Molecular diagnosis of Haemorrhagic Septicaemia - A Review

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Abstract

Pasteurella multocida is associated with hemorrhagic septicaemia in cattle and buffaloes, pneumonic pasteurellosis in sheep and goats, fowl cholera in poultry, atrophic rhinitis in pigs and snuffles in rabbits. Haemorrhagic septicaemia is caused by Pasteurella multocida type B:2, B:2,5 and B:5 in Asian countries and type E:2 in African countries. Pasteurella multocida have five types of capsular serotype i.e. type A, B, D, E and F. Diagnosis of the disease is mainly based on the clinical sign and symptom, post mortem findings. Confirmatory diagnosis is done by isolation and identification of causative agent. A variety of laboratory diagnostic techniques have been developed over the years for pasteurellosis and used routinely in the laboratory. Among these techniques molecular techniques of diagnosis is most important. This technique not only gives diagnosis but it also provides information regarding capsular type of Pasteurella multocida. Techniques which are used for molecular diagnosis of haemorrhagic septicaemia are PCR based diagnosis, Restriction endonuclease analysis (REA), Ribotyping, Colony hybridization assay, Filled alternation gel electrophoresis (FAGE), Detection of Pasteurella multocida by Real Time PCR. Among these techniques real time PCR is most sensitive and specific.

Keywords: Haemorrhagic septicaemia, Molecular diagnosis, Laboratory diagnostic technique, Real time PCR.

Introduction

Pasteurellosis is a major disease of cattle and buffalo occurring as catastrophic epizootics in many Asian and African countries resulting into high mortality and morbidity (AHIS, 1997; Mustafa et al., 1978; Singh et al., 1996). The disease has been also recorded in poultry, rabbit, pig and wild mammals (Carigan et al., 1991; Rosen, 1981). *Pasteurella multocida* is associated with hemorrhagic septicaemia in cattle and buffaloes, pneumonic pasteurellosis in sheep and goats, fowl cholera in poultry, atrophic rhinitis in pigs and snuffles in rabbits (De Alwis, 1996). An annual economic loss in India due to *Pasteurella multocida* is Rs. 225/- millions (Singh, 2008). Fowl cholera has been recognized as an important disease in domestic poultry for more than 200 years that causes devastating economic losses to poultry industry worldwide (Aye et al, 2001).

Haemorrhagic septicaemia is caused by *Pasteurella multocida* type B:2, B:2,5 and B:5 in Asian countries and type E:2 in African countries. *Pasteurella multocida* have five types of capsular serotype i.e. type A, B, D, E and F. *Pasteurella multocida* type A produce chloral in fowl; pneumonia in cattle, sheep, and pig. Capsular type D of *Pasteurella multocida* produces atrophic rhinitis in pig and snuffles in rabbits.

Two typing systems for serotyping of *Pasteurella multocida* isolates are adopted. One for capsular typing by Carter's IHA system and other somatic typing by the method of Namioka and Murata or Heddleston. Diagnosis of the disease is mainly based on the clinical sign and symptom, post mortem findings. Confirmatory diagnosis is done by the isolation and identification of causative agent. A variety of laboratory diagnostic techniques have been developed over the years for pasteurellosis and used routinely in the laboratory. The organism is identified directly through examination of blood smear from affected animal and can be isolated in suitable culture medium in the laboratory. Various biochemical and serological tests are used for the identification and serotyping of the organism. Rapid slide agglutination test is performed on slide for rapid diagnosis; in which floccular agglutination appear within 30 seconds in the positive cases. Indirect haemagglutination test is carried out for the determination of capsular types of *Pasteurella multocida*.

With development in biotechnological techniques for the detection of nucleic acid, the identification and characterization of etiological agents has become quick, easy and accurate (Dutta et al., 2005).

1. PCR based diagnosis and typing: Numerous studies for diagnosis and characterization of

Pasteurella multocida have been carried out with variable results. The phenotypic characterization systems by means of morphology, biochemical typing, serotyping etc. are very much laborious and time consuming. Even after capsular and somatic antigen determination, still few isolates react similarly in both the antigens. The PCR based techniques have provided the alternative methods of characterization to overcoming the limitations of phenotyping.

a. Pasteurella multocida specific PCR assay: The species specific PCR assay can be applied for detection of *Pasteurella multocida* by using template as either genomic DNA or bacterial colony or by using the direct field samples such as nasal swab, morbid materials like spleen, one marrow, and heart blood. Earlier the PCR needed additional hybridization step for increasing the specificity but later with improved PCR technique it became possible to detect as minimum as 10 organisms per reaction. The *Pasteurella multocida* can identify all subspecies of *Pasteurella multocida*. The sensitivity and specificity of this PCR offer the most compelling argument for the use of PCR technology in laboratory to investigate the suspected HS cases using the primer set as KMT1T7- 5'-ATC CGC TAT TTA CCC AGT GG-3'and KMT1SP6 5'-GCT GTA AAC GAA CTC GCC AC-3'(Townsend et al1998, 2000) by the amplification of a 460bp fragment of DNA. This technique has reduced the time for diagnosis of the disease and also it is specific than traditional one.

b. HS causing type B specific PCR assay: The PCR amplification can also detect the serotype B specific *Pasteurella multocida* directly HS causing type B specific PCR is 100 % specific for type B serotypes of *Pasteurella multocida* isolates. Serotype B cultures with the any combination of somatic antigen are identified by the amplification of a 620 bp fragment with the KT SP61: 5'- ATC CGC TAA CAC ACT CTC- 3' and KTT72: 5'- AGG CTC GTT TGG ATT ATG AAG- 3' primers (Townsend et al., 1998, 2001).

c. Pasteurella multocida type A specific PCR: Primers for typing of serogroup A strains which causing number of infection in livestock and poultry with several somatic types have been reported to be useful in specific identification of isolates. The primers RGPMA5: 5'- AAT GT TTG CGA TAG YCC GTT AGA- 3' and RGPMA6: 5'- ATT TGG CGC CAT ATC ACA GTC- 3' gives PCR amplicon size of 564 bp which confirms the presence of *Pasteurella multocida* serotype A.

d. Multiplex PCR for Pasteurella multocida capsular typing: A multiplex PCR assay is a rapid alternative to the conventional capsular serotyping system and used for capsular types determination. The serogroup specific primers used in this assay were designed following identification, sequence determination and analysis of the capsular biosynthetic loci of each capsular group. The multiplex capsular PCR assay is highly specific and its result correlated well with conventional serotyping results with the exception of those for some serogroup F strains (Townsend et al., 1998, 2001).

The capsular typing of all the isolates were determined by multiplex PCR using capsular types A, B, D, E and F specific primers as mentioned below:

1.CAP A- F 5'- 3' TGCCAAAATCGCAGTCAG
2.CAP A- R 5'- 3' TTGCCATCATTGTCAGTG
3.CAP B- F 5'- 3' CATTTATCCAAGCTCCACC
4.CAP B- R 5'- 3' GCCCGAGAGTTTCAATCC
5.CAP D- F 5'- 3' TTACAAAAGAAAGACTAGGAGCCC
6.CAP D- R 5'- 3' CATCTACCCACTCAACCATATCAG
7.CAP E- F 5'- 3' TCCGCAGAAAATTATTGACTC
8.CAP E- R 5'- 3'GCTTGCTTGATTTTGTC
9.CAP F- F 5'- 3' AATCGGAGAACGCAGAAATCA
10. CAP F- R 5'- 3' TTCCGCCGTCAATTACTCTG

Sizes of the multiplex PCR amplicons are as follows:

Amplicon size	Capsular type
1044 bp	A
760 bp	B
657 bp	D
511 bp	E
851 bp	F

e. REP- PCR and ERIC- PCR: Recently Repetitice Extragenic Palindromic (REP) and Enterobacterial Repetitive Insertion Consensus (ERIC) PCR have been developed for the characterization of *Pasteurella multocida* isolates. REP elements (33 to 40 base pair repeats) are present in 500- 1000 copies accounting for upto 1% of the genome (Stern et al., 1984) and are present in a wide range of bacteria (Olive and Bean, 1999). As the REP elements are distributed widely across the genome, it produces a multiple banding pattern. ERIC- PCR has been successfully used to differentiate strains of *Pasteurella multocida*. The visual analyses of banding pattern were in range of 100- 900 bp. The band patterns provide DNA fingerprints which allows distinction between species and between strains within species.

f. Detection of toxigenic Pasteurella multocida: The *Pasteurella multocida* capsular type D strain has been identified as causative agent of atrophic rhinitis in pigs and snuffles in rabbits. The toxA gene of *Pasteurella multocida* encodes the dermanecrotic toxin responsible for atrophic rhinitis. The toxA gene based PCR can be used for direct analysis of toxigenic *Pasteurella multocida* without additional hybridi-

zation. The assay appears to be the most sensitive and effective method for large scale analysis of nasal and tonsillar swabs (Kamp et al., 1996).

2. Restriction endonuclease analysis (REA): Restriction endonucleases cleave the DNA at specific nucleotide sequences and produce a set of DNA fragments which, upon electrophoresis separate into a characteristic banding pattern or fingerprint of the respective genome. Restriction endonuclease analysis has been successfully used as a tool for differentiation of strains in a variety of bacterial infections including that cause by *Pasteurella multocida*. Several restriction enzymes (HhaI, HpaII, SmaI BglII, PstI, EcoRI) have been taken into consideration for characterization the different isolates of *Pasteurella multocida* (Zhao et al., 1992).

3. Ribotyping: Ribotyping in conjunction with REA has been widely used to characterize and differentiate the Pasteurella multocida isolates (Blackall et al., 1995). REA followed by additional hybridization with a labeled DNA probe made easy to read the banding pattern and give the necessary interpretation. The probe may be labeled either by radio active or non radioactive materials. rRNA probe is widely accepted for hybridization and subsequent interpretation (Blackall, 2000).

4. Colony hybridization assay: A colony lift hybridization assay using a commercially available multicolour detection kit was recently developed for rapid and simultaneous detection of toxigenic Pasteurella multocida and Bordetella bronchiseptica (Register et al., 1998). The major advantage of this assay is the ability to screen the suspect colonies in primary isolation plate so, there is no need of pure cultures and it can analyze the large number of samples in a very short period.

5. Filed alternation gel electrophoresis (FAGE): This technique is also known as 'Pulsed Field Gel Electrophoresis' (PAGE) and it is a method of fingerprinting with high specificity and precision. Conventional electrophoresis, which used a constant current that cannot resolve the large fragments generated by rare cutting restriction enzymes. But in PFGE, where the electric field across the gel is changed periodically can effectively separate the large size DNA fragments on size basis. PFGE analysis has consistently shown the greater discrimination in identification of bacterial species than ribotyping but, it has limited application in the typing of *Pasteurella multocida* isolates (Townsend et al., 1997a).The major drawbacks of this technique are the requirements of highly purified intact DNA and specialized and expensive electrophoresis equipment, which is generally not available in normal diagnostic laboratories (Dutta et al., 2005).

6. Detection of Pasteurella multocida by Real Time PCR: This latest method for detection of Pasteurella multocida in field sample. This highly sensitive and specific test than PM PCR and Multiplex PCR.

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