F2 5'ACCTCGGTTTTATGGCATTG3′ R2 5'CAGATGGCTGGTTAGACTA3′	2627 2671	56 56	Singh et al. (2018) Accession number U60439.1

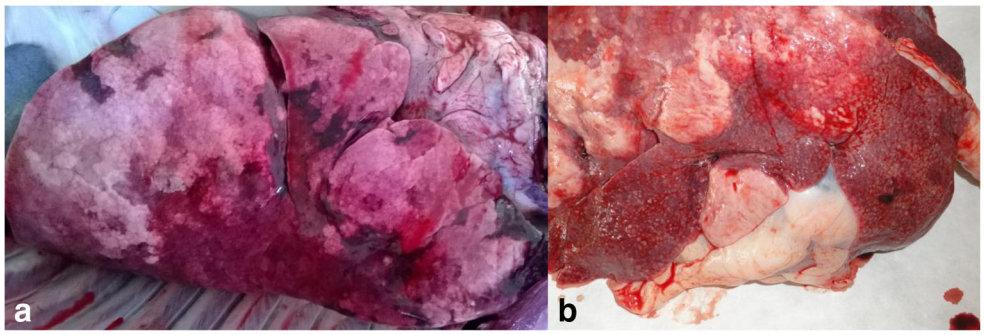

Fig. 1 **a**, **b** Pneumonic lungs showing haemorrhagic lesions (**a**) and consolidation (**b**)

predominantly with the pneumonic changes. Consolidation was frequently seen in the apical lobe. Acute bronchopneumonia was grossly characterised by severe congestion in the lungs along with focal to diffused consolidation and hepatisation of their lobes. The straw-yellow coloured mucus mixed with the frothy-fluid was found to be trapped in the internal tissues of the lung, mainly the lumens of the bronchi and bronchioles. A few numbers of chronic and complicated cases showed lung suppuration with small- to large-size abscesses in the parenchyma of lobes. Lung adhesions with the diaphragm and thoracic wall were commonly witnessed in chronic and suppurative pneumonic cases. There was generalised haemorrhage in the tracheal mucosa and the tracheal lumen was found to be filled with a large volume of mucus and froth. The mucus was seen with discrete haemorrhagic clots. Moreover, the blood-tinged frothy exudate was evident in the trachea (Fig. 2a). Apart from the lung and trachea, the petechial haemorrhages were also seen on the epicardium (Fig. 2b), spleen and kidneys. In some pneumonic sheep, the liver and intestine were spotted with the congestive changes.

The pneumonia was pathologically marked as acute fibrinous-bronchopneumonia ($n = 36$) (Fig. 3a, b), suppurative chronic bronchopneumonia ($n = 10$) (Fig. 4a), acute interstitial pneumonia ($n = 32$) (Fig. 4b) and acute pneumonic congestion ($n = 18$). The acute fibrinous-bronchopneumonia was characterised by pinkish sero-fibrinous exudates and trapped mucus in the bronchiolar lumen and heterophilic infiltration beneath the mucosa of the bronchi and bronchioles. Inflammatory exudate consisting of infiltrated polymorphs and macrophages was seen in alveolar mass. There was thickening of the pleura with neutrophilic and fibrinous exudate. Chronic suppurative bronchopneumonia revealed a distinct central caseo-necrotic zone surrounded by a pyogenic membrane and neutrophilic and mononuclear cellular infiltration.

Acute interstitial pneumonia was evident with thickening of interalveolar septa and dense leucocytic infiltration in the pulmonary interstitium. The epithelium in bronchi and bronchioles was hyperplastic and infiltrated with perivascular lymphocytes. In acute pulmonary congestion, the alveoli were distended and the alveolar capillaries were congested with a large number of erythrocytes.

Culturally, the pneumonic pasteurellosis was confirmed to 28 cases (29.1%) of pneumonia in which 18 cases (18.75%) showed the *M. haemolytica* recovery while 14 cases (14.58%) were positive for *P. multocida*. Fourteen cases (14.58%) signified the isolation of *Bibersteinia trehalosi*. *M. haemolytica* along with *P. multocida* was recorded from eight sheep. *M. haemolytica* was co-isolated with *B. trehalosi* from six cases. Two cases were complicated by all three types of bacteria together. Eighteen heart blood samples (18.75%) noticed the *P. multocida/M. haemolytica/B. trehalosi* isolation which indicated the septicaemia and/or systemic pasteurellosis. Analysis of 16S rRNA sequence confirmed the *M. haemolytica* ($n = 18$), *P. multocida* ($n = 14$) and *B. trehalosi* ($n = 14$). Further identification was performed by species-specific amplification of the *PHSSA* (*M. haemolytica*) or the *KMT1* (*P. multocida*) gene.

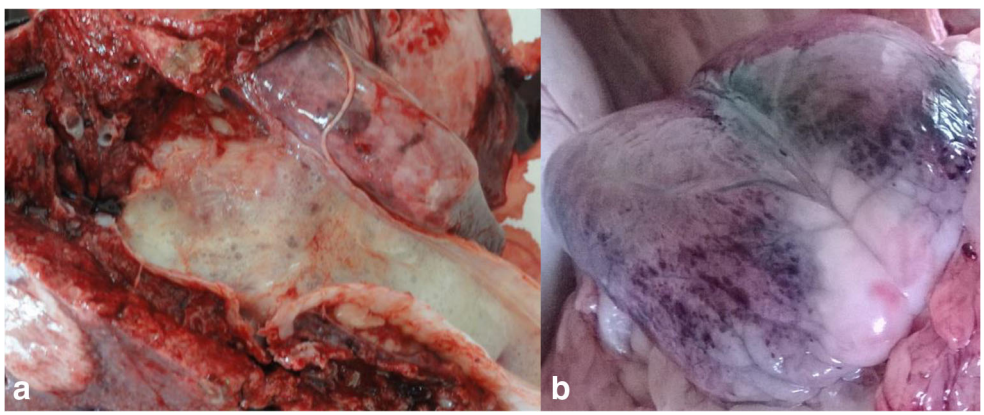

Fig. 2 **a**, **b** Pneumonic lungs showing accumulation of froth in bronchi and bronchioles (**a**) and characteristic haemorrhagic lesions on the epicardium (**b**)

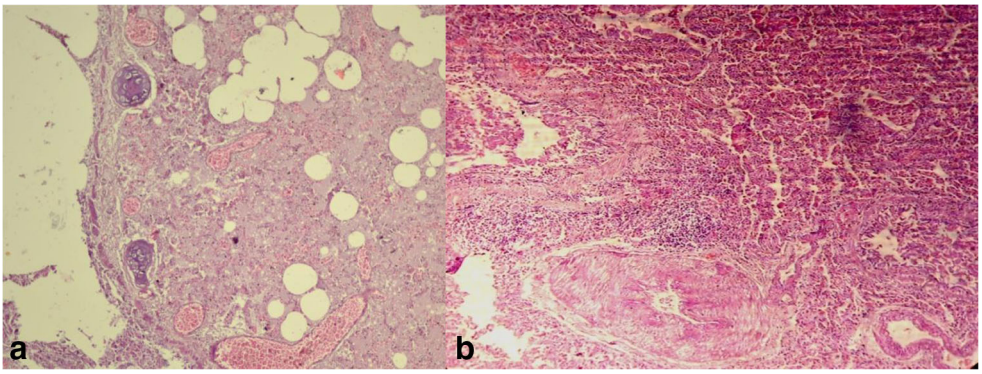

Fig. 3 **a**,**b** Pneumonic lungs showing acute fibrinous-bronchopneumonia (**a**) and acute bronchopneumonia with mononuclear cells and thickened blood vessels (**b**)

The isolates showed susceptibility to all tested antimicrobials. Complete susceptibility (100%) was recorded against amoxicillin-clavulanic acid, tetracycline and chlortetracycline. However, an intermediate susceptibility was evident against amikacin (31.25%), gentamicin (15.6%), amoxicillin (9.4%), cefotaxime (9.4%), ciprofloxacin (9.4%), enrofloxacin (9.4%), ofloxacin (9.4%) and norfloxacin (9.4%).

Further molecular characterisation revealed amplification of *plpE* (~1.3 kb) and *Rpt2* (~1022 bp) genes specific to *M. haemolytica*. We observed three genotypic variants of *M. haemolytica* based on the nucleotides variability in *Rpt2* gene. The quantum of CACAG repeats was varied among these variants (CSWRI/AH/MhA.16, CSWRI/AH/MhA1.16 and CSWRI/AH/MhA2.16) which showed 19, 28 and 25 repeats, respectively. One of the three strains (CSWRI/AH/MhA.16) was homologous (based on the similarity in Rpt2 gene sequence) to the previous Indian *M. haemolytica* strains (KJ566123.1: strain CSWRI/AH/11/12 and KJ534630.1: strain CSWRI/AH/12/12) circulated during 2011–2012. Another two strains of *M. haemolytica* differed (based on the variability in Rpt2 gene sequence) with the strains reported earlier from the USA, Canada, Germany and India (Fig. 5).

Capsule typing of *P. multocida* showed A ($n = 16$) and D ($n = 02$) type of capsules. We did not observe the type B capsule in any of these isolates. All *P. multocida* isolates of serogroup A viewed the specific amplification of the *hyaD-hyaC* gene.

All *P. multocida*-specific isolates revealed amplification of the *ompH* gene (~1.2 kb) with an open reading frame (ORF) ranging from 1023 to 1059 bp and the *omp87* gene (2627 bp) with an ORF of 2376 bp. There was high variability in nucleotide sequences of *ompH* and *omp87* genes. Phylogenetic analysis of the *ompH* gene showed a remarkable difference but a similarity with a few Chinese, USA, Iranian and Malaysian strains of *P. multocida* (Fig. 6). The *omp87* gene sequence reflected a similarity with the nucleotide sequence of previously described Indian, Iranian and Malaysian strains of *P. multocida* (Fig. 7). The individual accession numbers of all variant sequences have been received from the NCBI GenBank database and shown in Table 2.

Discussion

Sheep farming plays an important role in sustaining the livelihood of various rural families in Rajasthan, India. Globally, the sheep is reared as a food animal and adapted mainly to the arid or semiarid climate. However, a confluence of important factors like production/reproduction stress in the prevailing climate of a particular geographical area and dynamic weather patterns predispose these animals to pasteurellosis (mannheimiosis) (Kumar et al. 2015). International surveillance studies have proved that the epizootic or enzootic pasteurellosis or mannheimiosis is a major animal disease

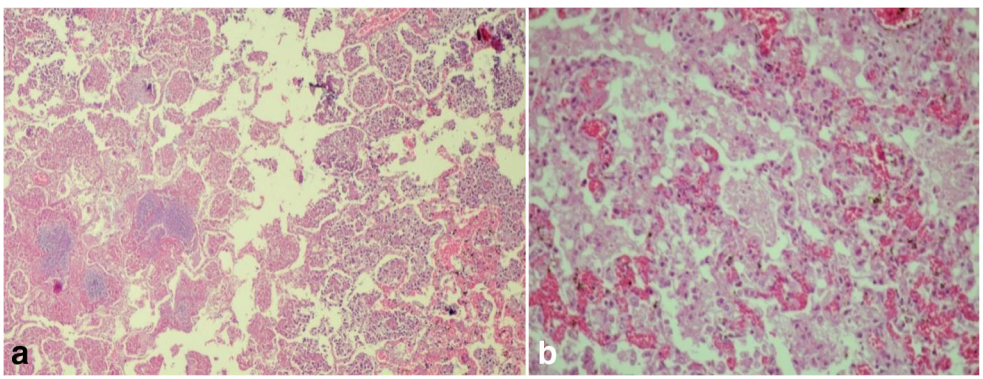

Fig. 4 **a**, **b** Pneumonic lungs showing chronic suppurative bronchopneumonia with multiple foci of neutrophil cells and deposition of exudate (**a**) and acute interstitial pneumonia with infiltration of mononuclear cells (**b**)

Fig. 5 Phylogenetic relationships of different *M. haemolytica* strains based on the variability in Rpt2 gene

influenced by changing climate (Black and Nunn 2009). Furthermore, the huddling behaviour and group grazing habits of sheep predispose them to many infections (Chakraborty et al. 2014).

Gross pathological changes in the lungs and trachea were corroborated by similar studies reported earlier (Marru et al. 2013; Dar et al. 2014). There was presence of petechial haemorrhages on different organs viz. heart (epicardium), spleen

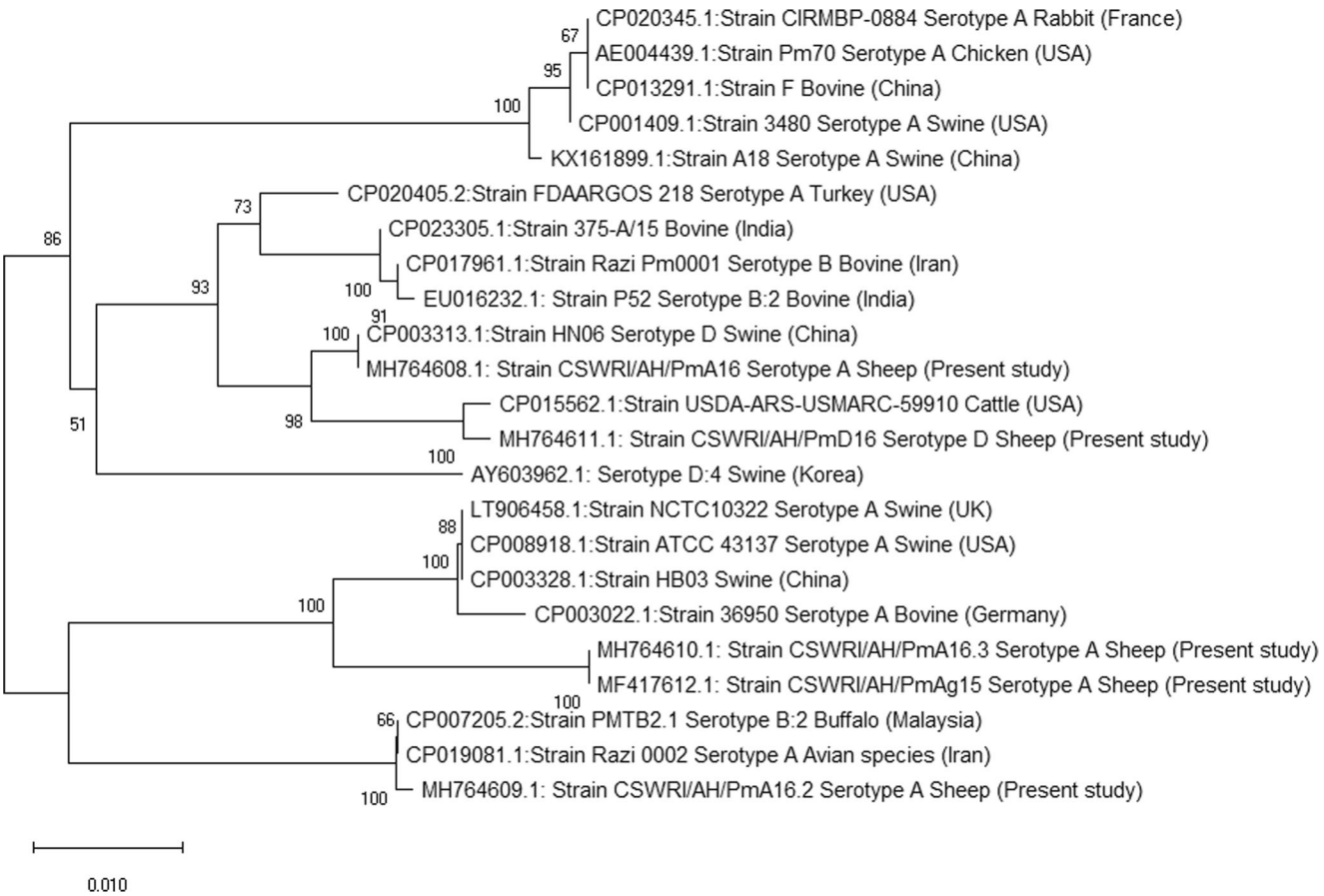

Fig. 6 Phylogenetic relationships of different *P. multocida* strains based on the variability in *ompH* gene

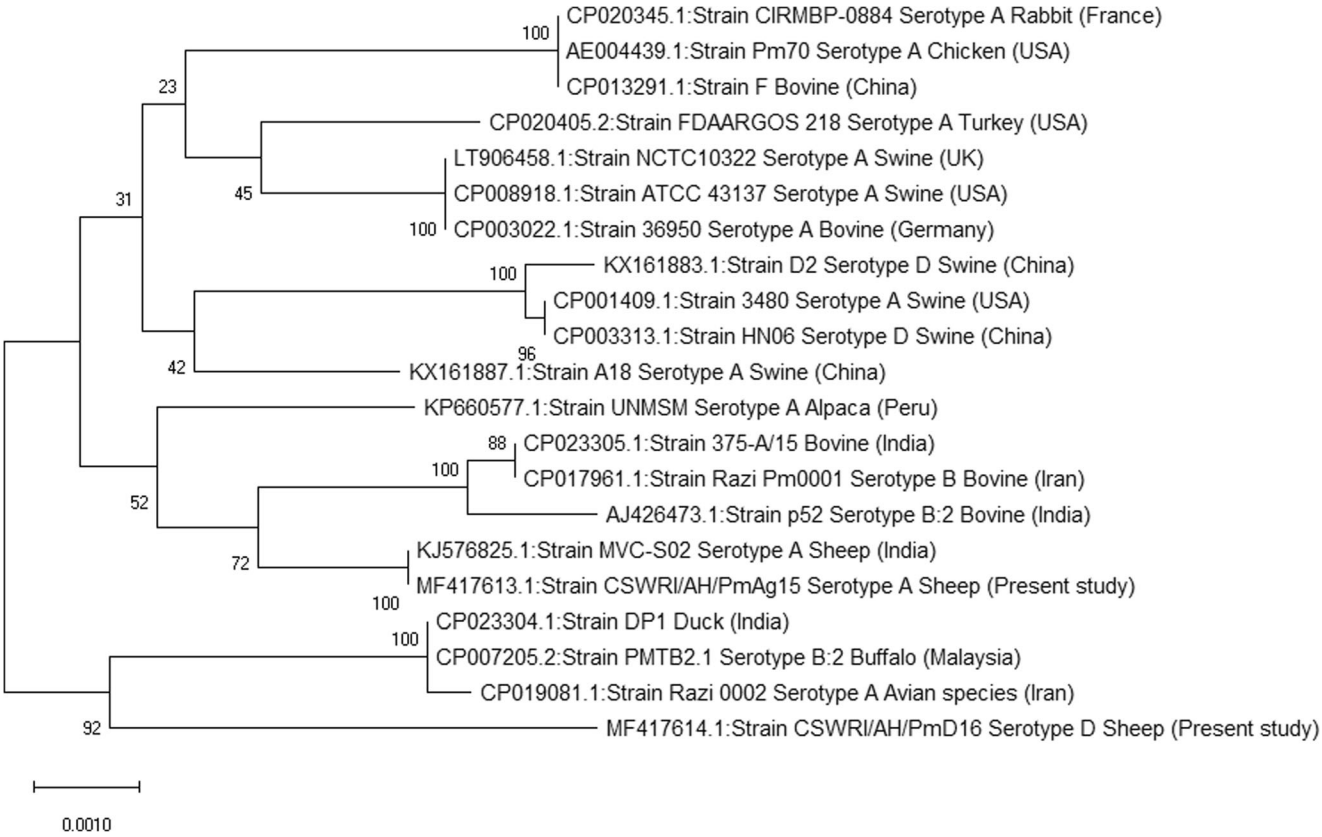

Fig. 7 Phylogenetic relationships of different *P. multocida* strains based on the variability in *omp87* gene

Table 2 Accession numbers of the nucleotide sequences of *P. multocida* and *M. haemolytica* strains

Bacterial strain	Gene target	Accession number
Pasteurella multocida	16S rRNA	MF417604.1
CSWRI/AH/PmA16	*KMT1*	KY825086.1
CSWRI/AH/PmD16	*KMT1*	KY825087.1
CSWRI/AH/PmA16	*hyaD-hyaC* (*capA*)	MF417606.1
CSWRI/AH/PmAg15	*hyaD-hyaC* (*capA*)	MF417608.1
CSWRI/AH/PmA16	*hyaD-hyaC* (*capA*)	MF417609.1
CSWRI/AH/PmAg15	*hyaD-hyaC* (*capA*)	MF417610.1
CSWRI/AH/PmD16	*dcbF* (*capD*)	MF417611.1
CSWRI/AH/PmAg15	*ompH*	MF417612.1
CSWRI/AH/PmA16	*ompH*	MH764608.1
CSWRI/AH/PmA16.2	*ompH*	MH764609.1
CSWRI/AH/PmA16.3	*ompH*	MH764610.1
CSWRI/AH/PmD16	*ompH*	MH764611.1
CSWRI/AH/PmAg15	*omp87*	MF417613.1
CSWRI/AH/PmD16	*omp87*	MF417614.1
Mannheimia haemolytica	16S rRNA	MF417605.1
CSWRI/AH/MhA.16	*Rpt2*	MF417615.1
CSWRI/AH/MhA1.16	*Rpt2*	MF417616.1
CSWRI/AH/MhA2.16	*Rpt2*	MF417617.1
CSWRI/AH/MhA.16	*PHSSA*	MF417618.1

and kidneys, which indicated septicaemia. Histopathological changes of acute fibrinous-bronchopneumonia agreed with the previous reports on ovine pneumonia (Brogden et al. 1998).

Recovery of *P. multocida*/*M. haemolytica*/*B. trehalosi* isolates from the lungs or heart blood confirmed their role in the causation and/or complication of ovine pneumonia. This finding was further corroborated by Brogden et al. (1998) who isolated the *M. haemolytica*/*P. multocida*/*B. trehalosi* from the clinical cases of fatal pneumonia in sheep. *B. trehalosi* (previously *P. trehalosi*) primarily causes the acute septicaemia in young sheep (Mohamed and Abdelsalam 2008). We have also isolated *B. trehalosi* alone or along with *M. haemolytica* and/or *P. multocida*.

Though the ovine pneumonia is primarily caused either by parainfluenza 3 virus or respiratory syncytial virus or *Mycoplasma ovipneumoniae* (Dassanayake et al. 2010) and predisposed by extreme of environmental insults, *M. haemolytica* has been considered as the main culprit behind the lung infections (Miller 2001). Conversely, it has been reported that the respiratory syncytial virus and the parainfluenza 3 virus can develop non-fatal pneumonia but they do not predispose pneumonia due to *M. haemolytica* infection in bighorn sheep (Dassanayake et al. 2013). However, *M. ovipneumoniae*, a cause of non-fatal pneumonia in bighorn sheep, can develop the fatal pneumonia due to

M. haemolytica infection (Dassanayake et al. 2010). The presence of virulent *M. haemolytica* and/or *P. multocida* reflected their role with the pathogenesis and pneumonia progression among affected lambs or adult sheep. Lung colonisation by two or more bacteria was seen in the severe and complicated pneumonia. The *M. haemolytica* infection induces *P. multocida* with high multiplicity growth and tissue invasion leading to the occurrence of fatal pneumonia of high severity and complexity (Dabo et al. 2007).

The *P. multocida/M. haemolytica* could be identified by phenotypic characters such as cultural or biochemical features (Kumar et al. 2015; Sarangi et al. 2015). Detection of *M. haemolytica* (27%) and *P. multocida* (8%) has been reported in association to the bronchopneumonia of acute nature from Ethiopian sheep (Deressa et al. 2010). We observed *M. haemolytica* as the most common isolate which had its tropism to the ovine lung tissue (Deressa et al. 2010; Marru et al. 2013). Though *P. multocida* develops less severe pasteurellosis, it accounts for the incidence of this disease in unorganised and field flocks (Odugbo et al. 2006). Detection of diverse serotypes of *B. trehalosi* has been attained from sheep with systemic pasteurellosis (Bell 2008).

Susceptibility findings of pasteurellae were more or less similar to the findings reported as earlier (Sarangi et al. 2015). Reduced responsiveness of aminoglycosides (amikacin and gentamicin) might be due to their selective therapeutic uses for clinical cases.

Virulence-associated genetic elements among *M. haemolytica* might be homologous to their *PHSSA* gene (Gonzalez et al. 1991). In stressful milieu, the *PHSSA* gene after expression induces the commensal or non-pathogenic *M. haemolytica* to be pathogenic to sheep, and hence, the PHSSA is reportedly supposed to be a species-specific and virulence-linked component of these bacteria (Gonzalez et al. 1995; Kumar et al. 2015). The gene *Rpt2* encoding methyltransferase has been analysed and found with the multiple and variable quantum repeats of CACAG which could have potentiated the pathogenic ability of carrier bacterial serotypes (Ryan and Lo 1999). Variability in *Rpt2* sequences might have resulted in the genesis of functionally diverse methyltransferase which possibly promoted the virulence of *M. haemolytica*.

Proteolytic enzymes and outer structures such as adhesin, capsular-lipopolysaccharide and the OMPs peculiar to these important microbes help their colonisation and exhibit the host defence evasion to initiate the lung pathology (Mohamed and Abdelsalam 2008). Thus, *Rpt2*, *PHSSA* and *plpE* are reportedly the crucial genetic components that supplement to the infection progression ability of the carrier bacterium and these bacterial genetic constituents aid to encounter the sheep with advanced and overt pneumonia. Likewise, the lipopolysaccharide moiety of the capsule produced by *P. multocida* resists the phagocytosis and exhibits the pneumonia progression by lowering the host immunocompetency (Harmon et al. 1991). Thus, *P. multocida* requires a unique lipopolysaccharide moiety of the capsule for its expansion in vivo to establish the clinical bronchopneumonia in sheep. However, the pasteurellae lipopolysaccharide constitutes a shielding antigen and stimulates the humoral immunity of the diseased or infected animals (Harper et al. 2006).

Diverse sequences of OMP genes might have terminated into the translation of altered surface-exposed epitopes. The *omp87* gene encodes a structurally and functionally significant outer protein specific to *P. multocida* (Chaudhuri and Goswami 2001). This protein also acts as an adhesin needed for bacterial colonisation on the mucosa of the pulmonary system (Dabo et al. 2005). By potentiating the pathogenic capability of *P. multocida* and their colonisation on the pulmonary mucosa, these OMPs lead to the progression of bacterial enzootic pneumonia in sheep. Bacterial OMPs enable the ionic transport or entry of energy molecules across their membrane, in summation to their important role in colonisation, host defence evasion and productive infection (Lin et al. 2002). OmpH is a unique protein specific to all serovars of *P. multocida* (Harper et al. 2006). Variable sequences of the *ompH* gene and their translation into the diverse surface epitopes are significantly paramount to *P. multocida* adaptation to its host environment (Singh et al. 2011). These bacterial surface markers are exposed to different selection pressures or environmental insults and modulate themselves to safeguard the host bacterium under harsh environment or changing ecological niches. Therefore, the genes encoding pathogenicity markers were targeted to assess the intra-species heterogeneity and for establishing the epidemiological relationship (Davies et al. 2004).

Conclusions

Acute fibrinous-bronchopneumonia was predominantly found to be connected with high mortality of lambs and adult sheep. Virulent *M. haemolytica* or *P. multocida* isolates sensitive to commonly used antimicrobials were recovered from the pulmonary tissues or the heart blood of sheep and lambs with divergent pneumonia. Different molecular elements accountable for the invasive role of the causative bacterial agents were detected which might have affected the prognosis of ovine fatal pneumonia.

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Compliance with ethical standards

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