Isolation, Identification and Antibiogram of Pasteurella multocida Isolates of Rabbits Suffering from Pasteurellosis

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Abstract

Isolation of Pasteurella multocida was attempted from the heart blood, spleen, liver and lung collected from rabbits (4 No,s) suspected to have died of pasteurellosis. A total of four P. multocida isolates were isolated and identified on the basis of biochemical characteristics, pathogenicity studies in mice and PM-PCR. The In vitro antibiotic sensitivity test of the organisms was conducted which revealed that all the four isolates showed sensitivity to Gentamicin and Ofloxacin. Three of them were sensitive to Enrofloxacin and Ciprofloxacin. Two isolates showed sensitivity to Doxycycline and Chloramphenicol. Only one isolate was found sensitive to Nalidixic acid. All the isolates were resistant to Doxycycline, Erythromycin, Neomycin, Ampicillin, Penicillin, Cephalexin and Polymyxin B. Based on the antibiotic sensitivity test, Gentamicin was recommended as a drug of choice for the treatment of Pasteurellosis in rabbits and the disease was controlled.

Key words: Isolation – Identification – Antibiogram – Pasteurella multocida - Rabbits

Introduction

Pasteurellosis is a highly contagious disease of rabbits caused by Pasteurella multocida. Rabbits can become infected with P. multocida immediately after birth and the prevalence of P. multocida colonization increases with age until about 5 months. P. multocida causes a spectrum of conditions including rhinitis (snuffles) with purulent nasal discharge, pneumonia, otitis media, pyometra, orchitis, abscesses, oculoconjunctivitis and septicaemia. It is considered to be a predominant cause of death in rabbits which in turn result in considerable economic losses to the rabbit industry.
Materials and methods

Specimen

A total of 4 adult Soviet chinchilla rabbits of both sexes to have died of pasteurellosis were received from an organized farm in Chennai during 2010. All the rabbits were subjected to Post mortem examination and heart blood, tissue pieces from trachea, lung, spleen and liver were collected for bacterial isolation and identification. Heart blood smears, tissue impression smears from liver, spleen and lung were prepared and subjected to Leishman’s staining.

Bacterial isolation and identification

The heart blood and tissue samples were inoculated into brain heart infusions agar, Blood agar, MacConkey agar and Nutrient broth and incubated at 37 °C with 5 % CO₂ for 24 h for the isolation of P. multocida. The colonies suggestive of P. multocida were subjected to biochemical tests for identification. The biochemical test included IMViC tests, sugar fermentation tests, catalase and oxidase test.

Pathogenicity studies

The pure cultures of the P. multocida isolates in the present study were subjected to pathogenicity studies. Two albino swiss albino mice for each isolate were inoculated intraperitoneally with one hundred microlitre of 18 h broth culture and observed for 48 h. The dead ones were subjected to post mortem examination and heart blood, lung, spleen, liver and bone marrow were collected and subjected to reisolation of P. multocida. Impression smears prepared from heart, liver, spleen and lung from the dead mice were stained with Leishman’s stain and examined microscopically.

Pasteurella multocida specific Polymerase chain reaction (PCR)

DNA was extracted from the overnight culture by boiling method. P. multocida polymerase chain reaction (PM-PCR) was carried out using species specific primers KMT ISP 6 and KMT 177 designed by Townsend et al. to amplify KMT1 gene. The thermal cycle protocol was followed as per the method of Townsend et al. The analysis of PCR product was carried out in 1.3 per cent agarose gel stained with ethidium bromide (0.5 µg/ml). 100 bp DNA ladder (Genei, Bangalore) and appropriate controls were incorporated to rule out false positive and false negative results. The gel was viewed under UV transillumination.

Antibiotic sensitivity tests

Laboratory, Mumbai and the antibiotic sensitivity plates were incubated aerobically at 37°C for 24-48h.

Results and Discussion

Bacterial isolation and identification

On post mortem examination, severe congestion of trachea with accumulation of frothy fluid and congestion of lung with dark depressed areas were observed. On Leishman’s staining, Heart blood smears, tissue impression smears prepared from liver, spleen and lung revealed characteristic bipolar organisms suggestive of *P. multocida*. The heart blood and tissue samples from Rabbits (4 No’s) were subjected to bacterial isolation. A total of four *P. multocida* isolates could be isolated from all the samples. All the four isolates showed typical cultural characteristics of dew drop, mucoid, non haemolytic colonies in blood agar. No growth was observed in MacConkey agar. Grams staining of the smears revealed characteristic gram negative coccobacillary organisms. These findings are in accordance with Purushothaman *et al.*

The isolates subjected to biochemical tests were positive for indole production, nitrate reduction, oxidase and catalase production and coincide with earlier findings.

Pathogenicity studies

All the four isolates subjected to mice inoculation tests killed the mice in 24 – 48 h. Similar results are obtained with the *P. multocida* isolates of avian origin.

*Pasteurella multocida* specific polymerase chain reaction (PM-PCR)

PCR has been proved to be useful in the detection of DNA of *P. multocida*. The success of PCR depends on the method of DNA extraction. The addition of Guanidine thiocyanate, Cetyl trimethyl ammonium bromide, Phenol extraction to the specimen is some of the options for DNA extraction. In the present study, simple boiling method was used to extract the DNA of *P. multocida* which makes the PCR technique an even more rapid and cost effective technique.

*P. multocida* species specific polymerase specific PCR (PM - PCR) assay developed by Townsend *et al.* was used in this study to identify *P. multocida* isolates by amplifying 460 bp DNA fragment within KMT I gene using the Primers KMTISP6 and KMTIT7. In comparison with standard molecular weight marker (100bp), the molecular weight of the PCR products of all the isolates were found to be 460bp specific for *P. multocida* and confirmed the isolates as *P. multocida* and was in total agreement with Townsend *et al.*

Antibiotic sensitivity tests

Antibiotic sensitivity tests revealed that all the four isolates showed sensitivity to Gentamicin and Ofloxacin. Three of them were sensitive to Enrofloxacin and Ciprofloxacin. Two isolates showed sensitivity to Doxycycline and Chloramphenicol. Only one isolate was found sensitive to Nalidixic acid. All the isolates were resistant to Doxycycline, Erythromycin, Neomycin, Ampicillin, Penicillin, Cephalexin and Poymixin B.

References