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Research Article

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF *PASTEURELLA MULTOCIDA* ISOLATED FROM PIGS OF MIZORAM, MANIPUR AND ASSAM

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Abstract- Total 171 trachea/ nasal swab and lungs samples of pigs collected from Mizoram (40 samples), Manipur(51 samples) and Assam (80 samples), 17 (9.94 %) were found to be positive for *P. multocida*. Fourteen of the 17 isolates subjected to PCR targeting species-specific KMT 1 gene revealed a distinct band of 460 bp, which was a confirmation of the isolates as *P. multocida*. Among the confirmed *P. multocida* isolates, nine were identified as capsular type A (1044 bp), while the remaining five were capsular type D (657 bp), based on multiplex cap-PCR results, targeting *hyaD-hyaC* and *dcbF* genes, respectively.

Keywords- *Pasteurella multocida, Pig, pm-PCR, Cap-PCR*

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Introduction

Pig population of India is about 11.13 million as per Livestock Census [1] out of which 2.3 and 8.7 million are crossbred and indigenous. The North Eastern Region of India possesses the highest pig population of 4.45 million which comprises almost 40 per cent of the total pig population of India. Among the North Eastern states of India, Assam possesses the highest population with 2.0 million pigs followed by Nagaland with 0.6 million [2].

Swine pasteurellosis can be controlled by proper vaccination and good management practices [3-5]. To control swine pasteurellosis different vaccines prepared from specific serotype of *P. multocida* can be used against the various forms of pasteurellosis. Different extracts of Pasteurella organism like saline and Potassium thiocyanate (KSCN) has also been tried as vaccines against pasteurellosis in animal [6,7]. Vaccine containing sub-cellular components of *P. multocida* has been introduced recently [8].

Materials and Methods

A total of 171 *P. multocida* numbers of samples were collected from 3 different locations comprising 40 samples (Mizoram), 51 samples (Manipur) and 80 samples (Assam) respectively. The samples were collected from slaughtered pigs, apparently healthy pigs and ailing and dead pigs showing different pathological conditions suggestive of swine pasteurellosis. The details of the samples collected are given in [Table-1]. Samples were collected aseptically and were processed as early as possible in the laboratory for isolation of *P. multocida*.

All the samples collected during the period were inoculated in to 10 percent Sheep Blood Agar (BA) by streak plate technique as described by Collins and Lyne (1970) [9]. The inoculated plates were incubated at 37°C for 16 hours in aerobic environment. After 16 hrs of incubation, the plates were checked for development of bacterial colonies suggestive of *P.multocida*. The colonies suspected to be of Pasteurella spp. were initially examined for their Gram's staining and bipolar staining reactions with Gram's and methylene blue stains, respectively [10,11].

All the *P. multocida* isolates recovered during the present study were subjected to *P. multocida* species specific polymerase chain reaction (pm-PCR) for reconfirmation of the isolates and were further subjected to capsular typing by multiplex Cap-PCR. All the isolates of *P. multocida* were further subjected to molecular confirmation, in respect to their species identification and capsular type by pm-PCR and Cap-PCR respectively, as per the method described by Townsend *et al.* (1998;2001) [12,13]. All the research was conducted as per the approval of Institutional Animal Ethics Committee Ref. No 770/ac/CPCSEA/FVSc/AAU/IAEC/15-16/311 dated 10.04.2015

Result

Primary identification of the seventeen isolates as *P. multocida* was done on the basis of cultural, morphological and staining characteristics. All the isolates produced small, smooth and circular colonies without haemolysis on the Blood Agar (BA) plate following incubation at 37°C for 24 hrs. The colonies were found to be watery, discrete and translucent with a characteristic odour of the culture. None of the isolates could be grown on MacConkey's Lactose Agar.

All the isolates were found to be Gram-negative, cocco-bacilli arranged singly or in pairs. The bipolar reaction exhibited by all the isolates following methylene blue staining was an indication for *P. multocida*.

All the 17 isolates identified to be *Pasteurella multocida* were further subjected to molecular identification targeting *KMT1*, the species-specific gene (460 bp), by pm-PCR. Out of the 17 isolates subjected to pm-PCR, and 14 isolates were found to possess *KMT1* gene indicating the isolates to be of *P. multocida* [Fig-1].

The multiplex capsular typing of all the 14 isolates *P. multocida* revealed presence of *hyaD-hyaC* gene with an amplified product of 1044 bp in nine isolates. This indicated the isolates to be Capsular type A. The remaining five isolates showed presence of *dcbF* gene with 657 bp amplified product and were identified as Capsular type D. The reference strain gave an amplified product of 760 bp band size corresponding to capsular type B.

Table-1 *Details of samples collected for isolation of P. multocida*

Place of collection	Samples collected	Health condition	No. of samples	Total
Selesih (Mizoram)	Trachea/ nasal swab and lungs	Apparently healthy	18	40
		Slaughtered for human consumption	10	
		Ailing and death animals	12	
Imphal (Manipur)	Trachea/ nasal swab and lungs	Apparently healthy	14	51
		Slaughtered for human consumption	10	
		Ailing and death animals	27	
Khanapara (Assam)	Trachea/ nasal swab and lungs	Apparently healthy	25	80
		Slaughtered for human consumption	30	
		Ailing and death animals	25	
Total				171

The 17 samples found positive for *P.multocida* were further processed for molecular diagnosis. Molecular identification 14 (8.18%) samples to be positive for *P. multocida*. Out of these 14 isolates, 3 (7.50%) were from Selesih, Mizoram; 6 (11.76%) from Imphal district, Manipur and 5(6.25%) from Khanapara, Assam respectively

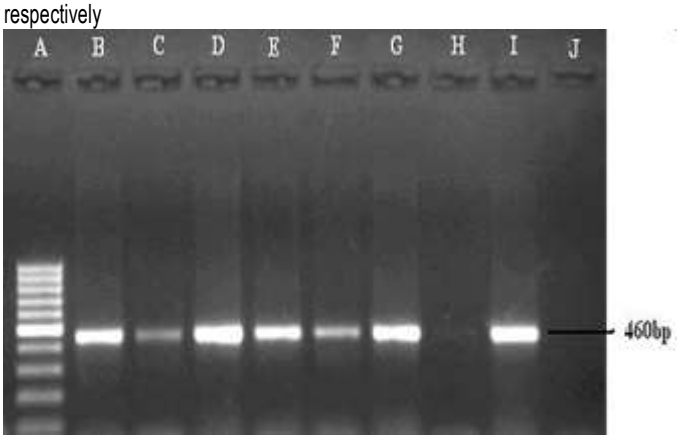

Fig-1 PM-PCR for detection of *KMT 1* gene (460 bp) of *P. multocida*
Lane B to G = Samples positive for *KMT 1* gene
Lane I = Positive control (P52); Lane J = Negative control
Lane A = 100bp DNA Ladder; Lane H = Sample Negative for *KMT 1* gene

Discussion

The isolates identified as *P. multocida* on the basis of cultural, staining and biochemical characteristics were further subjected to *P. multocida* specific polymerase chain reaction (pm-PCR) and capsular typing by Cap-PCR. Out of 17 isolates, 14 were confirmed as *P. multocida* by pm-PCR, which yielded a 460 bp amplified products. Of the present finding colerate the finding Townsend *et al*. (1998), Balakrishnan *et al*. (2012) [14], Prabhakar *et al*. (2012) [15]. Dutta *et al*. (2001) [16] has also confirmed 18 isolates as *P. multocida* of various serotypes from mixed cultures by pm-PCR. and also identified the isolates as *P. multocida* on the basis of amplified product of approximately 460 bp. They have also reported that detection of *P. multocida* by species-specific PCR (pm-PCR) have many advantages over the traditional diagnostic methods which are laborious and time consuming. Similar observation was also reported by Varte *et al*. (2014) [17], where they have identified *P. multocida* by pm-PCR (3.75%) from nasal swabs collected from both apparently healthy pigs and diseased pigs suspected for swine pasteurellosis. Based on present findings, a conclusion can be drawn in respect to the species-specific pm-PCR as a suitable molecular tool for identification of *P. multocida* from contaminated environment.

The capsular typing of the 14 isolates by Cap-PCR during the present investigation revealed that nine of the 14 isolates of *P. multocida* belonged to capsular type A bearing *hyaD-hyaC*gene (1044 bp), while the remaining five isolates indicated the presence of *dcbF* gene (657 bp), corresponding to capsular type D. Similar findings were reported by Townsend *et al*. (2001) and Keenan *et al*. (2008) [18], who have recorded the PCR-based capsular typing assay as extremely reliable method for determination of the capsular types of *P. multocida* strains. Whereas Robert *et al*. (2003) recorded a comparatively higher distribution of *P. multocida* capsular type A (75.0 %) in pigs affected with pneumonic pasteurellosis and atrophic rhinitis. They have also observed association of capsular type D (23.0%) and type E (1.0%) along with untypable isolates in the affected pigs. Kumar *et al*. (2007) [19] could record isolation of *P. multocida* B:2 from two outbreaks of swine pasteurellosis from Punjab with a concurrent infection with classical swine fever. Wide prevalence of *P. multocida* capsular types A and D in pig farms of China was reported by Tagga *et al*. (2009) [20], which was in agreement with the findings observed during the present study. It is also evident from present study and earlier report that pm-PCR and Cap-PCR could be used for detection and typing of *P. multocida* from field samples. It can also be opined that both capsular types A and D are prevalent, either as commensal or as pathogen in pigs. Capsular type A of *P. multocida* was proved to be the prime pathogen (64.29%) associated with pneumonic pasteurellosis in pig population.

Conclusion

A total of 14 isolates of *P. multocida* field isolates were confirmed to be *P. multocida* based on detection of *KMT1* gene (460 bp) by pm-PCR. The capsular typing of the isolates by multiplex Cap-PCR revealed both capsular type A (9) and type D (5) among the isolates. During the mouse pathogenicity trial, variable pathogenicity was observed among the *P. multocida* isolates.

Application of research: The mortality induced by the inoculated strains in mice varied from 50 to 100 percent. One of the isolates (KP10) of capsular type A produced 100 percent mortality in the inoculated mice between 24 to 48 hours post-inoculation and re-isolation was possible from internal organs as well as from heart blood of all the dead mice.

Research Category: Veterinary Sciences

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Study area / Sample Collection: Mizoram, Manipur, Assam

Breed name: Pig

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
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