ORIGINAL ARTICLE

Molecular characterization of the capsular antigens of *Pasteurella multocida* isolates using multiplex PCR

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**Abstract** The use of molecular techniques for detection and characterization of the *Pasteurella multocida* is very important for rapid and specific detection and characterization of the organism. During the period from 15th February, 2014 to 15th April, 2015, 425 nasopharyngeal swabs and 175 lung and spleen samples were collected and examined by conventional methods, 80 strains (18.82%) of *P. multocida* were isolated from the calves, sheep and goat with respiratory manifestation. Meanwhile, 77 strains (44%) were isolated from emergency slaughtered animals. All the recovered strains were positive for specific PCR for detection of *P. multocida* strains previously identified as *P. multocida* by standard microbiological techniques. Multiplex PCR for molecular typing of the capsular antigens of the recovered *P. multocida* revealed positive amplification of 1044 bp fragments specific to the capsular antigen type A with 105 strains (66.88%), and amplification 511 bp fragments of the capsular antigen type E with 52 strain (33.12%) and absence of B, D and F antigens.

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1. Introduction

*Pasteurella multocida* is a Gram-negative bacterium responsible for Pasteurellosis (acute septicaemic disease characterized by high morbidity and mortality rate as well as severe economical losses) in cattle, sheep, goat and poultry (Prabhakar et al., 2012; Jakeen et al., 2016). It has a zoonotic impact in human; it can cause soft tissue infection after animal bites or via inhalation causing respiratory tract infection.

Based on its capsular antigens *P. multocida* had been classified into five groups (capA, B, D, E and F) (Espinosa et al., 2012; Prabhakar et al., 2012). *P. multocida* is incriminated in many other disease conditions such as mastitis, meningitis, peritonitis, atrophic rhinitis and ear infections due to the effect of *P. multocida* toxins (Atashpaz et al., 2009; Prabhakar et al., 2010).

The outbreak of *P. multocida* showed acute respiratory disease manifested with high fever, nasal discharge, respiratory distress, polypnoea and death within few days. The post-mortem findings showed severe congestion in the lung, trachea, liver and small intestine (Azizi et al., 2011). The diagnosis of Pasteurellosis in farm animals is still under taken by the clinical manifestation, post mortem finding and by conventional bacteriological methods for identification of the causative agents, such methods are not reliable and time consuming and at the same time it is very difficult to differentiate between *P. multocida* and the other Pasteurella species specially the *Pasteurella haemolytica* (*Mannheimia haemolytica*) (Berge et al., 2006; Bell, 2008; Rajeev-Gautam et al., 2006).

Therefore, the use of molecular techniques, specially the polymerase chain reaction (PCR) for molecular detection and characterization of the capsular antigens of the *P. multocida* is very important for rapid and specific detection and characterization of the organism which play an important role in the control of the disease among the farm animals and reducing the economical losses (Rajeev-Gautam et al., 2006; Jakeen et al., 2016). Therefore, the current study is aimed to detect and characterize the recovered strains of *P. multocida* from samples collected from different abattoirs located in Riyadh, Kingdom of Saudi Arabia and transferred under complete aseptic condition to the College of science laboratory at King Saud University for standard bacteriological techniques and for molecular detection and characterization of *P. multocida*.

2. Materials and methods

2.1. Samples

During the period from 15th February, 2014 to 15th April, 2015, 425 nasopharyngeal swabs were collected under aseptic conditions from calves (*n* = 200) from 6 to 12 months age, sheep (*n* = 125) 6 to 12 months and goat (*n* = 100) from 1 to 2 years age suffering from respiratory manifestations. Also 175 lung samples were collected from emergency slaughtered calves (*n* = 75), sheep (*n* = 50) and goat (*n* = 50). The samples collected from different abattoirs located in Riyadh, Kingdom of Saudi Arabia and transferred under complete aseptic condition to the College of science laboratory at King Saud University for standard bacteriological techniques and for molecular detection and characterization of *P. multocida*.

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and spleen of emergency slaughtered animals as shown in Table 1. The characteristic colonial morphology of *P. multocida* had been observed with all the recovered strains. Moreover, all the recovered strains showed severe pathogenicity and killed all the inoculated mice within 24–72 h and the characteristic bipolarity could be observed in the stained blood and spleen smear prepared from the dead mice with Leishman’s stain. The results of ELISA test using somatic and capsular antigens as coating antigens to test the serum samples collected from all animals with respiratory manifestation and emergency slaughtered animals were 64% and 68% respectively.

| Types of samples          | Samples from animals with respiratory manifestation | Number of samples | Percent of isolation of *P. multocida* |
|---------------------------|------------------------------------------------------|-------------------|--------------------------------------|
|                           | Number                                               | Percent           | Percentage (%)                       |
| Sterile nasopharyngeal swabs | Calves                                                | 200               | 35                                   | 17.5                                   |
|                           | Sheep                                                 | 125               | 26                                   | 20.8                                   |
|                           | Goat                                                  | 100               | 19                                   | 19                                     |
|                           | Total number                                          | 425               | 80                                   | 18.82                                  |
| Lung and spleen           | Calves                                                | 75                | 30                                   | 40                                     |
|                           | Sheep                                                 | 50                | 22                                   | 44                                     |
|                           | Goat                                                  | 50                | 25                                   | 50                                     |
|                           | Total number                                          | 175               | 77                                   | 44                                     |

Figure 1  Agarose gel electrophoresis showing amplification of 460 bp fragments specific for *P. multocida* field isolates.

Figure 2  Agarose gel electrophoresis showing amplification of 1044 bp fragments specific to the capsular antigen type A and 511 bp fragments specific to the capsular antigen type E.

Molecular identification of the recovered strains with the specific primers KMT1T7 and KMT1SP6 revealed positive amplification of 460 bp fragments with all the recovered strains previously identified as *P. multocida* by standard microbiological techniques as shown in Fig. 1. Multiplex PCR for molecular typing of the capsular antigens of the recovered *P. multocida* revealed positive amplification of 1044 bp fragments specific to the capsular antigen type A with 105 strains (66.88%), and amplification 511 bp fragments of the capsular antigen type E with 52 strain (33.12%) as shown in Fig. 2 and absence of B, D and F antigens.
4. Discussion

The infections with *P. multocida* most commonly occur as secondary infections to the viral infection and other diseases conditions (Worarach et al., 2014). The interaction between bacterial infection and viral affection in cases of pneumonia of sheep and goats is usually occurring. A primary viral pneumonia may be an insignificant disease until the intervention of a secondary pasteurellosis which converts it into an outbreak of pneumonia of major economic importance (Rad et al., 2009).

The present study is aimed to detect and characterize the recovered strains of *P. multocida* from samples collected from different abattoirs located in Riyadh, Kingdom of Saudi Arabia on molecular basis. 157 strains of *P. multocida* were isolated from the examined samples all of them showed positive results with, catalase, oxidase, ornithine decarboxylase, indole and negative for urea hydrolyze, gelatin liquefactaion, and cannot ferment lactose and mannitol sugar (Jakeen et al., 2016). To overcome the disadvantage of the conventional methods used for diagnosis of *P. multocida* PCR assay reported by Townsend et al. (1998) using KMT1T7 and KMT1SP6 primers has been used to rapidly confirm the results of the standard bacteriological methods. All the recovered strains showed positive amplification of 460 bp fragments specific for *P. multocida* within few hours which indicate the higher sensitivity and specificity of the molecular techniques (Kumar et al., 2009; Jakeen et al., 2016). Multiplex PCR has been carried out in the current study for molecular characterization of the capsular antigens to overcome the disadvantage of the serological methods used for the typing of *P. multocida*. Positive amplification of 1044 bp fragments specific to the capsular antigen type A with 105 strains (66.88%), and amplification 511 bp fragments of the capsular antigen type E with 52.

5. Conclusion

Molecular techniques based on multiplex PCR for molecular typing of the capsular antigens of *P. multocida* can be used as a simple, sensitive, rapid, reliable technique instead of the serological techniques used for identification of the capsular antigens of *P. multocida*.

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