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# PCR detection of *Pasteurella multocida* in eagle PCR based detection, characterization and antibiogram of *Pasteurella multocida* from wild eagle (*Aquila rapax*) in Jammu and Kashmir

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The present study was carried out using conventional and PCR based technique for identification and characterization of *Pasteurella multocida* from an eagle suspected with pneumonic pasteurellosis and antibiogram of the isolate to record its antimicrobial sensitivity pattern.

A dead eagle received from Manda mini zoo, Jammu, J&K was subjected to post-mortem examination. A piece of lung, liver, spleen and few drops of heart blood were collected under aseptic conditions. All the materials were prepared for bacteriological examination and isolated pure colonies were identified as per Cowan and Steel (1970) and Cruickshank *et al.* (1975).

Isolated organism was subjected to *P. multocida* species and serotype B:2 specific PCR assays using oligonucleotide primer sets of KMT1T7 – KMTT1SP6 and KTT72 – KTSP61, respectively, as per Townsend *et al.* (1998). Amplimers were analyzed by gel electrophoresis (Sambrook *et al.* 1989) in 1.5% agarose containing ethidium bromide (0.5 µg/ml). The products were visualized and photographed with gel documentation system.

The isolate was tested for its antimicrobial sensitivity against 20 commonly used antimicrobial agents. Muller Hinton agar No. 2 plates were prepared and incubated overnight to avoid any possibility of contamination. Two hundred micro liters of 18 h-old culture was spread evenly on blood agar plates. The culture was allowed to absorb on plate for 10 min and then antimicrobial discs were placed at an appropriate distance from each other. The plates were then incubated at 37°C for 24 h.

Diameter of clear zone surrounding the disc was measured and matched with respective standard zone diameter to interpret the test culture as resistant, intermediate or sensitive.

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Post mortem examination revealed general hyperemia, swollen liver with necrotic foci and petechial hemorrhages. Congestion of lungs and mild enlargement of kidneys with distended tubules were recorded. Bacterial isolation from all the morbid materials analyzed was identified as *P. multocida*. They were gram negative, coccobacilli with characteristic bipolar staining property by Loeffler's alkaline methylene blue stain. Isolates were also positive for indole, catalase, oxidase and nitrate reduction tests whereas, negative for citrate, methyl red, voges – proskauer and gelatin liquefaction tests. No growth was recorded on Mac Conkeys agar and all the isolates were non hemolytic on sheep blood agar.

Multiplex PCR assay gave an amplified product of ~460 bp only, indicating the presence of *P. multocida* other than serotype B:2/B:2,5/B:5. For further serotyping, the isolates were sent to reference serotyping laboratory in Sri Lanka and result is awaited.

Results of antimicrobial sensitivity/resistance patterns of isolated *P. multocida* against 20 commonly used antimicrobial agents were interesting. It was completely resistant to sulphadiazine, a drug of choice for pasteurellosis. The resistance percentage indicates that *P. multocida* strains were most sensitive to gentamicin followed by ampicillin, lincomycin, oxytetracycline, nitrofurantoin and co-trimexazole. The resistance percentage against amikacin, streptomycin, spectinomycin, penicillin-G and vancomycin was more than 75% and considered to be non-effective against animal pasteurellosis.

Our results indicate the direct association of *P. multocida* for the death of the wild eagle. Results of biochemical tests were in accordance with Shivachandra *et al.* (2006a) when they had analysed 123 avian *P. multocida* isolates to record their biovariations. Postmortem examinations revealed the fact that *P. multocida* infection produces to the pneumonic lesions in the bird leads to death without any treatment. Species specific PCR assay further confirmed the presence of *P. multocida*. This technique has been proved to be an

excellent method developed by Townsend *et al.* (1998) and it was successfully applied by Dutta *et al.* (2001, 2004) for rapid identification of *P. multocida* in India. It is highly specific, rapid, cost effective and easy to perform technique for rapid diagnosis of animal pasteurellosis. Presence of only ~460 bp amplicon by multiplex PCR assay indicates non-involvement of serotype B:2/B:2,5/B:5 because *P. multocida* of those serotypes produces 2 distinct amplicon of ~460 bp and ~620 bp (Townsend *et al.* 1998). Although accurate serotyping of the isolate could not be done, analyzing the result of present study and keeping in mind the result of previous workers (Kumar *et al.* 2004, Shivachandra *et al.* 2006b) it is assumed that the isolate is probably of serogroup 'A'.

The subject of study was a wild bird, which has never been exposed to any antimicrobial treatment regime. But isolation of multi-drug resistance *P. multocida* from a wild eagle indicates the transmission of pathogen from any domestic source, which is already highly resistant to the different antimicrobial agents. Earlier, the authors also reported the similar type of multi-drug resistance to *P. multocida* from livestock and poultry but not from wild birds (Dutta *et al.*2004). This is probably is the first report of such kind of organism from wild eagle.

## SUMMARY

*Pasteurella multocida*, a gram negative, coccobacilli was isolated and identified from a dead wild eagle in Jammu and Kashmir. Cultural and biochemical tests proved the association of *P. multocida*. Species and type specific multiplex PCR assay confirmed the presence of *P. multocida* other than serotype B:2/B:2,5/B:5. Isolate was recorded as a multi-drug resistant *P. multocida* by antimicrobial sensitivity assay using disc diffusion method. This is probably first report to isolate a multi-drug resistant *P. multocida* from a wild eagle in India.

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