

Isolation and *in Vitro* Antibiotic Susceptibility of *Pasteurella multocida* from Cattle Origin

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ABSTRACT

Pasteurellosis is one of the important economic diseases in ruminants, especially in cows and buffaloes. It is caused by *Pasteurella multocida* and occasionally by *Mannheimia haemolytica*. During the present study 6 *Pasteurella multocida* were obtained by cultural, biochemical methods from 26 suspected cases of haemorrhagic septicaemia from intestinal samples (lungs, liver and spleen) of cattle. *Pasteurella multocida* species specific polymerase chain reaction provides a rapid and specific of all the serotypes of *P. multocida* and can be used as rapid method for detection *P. multocida* infection in animal. Two of the isolates were detected to have somatic antigen 3,4 and one isolate 2,5 while in the remaining four, somatic antigens were not detected. Antibiotic susceptibility pattern of the isolates revealed that enrofloxacin, flumequin, Penicillin, chloramphenicol and cephalexin were most susceptible (100%), followed by pefloxacin (93.2%), gentamycin (90.1%), ampicillin (90 %), ciprofloxacin (45.5%), amoxicillin (29.76 %) and erythromycin (18.3%). Where all the *P. multocida* isolates were found to be resistant against sulphadiazine (100%). The study confirmed the presence of *P. multocida* with somatic antigen 3, 4 and *P. multocida* 2, 5 were identified for first time in Gombe state amongst the isolates. However more work needs to be carried out in other parts of the country to gather more relevant information with regards to somatic types.

Keywords: *Pasteurella multocida*, Cattle, PM-PCR, Antibigram.

INTRODUCTION

Pasteurella multocida is a non-motile, gram-negative, coccobacillus that is found in the nasopharynx and gastrointestinal tract of many wild and domesticated animals. It is divided into various serotypes on the basis of nomenclature. The serotypes are cause of pasteurellosis in cattle, buffalo, sheep, goat, wild animals and fowl cholera in poultry. The causative agent plays a role in the respiratory disease complex of cattle (Rimler and Glisson, 1997). It causes a number of diseases in various domestic and wild animals. The most important diseases are haemorrhagic septicaemia (HS) in cattle and buffaloes and fowl cholera (FC) or avian cholera in poultry/turkey resulting in heavy economic losses to livestock owners (Dutta *et al.*, 1990).

Laboratory diagnosis of *P. multocida* infection mainly based on isolation and identification by cultural and biochemical methods, this procedure has its own limitation. The development of *P. multocida* species specific polymerase chain reaction (PM-PCR) assay by

Townsend *et al.* (1998) has provided a rapid and specific detection of *P. multocida*. Over a period of time *P. multocida* changed its antibiogram and develop resistance to chemotherapeutic agents in use. Therefore for chemotherapy to be effective drugs to which the organism has been found sensitive need to be administered during the early phase of the disease before specific clinical signs appear (Gupta *et al.*, 1996). Keeping in view the above facts and magnitude of the problem to the livestock owners and veterinary clinicians present work was undertaken with the objective of isolation and identification of *P. multocida* by cultural, biochemical and molecular method from cattle in Gombe state.

MATERIALS AND METHODS

Sample: A total of 25 samples comprising of liver, lungs

and spleen were collected from suspected cases of haemorrhagic septicaemia in cattle in Gombe state Nigeria.

Isolation and biochemical characterization of *P. multocida*

Five percent sheep blood agar was used as a primary culture medium for preliminary isolation of *P. multocida* isolates. Titrated tissue sample was first inoculated on BA and incubated at 37°C for 24-48 hrs. The colonies suggestive of *P. multocida* were subjected to gram staining, catalase test, oxidase test, growth on MacConkey agar, indole test, nitrate test and citrate test for identification. All the isolates were identified by sugar fermentation test as per method described by Cowan and Steel (1985).

Identification by PM-PCR methods

The isolates identified by cultural, biochemical and sugar fermentation tests were confirmed by PM-PCR using species specific oligonucleotides primer (Townsend *et al.*, 1998) *P. multocida*-specific PCR: KMT1T7 5'-ATC-CGC-TAT-TTA-CCC-AGT-GG-3', KMT1SP6 5'-GCT-GTA-AAC-GAA-CTC-GCC-AC-3' (MWG, Bangalore, India). The genomic DNA of *P. multocida* isolates was carried out according to Wilson (1987). Template DNA was added to the PCR mixture (total volume of 25 µl) containing 1 × PCR buffer, 200 µM each deoxynucleotide triphosphate (dNTP), 2 mM MgCl₂, 3.2 pmol of each primer and 0.5 u *Taq* DNA polymerase. Cycling parameters for a Corbett FTS-320 Thermocycler (or similar) were as follows: initial denaturation at 95°C for 5 minutes; 30 cycles of 95°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute; with a final extension at 72°C for 7 minutes. The reaction was held at 4°C until required for electrophoresis; 5 µl of each sample was electrophoresed on a 2% agarose gel in 1 × Tris-acetate running buffer (TAE) at 4 V/cm for 1 hour. The gel was stained with 1% ethidium bromide. The amplified product was visualized as a single compact band of expected size under UV light and documented by gel documentation system (Syn Gene, Gen genius biolmaging System, UK).

Specificity of PCR

The specificity of PCR the standardized PCR was tested by screening standard reference strain of *P. multocida* (B standard strain) which was procured from National Veterinary Research Institute vom Nigeria.

Somatic typing of isolates

Determination of somatic serotypes by the agar gel immunodiffusion (AGID) test (Henddleston *et al.*, 1972)

was performed. The isolates were typed using the procedure of Henddleston *et al.* (1972), and the source of antisera used was from chicken. A panel of 16 reference antibodies made against (Henddleston *et al.*, 1972) reference serotypes 1-16 was used in the typing procedure. Antigen and antisera controls were used in each test.

Antibiotic susceptibility pattern

The in vitro antibiotic sensitivity pattern of *P. multocida* was conducted as per method of Bauer *et al.* (1966). Antibiotic disc (Hi Media Ltd, Mumbai, India) used in the present study were: chloramphenicol (30µg), amoxycilin (30µg), cephalaxine (30µg), ciprofloxacin (5µg), enrofloxacin (10 µg), pefloxacin (5µg), flumequine (5µg), sulphadiazine (300µg), Penicillin (10 i/u), Ampicillin (10 µg), Gentamicin (10 µg), and gentamycin (10 µg). The inhibition zone around each disk was measured independently and compared with standard interpretative charts: Clinical Laboratory Standards Institute (CLSI, 2009).

RESULTS

Of the 26 lungs, liver, spleen, samples tested, 6 isolates from the samples were Gram negative rods, non-haemolytic, round, grayish, smooth and mucoid colonies on Casein sucrose yeast agar (CSY) and blood agar. They were small, coccobacilli and non-motile. They were oxidase, catalase and ornithine decarboxylase positive, were urease negative and fermented glucose without production of gas, mannitol and sucrose positive but not lactose and showed no growth on MacConkey agar, reduced nitrate to nitrite, were indole positive and did not utilise citrate.

The identity of the strain of *P. multocida* were further confirmed by PCR amplification of the gene with size of 465 bp. Figure 1 show the amplicon sizes of the gene fragments amplified by PCR. All the samples gave positive bands for species specific amplicon. The set of primers, KMT1T7 and KMT1SP6 were species specific and amplified the expected products from all isolates of *P. multocida*.

Out of the six isolates two have somatic antigen 3, 4 and 2, 5 while in the remaining four, somatic antigens were not detected by the Henddleston *et al.*, (1972) scheme.

In the present study, all the 6 *P. multocida* isolates were tested for their antibiotic sensitivity pattern against 12 commonly used antibiotics and revealed sensitivity to chloramphenicol, flumequine, Penicillin, enrofloxacin and cephalaxin (100%). Followed by pefloxacin (93.2%), gentamycin (90.1), ampicillin (90%), ciprofloxacin (45.5.1%), amoxicillin (29.6 %), erythromycin (18.3%) and sulphadiazine was (100%) resistant.

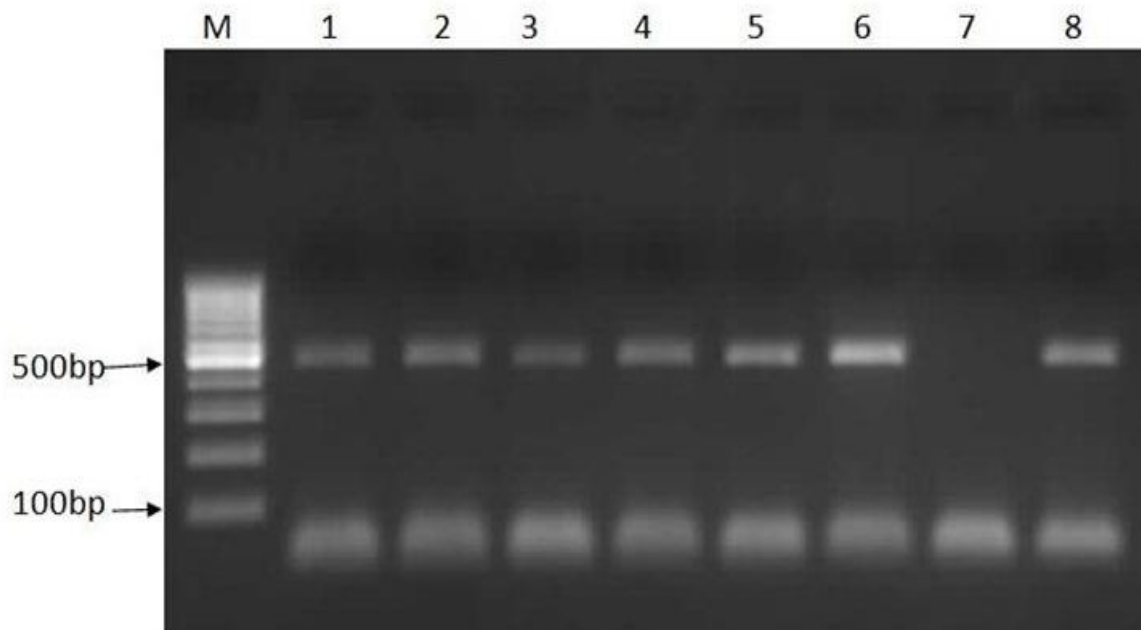


Figure 1. Species specific PCR.

Amplicons of *P. multocida* with size of 460 bp (Species specific). Lane 1- Gb1 isolate, lane 2-Gb2 isolate, lane Gb3 isolate, lane 4-Gb 4 isolate, Lane 5-Gb5 isolate, lane 6 Gb 6 lane7-negative control, lane 8- positive control. lane M- 100 bp DNA molecular marker (Fermentas®).

DISCUSSION

Isolation of *Pasteurella multocida* from cattle and has been the subject of this studies, particularly in Gombe State Metrapols. The study revealed the isolation of *P. multocida* most frequently from lungs. A similar study was reported by (Karimkhani *et al.*, 2011) where 64% of isolates were isolated from lungs. All the isolates were found to be gram-negative, coccobacilli on grams staining and were failed to grow on Mac Conkey agar, these findings were in accordance with De Alwis (1996). Theses isolates were further confirmed by various biochemical tests. All isolates found positive for oxidase, catalase, indole production and reduction of nitrat. Similar findings have also been reported by Kumar *et al* (1996). All the isolates were able to ferment glucose, manitol, sucrose and mannose but failed to ferment maltose, arabinose, lactose, ducitol, salicin, inositol and trehalose. All the isolates were negative for citrate utilization. *P. multocida* species specific PCR (PM-PCR) was performed using primer pair KMT1SP6 and KMT1T7 (Townsend *et al.*, 1998). All the isolates of *P. multocida* including vaccine strain (Standard B strain) produced an amplified product of approximately 465 bp size. Other bacterial culture which were used to check out specificity of primer used did not show any amplified product (Figure 1). These were in conformity to results obtained by Dutta

et al. (2001), who reported approximately 460 bp amplified product from all *P. multocida* isolates. It is evident from present results and earlier related reports, that PM-PCR provides rapid identification of all serotypes of *P. multocida* isolates and can be used as rapid method specific for detection of *P. multocida* infection in animal.

The determination of antibiotic sensitivity *in vitro* of particular *P. multocida* isolates would certainly increase the chances of successful therapy for HS. Of the drugs recommended in cattle and buffalo, *P. multocida* is highly sensitive to penicillin, ampicillin, cephalothin, enrofloxacin and oxytetracycline. *P. multocida* also exhibited a high sensitivity to chloramphenicol and neomycin, although these compounds are not recommended for parenteral use in the two species. Our observations are generally in agreement with previous reports by different investigators (Kalhor *et al.*, 2019).

In the study, somatic type 3,4 and 2,5 were identified among the isolates of *Pasteurella multocida* on using Hendleston *et al.*, (1972) scheme. *Pasteurella multocida* setrotype B:2 (6:B) and E:2 (6:E) are the principal causes of HS (Carter and De Alwis, 1989). Although serotype B:2 has been mainly reported in Asian countries and E:2 in African countries, both serotypes have been recovered from the disease in some African countries (Martrenchar and Njanpop, 1994; De Alwis, 1995; Kumar *et al.*, 2004).

CONCLUSION

The results of this study have contributed to knowledge on the biology of *P. multocida* associated with HS in Gombe state of Nigeria. *Pasteurella multocida* somatic type 3, 4 and *P. multocida* 2, 5 were identified among the isolates. Variation in the sensitivity to different antibiotics by the isolates showed the necessity of *in vitro* antibiotic sensitivity before treatment. It also emphasizes the need for judicious selection of antibiotic for effective treatment.

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