ISOLATION, IDENTIFICATION AND ANTIBIOMGRAM OF PASTEURELLA MULTOCIDA ISOLATES OF AVIAN ORIGIN

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ABSTRACT

Isolation of Pasteurella multocida was attempted from the heart blood, spleen, liver and lung collected from turkeys (4 No,s) and chicken (4 No,s) suspected to have died of avian pasteurellosis. A total of eight 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 isolates showed sensitivity to a wide range of antibiotics namely Enrofloxacin (100.00 per cent), Ciprofloxacin (100.00 per cent), Ofloxacin (100.00 per cent), Gentamicin (100.00 per cent), Amikacin (100.00 per cent), Ampicillin (100.00 per cent) and Penicillin (100.00 per cent). Both turkey isolates and chicken isolates showed sensitivity to Oxytetracycline (50.00 per cent) and Doxycycline (50.00 per cent) whereas all the turkeys and chicken isolates showed resistance to Chloramphenicol, Poyixin B, Trimethoprim, Erythromycin and Triple sulpha. Hence this study recommended to perform in vitro antibiotic sensitivity test prior to treatment in cases of fowl cholera. If not feasible, Fluoroquinolones, Gentamicin, Amikacin, Ampicillin and Penicillin must be given preferential consideration over other antibiotics particularly Chloramphenicol, Poyixin B, Trimethoprim, Erythromycin and Triple sulpha to contain the outbreak of Fowl cholera.

Key words: Isolation - Identification - Antiibiogram - Pasteurella multocida

INTRODUCTION

Fowl cholera is a contagious and economically important disease of poultry particularly chicken, turkeys, ducks and geese (Rhodes and Rimler, 1989; Rimler and Glisson, 1997). It is caused by gram negative bacteria, Pasteurella multocida which inhabit the upper respiratory tract of the many avian species as commensals. It has been reported 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). It usually occurs as primary disease that requires predisposing factors, but these factors may increase the severity of the disease. It may occur in different forms including peracute, acute and chronic infections (Rimler and Glisson, 1997). However, the disease usually occurs as septicaemia with high morbidity and mortality rates or as a chronic localized infection of joints and sinuses (Rimler and Glisson, 1997). The infected birds remain carriers upto 9 weeks after infection.
(Christiansen et al., 1992). It can affect birds of any age, but it rarely occurs in commercial poultry of less than 8 weeks of age (Rimler et al. 1998). Antimicrobial therapy has been used extensively in the treatment of fowl cholera with varying success depending mainly on the kind of drug used (Rimler and Glisson, 1997). Conventional approaches to combat fowl cholera in poultry by injudicious and indiscriminate use of antibiotics resulted in the emergence of multidrug resistant strains of P. multocida among Indian Poultry. The present communication deals with isolation, identification and antimicrobial sensitivity against P. multocida isolated from commercial chickens and turkeys.

**MATERIALS AND METHODS**

**Specimen**

Heart Blood and Tissue pieces from spleen, liver and lung were collected from turkeys (4 No's) and chicken (4 No's) suspected to have died of avian cholera in and around Chennai and Vellore during 2010. Heart blood smears, tissue impression smears from liver, spleen and lung were prepared and subjected to Leishman's staining. Heart blood and tissues were subjected to bacterial isolation and identification.

**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 oC with 5 % Co2 for 2 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 (Quinn et al., 1994).

**Pathogenicity studies**

The pure cultures of the P. multocida isolates in the present study were subjected to pathogenicity studies. One hundred microlitre of broth culture were inoculated intraperitoneally in swiss albino mice and observed for 48 h. The dead ones were subjected to post mortem examination and reisolation of P. multocida. Heart blood was aspirated from the dead mice and streaked directly onto blood agar and MacConkey agar and incubated at 37 oC with 5% CO2 for 24 h. 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. (1998) to amplify KMT1gene. The thermal cycle protocol was followed as per the method of Townsend et al. (1998). 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) and appropriate controls were incorporated to rule out false positive and false negative results. The gel was viewed under UV transillumination.

**Antibiotic sensitivity tests**

The In vitro antibiotic sensitivity test of the organisms was conducted on Mueller-Hinton agar as per the method of Bauer, et al. (1966) using 15 antibiotic discs (Ciprofloxacin - 5mcg/disc, Enrofloxacin - 10 mcg/disc, Ofloxacin - 5 mcg/disc, Oxytetracycline - 30 mcg/disc, Doxycycline - 10 mcg/disc, Trimethoprim, Ampicillin - 10 mcg/disc, Penicillin G - 10 units/disc, Chloramphenicol - 50 mcg/disc, Gentamicin - 10 mcg/disc, Amikacin - 30 mcg/disc, Erythromycin - 10 mcg/disc, Triple sulphax - 30 mcg/disc and Polymixin B - 300 mcg/disc ) supplied by M/s. Hi-Media Laboratory, Mumbai and the antibiotic sensitivity plates were incubated aerobically at 370 C for 24-48h.
RESULTS AND DISCUSSION

Bacterial isolation and identification

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 turkeys (4 No’s) and chicken (4 No’s) were subjected to bacterial isolation. P. multocida could be isolated from all the samples. All the eight isolates (Turkey-4, Chicken-4) 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 OIE (2004) and Purushothaman et al., (2008). The isolates subjected to biochemical tests were positive for indole production, nitrate reduction, oxidase and catalase production and coincide with earlier findings (Kawamota, 1990; OIE, 2004 and Quinn et al., 1994).

Pathogenicity studies

All the eight isolates subjected to mice inoculation tests killed the mice in 24 - 48h. These results are in agreement with the findings of Jaya Kumar (1998) and Balakrishnan and Mini (2001).

PM PCR

PCR has been proved to be useful in the detection of DNA of P. multocida (Townsend et al., 1998 and Purushothaman et al., 2008). 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 (Boom et al., 1997) 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. (1998) 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. (1998).

Antibiotic sensitivity tests

Antibiotic sensitivity tests revealed that all the isolates showed sensitivity to a wide range of antibiotics namely Enrofloxacin (100.00 per cent), Ciprofloxacin (100.00 per cent), Ofloxacin (100.00 per cent), Gentamicin (100.00 per cent), Amikacin (100.00 per cent), Ampicillin (100.00 per cent) and Penicillin (100.00 per cent). Both turkey isolates and chicken isolates showed sensitivity to Oxytetracycline (50.00 per cent) and Doxycycline (50.00 per cent) whereas all the turkey and chicken isolates showed resistance to Chloramphenicol, Poymixin B, Trimethoprim, Erythromycin and Triple sulpha. It is suggested that antibiotics should be used preferably after performing antibiotic sensitivity test in cases of fowl cholera. If not feasible, Fluoroquinolones, Gentamicin, Amikacin, Ampicillin and Penicillin must be given preferential consideration over other antibiotics particularly Chloramphenicol, Poymixin B, Trimethoprim, Erythromycin and Triple sulpha to contain the outbreak of Fowl cholera.

REFERENCES


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