

Isolation, Characterization and Antibigram Study of *Pasteurella multocida* Isolated from Ducks of Kishoreganj District, Bangladesh

M Kamruzzaman¹, M Islam^{2*}, MM Hossain³, MK Hassan², MHB Kabir², MS Sabrin² and MSR Khan⁴

¹Department of Livestock Services, Bangladesh

²Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

³Department of Anatomy, Histology & Physiology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

⁴Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh

*Correspondence: mislam.mipa@sau.edu.bd

ABSTRACT

A study was carried out to isolate and characterize *Pasteurella multocida* isolates from ducks with emphasis on antibiotic sensitivity and resistant pattern. A total of 100 fecal samples were aseptically collected from sick ducks during suspected duck cholera outbreak in Kishoreganj district, Bangladesh during the period from January to December, 2009. The processed samples were cultured on blood agar media for isolation of bacteria. Pure cultures were obtained after subsequent cultures. *P. multocida* isolates were identified according to their morphology, staining, motility, cultural and biochemical properties. The isolated bacteria were then subjected to antibiotic sensitivity test. The prevalence of isolated *P. multocida* from ducks was 34%. From cultural studies it was found that *P. multocida* showed small, glistening, mucoid and dew drop like colony on blood agar plate. They did not grow on MacConkey's agar and found to be non-haemolytic on blood agar plates. The isolates were found Gram negative with coccobacillary appearance, bipolar organisms and non-motile. All the isolates were found positive to catalase, oxidase, indole and nitrate reduction tests and negative to methyl red, Voges-Proskaur, citrate utilization and H₂S production tests. The isolates fermented dextrose, sucrose and manitol without production of gas but did not ferment lactose and maltose. Isolated *P. multocida* isolates were sensitive to ciprofloxacin (95%) and azithromycin (80%), intermediate sensitive to gentamycin (85%), tetracycline (75%), amoxicillin (75%) and erythromycin (70%), but resistant to penicillin G (100%).

Key words: *Pasteurella multocida*, isolation, characterization, antibiotic sensitivity, ducks

INTRODUCTION

Among poultry, ducks are considered as a second widespread species following the chickens in Bangladesh and are being considered as an asset to the poor farmers. According to Ramachandran and Ramakrisnan (1982) duck farming is still in the hands of socially and economically handicraft section of people. Duck cholera is a fatal, contagious and septicemic disease of ducks caused by *P. multocida*—a bacterium with a broad host range and ubiquitous distribution (Ramachandranpillai et al., 2012). It also causes severe economic losses not only in terms of mortality but also in terms of low productivity of egg and meat (Baki et al., 1991).

The causal agent, *P. multocida* is a Gram negative, non-motile, non-spore forming rod shaped organism occurs in single or in pairs or occasionally as chain forms or filament. A capsule can be demonstrated using indirect method of staining of freshly cultured bacteria by Hiss's stain (Calnek et al., 1997). The organisms show bipolar character in Leishman's stain (Buxton and Fraser, 1977). The bacterium grows well on blood agar, nutrient agar and nutrient broth media. However, their growth is enhanced in nutrient broth with the addition of yeast extract and beef extract. *P. multocida* produce whitish, opaque, circular and translucent colonies with no hemolysis on blood agar media (Buxton and Fraser, 1977).

Kishoreganj district is one of the most important duck rearing area of Bangladesh. The duck cholera is considered a major constraint and economically important diseases of ducks of different age and breeds in Bangladesh especially in Kishoreganj district. Antimicrobial therapy especially choice of effective antibiotics is still an effective tool for treatment of duck cholera. Though, *P. multocida* isolates are susceptible to most of the widely used commercial antimicrobial agents but their excessive and unjustified use accelerates the emergence of resistant strains. Proper isolation and identification of the disease is prerequisite for adapting successful control measure of the disease. Moreover the antibiotic resistance pattern increases the incidence of disease and subsequently affects the economy of Bangladesh. Previously very few studies were conducted in Bangladesh regarding antibiotic sensitivity of *P. multocida* of duck origin. Therefore, the present study was undertaken to isolate, characterize and to find out antibiotic sensitivity pattern of *P. multocida* from duck in Kishoreganj district of Bangladesh.

MATERIALS AND METHODS

Sample collection

A total of 100 fecal samples were aseptically collected from sick ducks with clinical signs of duck cholera (showing high morbidity and mortality, depression, anorexia, mucoïd discharge from the mouth, ruffled feathers, yellow to greenish watery diarrhea, increased respiratory rate, etc.) during suspected duck cholera outbreaks in Kishoreganj district, Bangladesh during the period from January to December, 2009. Freshly defecated fecal samples were collected using sterile cotton swabs in a view to prevent extraneous contamination. The collected samples will then put into sterile test tubes containing nutrient broth. Immediately after collection the samples were transferred to the Laboratory of Microbiology & Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh by using ice box. The samples were then preserved in refrigerator before cultivation.

Isolation and Characterization of *P. multocida*

The collected samples were processed as per the procedure of Cheesbrough (2006). For isolation and identification of *P. multocida* organisms, the procedures suggested by Cowan

(1985) were followed throughout the experiment. Briefly, the processed samples were inoculated into nutrient broth followed by inoculation onto blood agar media from the broth culture. The inoculating media was incubated at 37⁰C in bacteriological incubator for characteristic colony formation. Subsequent subculture was done for getting pure culture. Stock cultures were maintained in both Agar slant and 20% sterile buffered glycerin (Merchant and Packer, 1967).

The isolated organisms were identified based on colonial morphology by Gram's staining technique, Leishman's staining technique and motility test (Kumar et al., 2004). For biochemical characterization Carbohydrate fermentation tests, catalase test, Methyl Red test, Voges Proskauer test, Indole test, oxidase test, nitrate reduction test, citrate utilization test and H₂S production test were performed according to standard laboratory procedures (Bhattacharya, 2005).

Antibiotic sensitivity tests

Antibiotic sensitivity tests were performed using disc diffusion test of the method described by Kirby-Bauer (Bauer et al., 1966). Commercially available antimicrobial discs (Becton, Dickinson and Company, USA) were used for the determination of the drug sensitivity. The concentration of antimicrobial agent per disc was: Azithromycin (15 µg), Tetracyclin (30 µg), Penicillin G (10 µg), Amoxycillin (10 µg), Ciprofloxacin (5 µg), Ampicillin (10 µg), Erythromycin (15 µg) and Gentamycin (10 µg). For this purpose, at first broth cultures were prepared from stock cultures using nutrient broth. Then, 0.1-0.2 ml of freshly growing broth cultures were poured on Blood agar plate and spread uniformly. Antibiotic discs were placed apart onto the surface of the inoculated plates aseptically with the help of a sterile forceps and incubated at 37°C for 24 hours. After incubation, the plates were examined and the diameters of the zone of inhibition were measured and were interpreted with the standard diameters of NCCLS, (1999) and recorded as Sensitive (S), Intermediate sensitive (I) and Resistant (R).

RESULTS AND DISCUSSION

Isolation and characterization of P. multocida from suspected ducks

Upon morphological, cultural and biochemical tests a total of 34 isolates (34%) of *P. multocida* were obtained from 100 suspected ducks of Kishoreganj district, Bangladesh. In Gram staining technique *P. multocida* revealed Gram negative coccobacillary appearance and in Leishman's staining technique revealed bipolar organisms. All the isolates were found to be non-motile when examined under the hanging drop preparation. The results of cultivation and biochemical tests of isolated *P. multocida* are presented in Table 1 and Table 2 respectively. These findings were confirmatory with the findings of Heddleston (1976), Das et al. (1991), Kumar et al. (1996), Kumar et al. (2004) and Bhattacharya (2005).

Table 1 Results of cultivation of isolated *P. multocida*

Colony characteristics			
Blood agar media	Nutrient agar media	Eosine- Methylene Blue agar media	Mac Conkey agar media
Whitish, opaque, circular, translucent appearance and no hemolysis	Whitish, opaque, circular, translucent appearance	Small, circular, convex, glistening colonies, no metallic sheen	No colony Appears

Table 2 Results of biochemical tests of isolated *P. multocida*

Name of biochemical tests	Results
Carbohydrate fermentation test	Dextrose Fermentation with production of acid Maltose No fermentation Lactose No fermentation Sucrose Fermentation with production of acid Mannitol Fermentation with production of acid Control No fermentation
Methyl Red test	Negative
Voges Proskauer test	Negative
Indole test	Positive
Catalase test	Positive
Oxidase test	Positive
Nitrate reduction test	Positive
Citrate utilization test	Negative
H ₂ S production test	Negative

Antibiotic sensitivity test of isolated *P. multocida*

Antibiotic sensitivity testing of bacteria has both laboratory and clinical significance. In the present study a total of 20 isolated *P. multocida* isolates were randomly selected among 34 isolates and performed antibiotic sensitivity tests against commonly used antibacterial agents of different groups. The results of antibiotic sensitivity test are represented in Table 3.

Table 3 Results of antibiotic sensitivity tests of the isolated *P. multocida* from samples of suspected sick and dead ducks

Name of antibiotics	Number of isolates	Number of positive (percentage)		
		Sensitive	Intermediate Sensitive	Resistant
Azithromycin	20	16 (80%)	4 (20%)	0 (0%)
Tetracyclin		3 (15%)	15 (75%)	2 (10%)
Penicillin G		0 (0%)	0 (0%)	20 (100%)
Amoxycillin		0 (0%)	15 (75%)	5 (25%)
Ciprofloxacin		19 (95%)	1 (5%)	0 (0%)
Ampicillin		0 (0%)	9 (45%)	11 (55%)
Erythromycin		6 (30%)	14 (70%)	0 (0%)
Gentamycin		0 (0%)	17 (85%)	3 (15%)

%= Percentage

In present study 95% sensitivity was recorded to ciprofloxacin, 80% sensitivity to azithromycin, 30% sensitivity to erythromycin and 15% sensitivity to tetracycline. On the other hand 85% isolates were shown intermediate sensitivity to gentamycin, 75% to tetracycline, 75% to amoxicillin, 70% to erythromycin, 45% to ampicillin, 20% to azithromycin and 5% to ciprofloxacin, whereas 100% isolates were recorded as resistant to penicillin G, 55% resistant to ampicillin, 25% resistant to amoxicillin, 15% resistant to gentamycin and 10% resistant to tetracycline. This study was supported by Rahman et al. (2004) who showed ciprofloxacin (100%) was found highly sensitive against *P. multocida*, followed by moderately sensitive to gentamycin (100%), ampicillin (60%),

cephradine (60%), penicillin G (60%), less sensitive to erythromycin (40%) and highly resistant to tetracycline (100%). Prabhakar et al. (2012) recorded *P. multocida* isolates revealed 100% sensitivity to Ciprofloxacin, 93% sensitivity to Enrofloxacin, 90% sensitivity to Gentamycin and this results correlate with our findings. Similar findings were also reported by Sarangi and Panda (2011) who reported that *P. multocida* isolated were sensitive to enrofloxacin (100%), gentamycin (85.7%), levofloxacin (85.7%), gatifloxacin (85.7%), chloramphenicol (71.4%) and resistant to penicillin G(85.7%), streptomycin (85.7%), sulfadiazine (85.7%), cephalexin (71.4%), cephotaxim (71.4%) and ampicillin (71.4%). On the other hand, Maity et al. (2012) reported that *P. multocida* were sensitive to amoxiclav, chloramphenicol, gentamicin and moderately sensitive to amikacin, cefotaxime, neomycin and norfloxacin and resistant to ciprofloxacin and lomefloxacin. The variation in the sensitivity grade among various studies may be due to over or limited previous exposure and/or indiscriminate use of antibiotics as feed additives and/or preventive or curative agents.

In conclusion, the isolated *P. multocida* isolates characterized by staining, morphological, and biochemical tests. The study comes out with antibiotic sensitivity pattern of *P. multocida* isolates from ducks. The antimicrobial sensitivity test revealed that isolated *P. multocida* were sensitive to ciprofloxacin and azithromycin, intermediate sensitive to gentamycin, tetracycline, amoxicillin and erythromycin, but resistant to penicillin G. This research data provide accurate information to the researchers and also help the veterinarian to choice their suitable antibiotics for successful treatment of duck cholera.

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