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Surveillance of mycoplasmosis in cased layer birds in the coastal region of Bangladesh

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ABSTRACT

The study was conducted for surveillance of mycoplasmosis in cased layer birds in the coastal region Patuakhali, Barguna and Vola districts of Bangladesh. The study was performed using cultural, morphological, biochemical, serological, agglutination test, pathogenicity and antibiotic sensitivity tests. A total of 75 tracheal swab samples were collected (25 from each breed group). Of the 75 samples, 45 were found positive for *Mycoplasma gallicepticum* isolation in PPLO ager, Heart infusion peptone broth and tested by serum agglutination plate. The rates of isolation of *Mycoplasma gallicepticum* were 27%, 23%, 22% from layer, sonali layer, and indigenous layer respectively. Over-all survillence of *Mycoplasma gallicepticum* was 72% (45 out of 75 samples). Area basis prevalence was found 64%, 71% and 65% at Patuakhali, Barguna and Vola respectively. In case of Toxin profile bacterial products (Bacteria, Toxin and Bacteria+ Toxin) of multidrug sensitive and resistant *Mycoplasma gallicepticum* isolates of three breeds of three different areas were inoculated orally to a total of 120 day-old layer chicks. No mortality found indicating that the isolates were non-pathogenic. The antibiogram profile revealed that, Gentamycin (89.55%) and Kanamycin (86.57%) were sensitive, Cephalexin was intermediately sensitive, Ciprofloxacin was less sensitive. Amoxicillin and Tetracycline were completely resistant, whereas Cephradin (79.10%) and streptomycin (80.60%) were more or less resistant. Considering all the matters it can be concluded non-pathogenic, multidrug resistant *Mycoplasma gallicepticum* is prevailing in apparently healthy layer birds, which may become a big threat for poultry industry and as well as for human health.

INTRODUCTION

The poultry industry plays a crucial role in economic growth and simultaneously, creates numerous employment opportunities (Shamsuddoha and Sohel, 2003). Layer and broiler birds are being reared in modern farming system. Beside the hybrid, indigenous breeds are also being reared as back yard poultry in villager's house. Recently Sonali (Rhode Island Red × Fayomi) breed becoming popular due to their adaptability and acceptability under the climatic conditions of Bangladesh (Anisuzzaman and Wahid, 1988).

Over the years commercial poultry farming has been developed introduce some high yielding strains of chicken are reared in intensive system. There are many types of microorganisms which reside as commensal, in layer, broiler, sonali and indigenous poultry. *Mycoplasma gallicepticum* are one of them. It may be found both in pathogenic and non-pathogenic forms, causing major losses of commercially produced poultry as a major pathogen of world-wide importance. The disease was first described in 1905. It was described as a respiratory disease that was found in domestic poultry. However, it wasn't for another 50 years that the causative agent, *Mycoplasma gallisepticum*, was cultivated.

The treatment of *Mycoplasma gallicepticum* infection in poultry is mediated by different kinds of antimicrobial agents, such as Ciprofloxacin, Erythromycin or Kanamycin etc. These antimicrobial agents are being used as an important therapeutic tool in poultry production. However, isolates of *Mycoplasma gallicepticum* from poultry are possessing resistance frequently to one or more of these antimicrobial agents (Jakaria, 2011). This resistance possesses two fold problems. Firstly the poultry industry has few antimicrobial agents to which *Mycoplasma gallicepticum* has not already resistant. Secondly, the public health community is concerned that humans eating poultry meat from flocks treated with antimicrobial agents may lead to acquire poultry bacteria resistant against their normal flora (Charles et al., 2001).

In Bangladesh antibiotics are randomly being used for treatment purpose. There is clear evidence in abuse of antibiotics, for which emergence of multi-drug resistant *Mycoplasma gallicepticum* are continuously increasing (Hussain et al., 1982). Most of the people are ignorant with the schedule of antibiotic course. As a result, the bacteria are possessing resistance to different antibiotics. Subsequently, more powerful and or new antibiotics are being used to defeat the altered bacterial population. In this way many bacteria are getting resistance to various antibiotics.

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Microbial characteristics associated with virulent avian *Mycoplasma gallicepticum* include production of respiratory distress such as coughing, sneezing, slight to marked rales, and difficulty breathing. Swollen eyelids, ocular discharge, and loss of sight are signs and symptoms that are very important for this disease as well, verotoxin, colicins, and siderophores, type 1 pili and motility, resistance to the lytic action of host complement and antibiotics (Dho and Lafont, 1984; Chulasiri and Suthienkul, 1989; Wooley et al., 1992).. Strains that cause infections are designated respiratory distress *Mycoplasma gallicepticum* a group that includes emergent pathogens with public health relevance worldwide (Nataro and Kaper, 1998). Five categories of *Mycoplasma gallicepticum* have been well associated with respiratory distress in several epidemiological studies (Nataro and Kaper, 1998).

In Bangladesh, Isolation and characterization of *Mycoplasma gallicepticum* performed from blood, catarrhal exudates in the nasal passages, infraorbital sinuses, trachea, and bronchi, Caseous exudates in the air sacs, fibrinous or fibrinopurulent pericarditis, perihepatitis and congestion in lungs etc (Choudhury et al., 1967; Nazir, et al., 2005 and Hasina, 2006). The study on cased layer birds *Mycoplasma gallicepticum* generally has been performed at PSTU campus and its surroundings coastal areas. There is no study on healthy layer, indigenous layer and sonali layer poultry related with *Mycoplasma gallicepticum*. The present study was undertaken to determine the surveillance of mycoplasmosis in cased layer at costal area like Patuakhali, Barguna and Vola districts.

Keeping in mind all the above facts, the present study was undertaken to diagnoseavian Mycolpasmosis in cased layer birds in the coastal region of Bangladesh. The antibiogram profile of *Mycoplasma gallicepticum* isolates of layer birds against different antimicrobial agents was also determined.

MATERIALS AND METHODS

Sample collection

Nasal and Tracheal swab sample were collected from apparently healthy layer from different layer farm and back yard poultry of Patuakhali and Vola districts of Bangladesh. Total number of samples was 75. Of them 25 samples were collected from each group (layer, sonali and indigenous poultry) (Table 1). The samples were transferred immediately with maintain cool chain to the laboratory of the Department of Microbiology and Hygiene, Patuakhali Science and Technology University (PSTU), Barisal. All samples were collected with the help of sterile cotton buds and transferring the cotton buds immediately to sterile nutrient broth. At each time of collection, precaution was taken to prevent or minimize cross-contamination of samples. The samples were subjected to cultural, morphological, biochemical, Serological, agglutination test, pathogenicity and antibiotic sensitivity tests.

Table 1: Number of cloacal swab samples collected from different areas

Sample area	Total no. of samples examined	Type of samples		
		Cased Layer	Sonali	Indigenous
Patuakhali	40	15	15	-
Barguna	10	10		10
Vola	25		10	15
Total	75	25	25	25

Detection of mycoplasmosis

Mycoplasmosis was diagnosed made on the basis of clinical signs of infected chickens and postmortem changes in dead chickens. The birds were examined systematically and the postmortem changes were recorded during necropsy.

For bacteriological analysis primary growth of all kinds of bacteria was performed in nutrient broth. The sample is given in the nutrient broth and incubated for overnight at 37^{o}C. Microscopic examination at 40-60X of inverted plates reveals the colony morphology of mycoplasmas. Organisms are recognized by typical tiny "fried egg" colonies or finely granular ("ground glass") colonies with a berry-like appearance that penetrate the agar surface. Colonies were ranged from 20-300µm.

Identification of the isolates

Physical test

Morphological characteristics of colony (shape, size, surface texture, edge, elevation, colour, opacity etc.) developed after 7 days of incubation were carefully studied and recorded. The motility test was performed according to the method described by Cowan, (1985) to differentiate motile bacteria from the non-motile one.

Biochemical studies

Isolation and identification of different organisms were performed by culturing, staining, different biochemical (sugar tests, indole test, methyl red (MR) test, Voge's-Proskauer (VP) test.

Serological analysis

In live birds, blood samples (2 mL) were collected from wing vein by using fresh disposable plastic syringe (5 mL) and collected blood was kept at room temperature for about 1 to 2 hours. A clean straw color serum was seen around the clotted clump and after centrifugation (252 g for 10 minutes) the serum was stored at -20°C until used.

Serum plate agglutination (SPA) test

The SPA test was conducted with crystal violet stained M. gallisepticum commercial antigen (Nobilis® MG) obtained from Intervet Company Ltd. (The Netherlands). Following the manufacturer's instruction, antigen (0.03 mL) and crude or diluted serum (0.03 mL) was placed side by side with pipette in a glass plate and mixed well by stirring with glass rod, followed by rocking. Results were read within 2 minutes. In positive cases granules were formed slowly which could be seen during rocking. In the negative case, no such granules were formed. All SPA results were recorded.

Postmortem changes

Diagnosis was confirmed by post-mortem examination of dead chickens. Lesions comprising catarrhal exudates in the nasal passages, infraorbital sinuses, trachea, and bronchi, Caseous exudates in the air sacs, fibrinous or fibrinopurulent pericarditis, perihepatitis and congestion in lungs were observed.

Toxin profile of *Mycoplasma gallicepticum*

Production of toxin

Culture filtrate was prepared for the production of toxin. For this, *Mycoplasma gallicepticum* cultures were inoculated into nutrient broth and incubated at 37°C for 24 hours. In the next morning, the overnight cultures were centrifuged for 15-20 minutes at 4000 rpm. The supernatants were collected and transferred into new vials. Then, Gentamycin was added to those vials at a concentration of 5 µg per ml and stored at room temperature for overnight. Crude toxin was prepared by 0.22 µm Millipore filters (Satorius Stedium, Germany). Overnight grown culture was allowed to pellet down by centrifugation at 4000 rpm for 30 minutes and washed with the Tryptose Soy Broth (TSB) then the pellet was re- suspended and diluted with TSB. For detection of purity of toxin, free from *Mycoplasma gallicepticum*, the supernatants were streaked on PPLO selective media with a red hot iron loop and incubated at 37°C for 2-3 wks. After incubation if no colony was observed the supernatant was used for detection of heat-stable (ST) toxin by oral inoculation to Day-old layer chicks (Table 2).

Determination of toxigenic effect on day-old layer chicks

Each bacterial isolates was divided into three categories which are bacteria, bacteria + toxin and toxin only. A total of 125 layer chicks were used. Day-old chicks were divided into 25 experimental groups; each group consisted of 5 chicks. The product was inoculated orally by using micropipette (Table 2). They were observed for 10 days.

Table 2: Oral inoculation of *Mycoplasma gallicepticum* and their products

Bacterial products	*Mycoplasma gallicepticum*						Control
	Layer		Sonali		Indigenous		
	S	R	S	R	S	R	
Bacteria	5	5	5	5	5	5	
Bacteria +Toxin	5	5	5	5	5	5	5
Toxin	5	5	5	5	5	5	

Legends: S= Multidrug sensitive, R= Multidrug resistant

Antibiotic sensitivity test

In vitro antibiotic sensitivity test was done according to the guidelines of Clinical and Laboratory Standard Institute (CLSI), 2007, formerly it was known as NCCLS using disc diffusion test.

Inoculum preparation

To standardize the inoculums density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent was used. The turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standards. This results in a suspension containing approximately 1-2 $x10^8$ CFU/ ml.

Inoculation of test plates

Optimally, within 15 minutes after adjusting the turbidity of the inoculums suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. The lid was kept for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

Application of discs to inoculated agar plates

The predetermined battery of antimicrobial discs was dispensed onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete contact with the agar surface. Whether the discs are placed down individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from centre to centre. The plates was inverted and placed in an incubator set to 35^{o}C within 15 minutes after the disc are applied. After 16 to18 hours of incubation each plate was examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. The diameters of the inhibition zones were measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate (Table 3). The results were recorded at 16-18 hours post incubation. Transmitted light was used to examine the zone of inhibition.

Table 3: Antimicrobial agents, their disc concentrations and interpretation standard (Clinical and Laboratory Standard Institute (CLSI), 2007)

SL No	Antimicrobial Agents	Disc concentration (µg/disc)	Interpretation of results (Zone diameter in mm)		
			S	I	R
1	Amoxicillin	30 µg	≥ 18	14-17	≤14
2	Cephradin	25 µg	≥16	13-15	≤12
3	Cephalexin	30 µg	≥17	12-15	≤11
4	Ciprofloxacin	5 µg	≥21	16-20	≤15
5	Gentamycin	10 µg	≥15	13-14	≤12
6	Kanamycin	30 µg	≥18	14-17	≤13
7	Streptomycin	10 µg	≥15	12-14	≤11
8	Tetracycline	30 µg	≥15	12-14	≤11

SL= Serial; No.= Number; µg= Microgram; mm= Millimeter; S= Sensitive; I= Intermediately sensitive; R=Resistant.

RESULTS

Isolation and identification of *Mycoplasma gallicepticum*

A total of 75 Tracheal swab samples were cultured on Nutrient broth and produced turbidity (Figure 1A). The organisms produced circular, smooth, colorless colony on NA (Figure 1B). Among them 45 were isolated by producing Red -yellow colonies with red on PPLO agar which is very much characteristic to *Mycoplasma gallicepticum* (Figure 1C). They also produced bright pink or red colonies on selective agar (Figure 1D). The organisms produced hemolysis on BA with discoloration of the media around the growth of the organisms (Figure 1E). On S-S agar suspected isolates produced pinkish colony after 24 hours of incubation (Figure 1F). In Giemsa staining the organism revealed Gram-negative, pink colour, small rod shaped and they were as single or paired under microscope (Figure 2). The strains of suspected *Mycoplasma gallicepticum* isolates were found to be mobile in hanging drop slide preparation. All the isolates

fermented the five basic sugars producing acid and gas (Figure 3). Acid production was indicated by the color change from reddish to yellow and the gas production was noted by the appearance of gas bubbles in the inverted Durham's tubes (Figure 3). All the isolates were MR Catalase, Oxidase and Indole tests positive but VP negative (Figure 4).

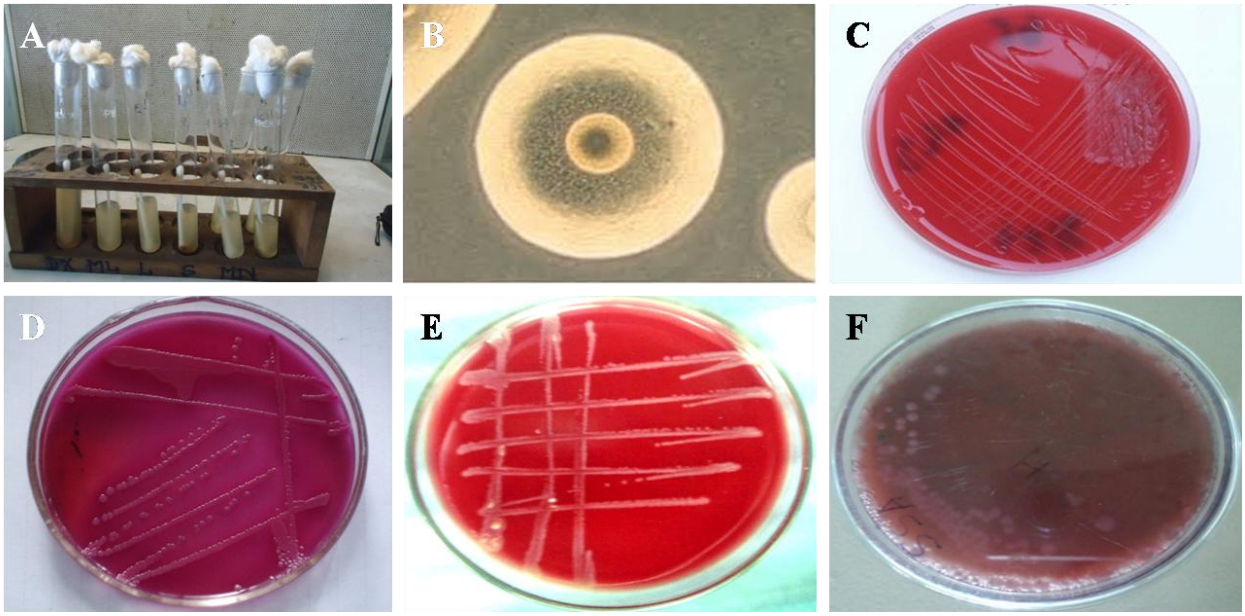


Figure 1: A. Growth of *Mycoplasma gallicepticum* on Nutrient broth; B. Colony showing smooth, circular, yellow colony on Nutrient agar; C. Colony showing pink PPLO agar; D. Growth of isolated *Mycoplasma gallicepticum* in heart infusion peptone agar showing pink colour colonies; E. Colorless colony with hemolysis on Blood agar; F. Pinkish, circular small colonies of *Mycoplasma gallicepticum* on Selective agar.

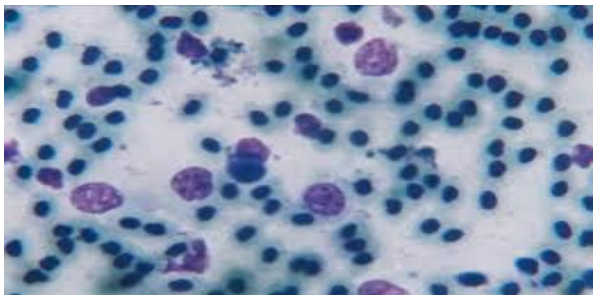
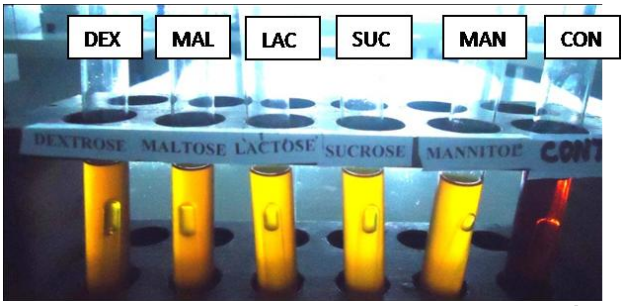
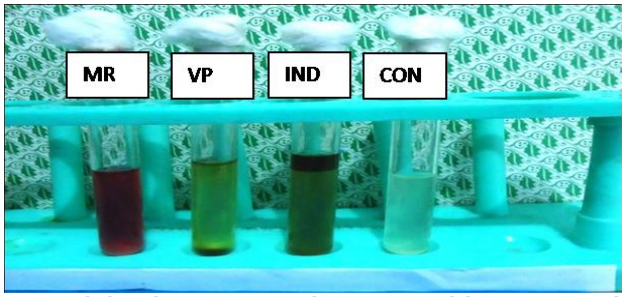


Figure 2: Giemsa staining of *Mycoplasma gallicepticum* isolates showing gram posative, pink coloured, small cocci-shaped, single or paired organisms (X1000)



DEX= Dextrose, SUC= Sucrose, LAC= Lactose, MAL= Maltose, MAN= Mannitol,

Figure 3: Carbohydrate fermentation test of *Mycoplasma gallicepticum*



MR= Methyle-red, VP= Voges-Proskauer, IND= Indole, CON= Control

Figure 4: MR positive, VP negative,Indole positive of *Mycoplasma gallicepticum*

Out of 75 samples, 45 samples were found to be positive for *Mycoplasma gallicepticum* isolates. The prevalence of *Mycoplasma gallicepticum* in the tracheal sample was 72% (Table 3).

Prevalence of *Mycoplasma gallicepticum*

The prevalence of *Mycoplasma gallicepticum* varies based on breed and localities. Highest prevalence was observed in layer birds (27%) followed by sonali (23%) and indigenous (22%). The prevalence *Mycoplasma gallicepticum* was higher in Borguna (71%) than Patuakhali (65%) and Vola (64%) (Table 4).

Table 4: Prevalence of *Mycoplasma gallicepticum* based on breed and locality of birds

		Total samples examined	Samples positive	Prevalence (%)
Breeds of birds	Cased Layer	25	20	27%
	Sonali layer	25	17	23%
	Indigenous layer	25	16	22%
	Total	75	53	72%
Locality	Patuakhali,	40	26	65%
	Barguna	35	25	71%
	Vola	25	16	64%
	Total	100	67	67%

Table 5: Area and breed basis prevalence of *Mycoplasma gallicepticum*

Area	Cased Layer		Sonali layer		Indigenous layer	
	Tested	Positive	Tested	positive	Tested	positive
Patuakhali	15	10 (67%)	15	9 (60%)	10	7(70%)
Barguna	10	10 (100%)	15	7(46.67%)	10	8 (80%)
Vola			10	8 (80%)	15	8 (53%)

Table 6: Colony morphology and number/ml of other organisms found in Total Viable Count

Name of organism	Colony on PCA	Morphology	No/ml
E. coli,	Smooth, circular, white to grayish white colony.	Gram negative, pink colored, small rod shaped organisms arranged in single, pairs or short chain.	$55X10^8$/ml
Salmonella spp	Circular, smooth, white to grayish or white colony	Gram- negative, pink color, small rod shaped organisms arranged in single or paired.	$32X10^8$/ml
Staphylococcus spp	Gray, white or yellowish colony	Gram positive, cocci arranged in grapes like clusters.	$47X10^8$/ml
Bacillus spp	Thick, grayish-white or cream colored colonies were produced.	Gram-positive, large rod shaped organisms arranged in chain.	$5X\ 10^8$/ml

Table 7: Oral inoculation of *Mycoplasma gallicepticum* in tracheal sample isolates and their products in Day-old- layer chicks

Products of *E. coli*	Multidrug sensitive isolates of *Mycoplasma*			Multidrug resistant isolates of *Mycoplasma*		
	No. of inoculation	Clinical signs	Mortality	No. of inoculation	Clinical signs	Mortality
Bacteria	20	0(0%)	0(0%)	20	0(0%)	0(0%)
Toxin	20	0 (0%)	0(0%)	20	2(5%)	0(0%)
Bacteria +Toxin	20	2 (10%)	0(0%)	20	8(20%)	0(0%)
Total	60	2(3.33%)	0(0%)	60	9(15%)	0(0%)

Toxin profile in day-old- layer chicks

Toxin profile was done by inoculating different concentration of bacterial products in day old broiler chicks. The concentration was determined by total viable count (Table 6). Only some clinical signs with diarrhea, loss of appetite and drowsiness were observed. No mortality found within they seven days of observation. The chicks recovered normally (Table 7).

Antibiotic sensitivity tests

The antibiogram study was performed with the *Mycoplasma gallicepticum* isolates to study their sensitivity and resistance pattern against the commonly used antibiotic discs (Figure 5, Table 8 - 11).

Table 8: Antibiotic sensitivity and resistance pattern of *Mycoplasma gallicepticum* isolates from layer birds

Sampling Area	Isolates	Sensitive	Intermediate	Resistant
Patuakhali	B-1	GEN, K, CN		Cip, S, CH, TE,AMX
	B-2	GEN ,K	CH, CN	Cip, S, TE,AMX
	B-3	GEN, K	S, CN	Cip CH, TE,AMX
	B-4	GEN, K, CN		Cip, S, CH, TE.AMX
	B-8	GEN, K	S,CN	Cip, CH, TE,AMX
	B-9	K, GEN	CN	Cip, S, CH, TE,AMX
	B-10	K, GEN,CN		Cip, S, CH, TE,AMX
Barguna	B-1	K, GEN	CN	Cip, S, CH, TE,AMX
	B-2	GEN	K, CN	Cip, S, CH, TE,AMX
	B-5	K GEN	Cip,S	CH,CN, TE,AMX
	B-7	K ,GEN	CN	Cip, S, CH,TE,AMX
	B-9	GEN, K,S	Cip, CN	CH, TE,AMX
	B-12	K, GEN	Cip, CN	S,CH, TE,AMX
	B-15		GEN, CN	Cip, S, K, CH, TE,AMX

Legends; AMX=Amoxicillin, CH= Cephradin,CN = Cephalexin, Cip = Ciproflo xacin, GEN= Gentamycin, K = Kanamycin, S = Streptomycin, TE = Tetracycline.

Table 9: Antibiotic sensitivity and resistance pattern of *Mycoplasma gallicepticum* isolates from layer birds

Sample Area	Isolates	Sensitive	Intermediate	Resistant
Patuakhali	L-1	GEN, K	CN	AMX, Cip, CH, S, TE
	L-2	GEN, K	CN	AMX, Cip, CH, S, TE
	L-3	GEN, K	CN	AMX, Cip, CH, S, TE
	L-5	GEN	K	AMX, Cip, CH, CN, S,TE
	L-6	GEN		AMX, Cip, CH, CN,K,S,TE
	L-8	GEN,K		AMX, Cip, CH, CN, S,TE
	L-9	GEN,K		AMX, Cip, CH, CN, S, TE
	L-12	GEN, K	CN	AMX, Cip, CH, S,TE
	L-14	GEN,K	CN	AMX, Cip, CH, S, TE
	L-15	GEN, K	CH, CN	AMX, Cip, S, TE
Barguna	L-1	GEN,K		AMX, Cip, CH, CN, S,TE
	L-2	GEN, K		AMX, Cip, CH, CN, S,TE
	L-3	GEN, K	CN	AMX, Cip, CH, S,TE
	L-4	GEN,K	CH,	AMX, Cip, CN, S, TE
	L-5	GEN,K		AMX, Cip, CH,CN, S,TE
	L-6	GEN	K	AMX, Cip, CH,CN,S,TE
	L-7	GEN, K		AMX, Cip, CH,CN,S,TE
	L-8	GEN, K	CN	AMX, Cip, CH, S,TE
	L-9	GEN,K	CH, CN	AMX, Cip, S,TE
	L-10	GEN, K	CH,	AMX, Cip, CN, S, TE

Table 10: Antibiotic sensitivity and resistance pattern of *Mycoplasma gallicepticum* isolates of Sonali layer bird

Sampling Area	*Mycoplasma gallicepticum* Isolates	Sensitive	Intermediate	Resistant
Patuakhali	S-1	GEN,K	CN,	AMX, Cip, CH, S, TE
	S-3	Cip, GEN, K,CN	Cip, CH	AMX, S, TE
	S-4	GEN, K, CN	Cip, CH	AMX, Cip, S, TE
	S-5	GEN, K	CN, CH	AMX, Cip,S, TE
	S-7	GEN,Cip, K	CN	AMX, CH, S, TE
	S-8	CN, GEN, K	CH	AMX, Cip,S, TE
	S-11	GEN, K	Cip, CH, CN	AMX, S, TE
	S-13	GEN, K	CN	AMX, Cip, CH, S, TE
	S-14	Gen, K, CN		AMX, Cip, CH, S, TE
Vola	S-2	K	CH, CN	AMX, Cip, GEN,S, , TE
	S-4	K	GEN, CN	AMX, Cip, CH, S, TE
	S-5	CN, GEN, K	CH,	AMX, Cip, S, TE
	S-6		CN,	AMX, Cip, CH, GEN, K, S, TE
	S-7	K	CN	AMX, Cip, CH, GEN, K, S, TE
	S-8			AMX, Cip, CH, CN, GEN, S, TE
	S-9			AMX, Cip, CH, CN, GEN,K,S,TE
	S-10	K	CH, GEN, CN	AMX, Cip, S, TE

Table 11: Antibiotic sensitivity and resistance pattern of *Mycoplasma gallicepticum* isolates of Indigenous chicken

Sample Area	Isolates	Sensitive	Intermediate	Resistant
Vola	I-1	GEN, K	Cip,	CH, CN, S, AMX, TE
	I-2	GEN, K	Cip, CN	CH, S, AMX, TE
	I-4	Cip, GEN, K		CH, CN, S, AMX, TE
	I-7	CN,GEN, K	Cip,	CH,S, AMX, TE
	I-9	Cip, GEN, K	CN	CH,S, AMX, TE
	I-11	CN,GEN, K	Cip,	CH,S, AMX, TE
	I-13	GEN, K	Cip, CN	CH,S, AMX, TE
	I-15	GEN, K	CN	Cip, CH, S, AMX, TE
Barguna	I-2	CN, GEN, K	Cip,	CH,S, AMX, TE
	I-3	GEN, K	Cip, CN,	CH,S, AMX, TE
	I-4	GEN, K	S, CN	CH,Cip, AMX, TE
	I-5	GEN, K	CN	CH, Cip, S, AMX, TE
	I-6	CN,GEN, K	S,	CH, Cip, AMX, TE
	I-7	GEN, K	Cip, S, CN	CH, AMX, TE
	I-8	CN,GEN, K	Cip, S,	CH,AMX, TE
	I-9	CN,GEN, K		CH, Cip, AMX, TE

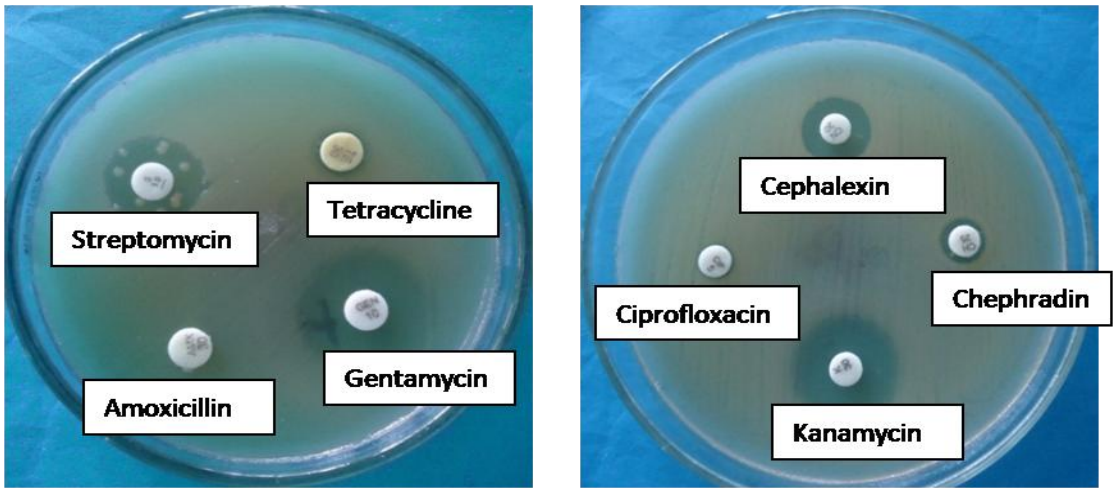


Figure 5: Diameter of zone of inhibition around antibiotic discs

Overall sensitivity pattern of *Mycoplasma gallicepticum*

Among the antibiotics Amoxicillin and Tetracycline were 100% resistant and 0% sensitive and intermediate sensitive. Ciprofloxacin was 4.48% sensitive, 20.90% intermediate and 74% resistant. Cephradin was 0% sensitive, 20.90% intermediate and 79.10% resistant. Cephalexin was 20.90% sensitive, 55.22% intermediate and 23.88% resistant. Gentamycin was 89.55%, 4.48% intermediate to 7.46% resistant. Kanamycin was 86.57% sensitive, 5.97% intermediate and 7.46 % resistant. Streptomycin was 1.49% sensitive, 0% intermediate and 80.60% resistant (Figure 6).

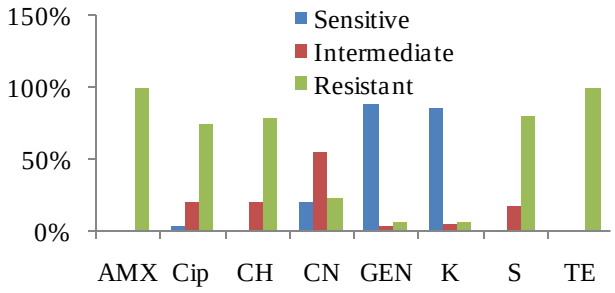


Figure 6: Over all sensitivity and resistance pattern of *Mycoplasma gallicepticum* to different antibiotics

Based on the breeds of chicken

100% of layer and indigenous were sensitive to Gentamycin. On the other hand 100% indigenous were sensitive to Kanamycin and 64.70% sonalilayer were sensitive to Gentamycin. 80% layer and 82.35% indigenous layer were sensitive to Kanamycin 29.42% and 37.5% indigenous layer were sensitive to Cephalexin. 12.5% indigenous layer and 5.88% sonali layer were sensitive to Ciprofloxacin and Streptomycin. No isolates of layer, sonali and indigenous layer were sensitive to Amoxicillin, Cephradin and Tetracycline. No isolates of layer were sensitive to Ciprofloxacin, Cephalexin and streptomycin. No isolates were of sonali and indigenous were sensitive to streptomycin. No isolates of other birds were sensitive to Ciprofloxacin (Table 12).

45% layer, 55.82% sonali and 50% indigenous layer isolates were intermediate sensitive to Cephalexin. 50% indigenous were intermediate to streptomycin. 20% layer, 47.06% sonali and 6.25% indigenous layer were intermediate to Cephradin 11.77% sonali and 56.25% indigenous layers were intermediate to Ciprofloxacin. 5.88% sonali were intermediate to Gentamycin and 15% layer were intermediate to streptomycin. No isolates of other birds, layer, sonali and indigenous were intermediate to Amoxicillin and tetracycline. No isolates of layer were intermediate to Ciprofloxacin, Gentamycin and Streptomycin. No isolates of sonali were intermediate to Kanamycin and Streptomycin. No isolates of indigenous were intermediate to Gentamycin and Kanamycin (Table 12).

100% isolates were resistant to Amoxicillin and Streptomycin. 100% of layer and sonali isolates were resistant to Streptomycin. 100% of layer isolates were resistant to Ciprofloxacin. Most of the layer and indigenous layer isolates (80% and 93.75% respectively) were resistant to Cephradin. In case of Ciprofloxacin 82.35% sonali and isolates were resistant. 50% of indigenous isolates and 64.29% broiler isolates to streptomycin, 52.94% sonali isolates to cephradin, 55% layer isolates to

Cephalexin were resistant 31.25% indigenous to Ciprofloxacin and 29.42% sonali to Gentamycin were resistant 17.65% sonali to Kanamycin, 12.50% indigenous and 11.76% sonali to Cephalexin were resistant. 5% layers were resistant to Kanamycin layer and indigenous layer isolates were resistant to Gentamycin. Whereas 0% indigenous isolates were resistant to Kanamycin (Table 12).

Table 12: Antibiotic sensitivity and resistance pattern of *Mycoplasma gallicepticum* from cased layer, sonali and indigenous layer

Source of *Mycoplasma gallicepticum*	Antibiotic	Sensitive	Intermediate	Resistant
		%	%	%
Cased Layer	AMX	0.00	0.00	100.00
	Cip	0.00	0.00	100.00
	CH	0.00	20.00	80.00
	CN	0.00	45.00	55.00
	GEN	100.00	0.00	0.00
	K	80.00	15.00	5.00
	S	0.00	0.00	100.00
	TE	0.00	0.00	100.00
Sonali	AMX	0.00	0.00	100.00
	Cip	5.88	11.77	82.35
	CH	0.00	47.06	52.94
	CN	29.42	58.82	11.76
	GEN	64.70	5.88	29.42
	K	82.35	0.00	17.65
	S	0.00	0.00	100.00
	TE	0.00	0.00	100.00
Indigenous	AMX	0.00	0.00	100.00
	Cip	12.5	56.25	31.25
	CH	0.00	6.25	93.75
	CN	37.5	50.00	12.5
	GEN	100.00	0.00	0.00
	K	100.00	0.00	0.00
	S	0.00	50.00	50.00
	TE	0.00	0.00	100.00

Based on study areas

*Mycoplasma gallicepticum*isolates of Patuakhali were sensitive 100% to Gentamycin, 88.46%to Kanamycin, 26.92%, to Cephalexin, 3.85% to Ciprofloxacin . In case of Gazipur, 96% to Gentamycin, 88% to Kanamycin and 4% to Cephalexin streptomycin. In case of Vola, 62.5% to Gentamycin, 75% to Kanamycin, 18% to Cephalexin and 12.5% to Ciprofloxacin (Table 13).

Table 13: Region basis antibiotic sensitivity and resistance pattern

Antibiotic	Patuakhali			Barguna			Vola		
	S %	I %	R %	S %	I %	R %	S %	I %	R %
AMX	O	0	100	0	0	100	0	0	100
Cip	3.85	7.70	88.46	0	28	72	12.5	31.25	56.25
CH	0	30.77	69.23	0	16	84	0	18.75	81.25
CN	26.92	57.70	15.38	16	52	32	18.75	56.25	25
GEN	100	0	0	96	4	0	62.5	6.25	31.25
K	88.46	7.70	3.85	88	8	4	75	0	18.75
S	0	7.70	92.30	4	36	60	0	6.25	93.75
TE	0	0	0	0	0	100	0	0	100

Legends: S = Sensitive, I = Intermediate and R = Resistant.

*Mycoplasma gallicepticum*isolates of Patuakhali were intermediate sensitive, 57.70 to Cephalexin, 30.77% to Cephradin and 7.70% to Ciprofloxacin, Streptomycin and Kanamycin. In case of Barguna, 52% to Cephalexin, 36% to Streptomycin, 28% to Ciprofloxacin, 16% to Cephradin, 8% Kanmycin and 4% Gentamycin. In case of Vola, 56.25% to Cephalexin, 31.25% to Ciprofloxacin, 18%.75% to Cephradin and 6.25% to Streptomycin and Gentamycin (Table 13).

Whereas, *Mycoplasma gallicepticum* isolates of Patuakhali were resistant 100% to Amoxicillin and Tetracycline, 92.30% to Streptomycin, 88.46% to Ciprofloxacin, 69.23% to Chephradin and 15.38% Cephalexin. In case of Barguna 1oo% to Amoxicillin and Tetracycline, 84% to Chephradin, 72% Ciprofloxacin, 60% to Streptomycin, 32% to Cephalexin and 4% to Kanamycin. In case of Vola, 100% to Amoxicillin and Tetreacycline, 93.75% to Streptomycin, 81.25% to Chephradin, 31.25% to Gentamycin, 25% to Cephalexin and 18.75% to Kanamycin (Table 13).

Comparative study

Among the *Mycoplasma gallicepticum* isolates from layer of Patuakhali, 100% were sensitive to Gentamycin and Kanamycin. Isolates of Barguna 86% and 72% sensitive to Gentamycin and Kanamycin where as 0% and 14% were resistant respectively and 14% intermediate to both antibiotics. 100% isolates of both areas were resistant to Amoxicillin, Tetracycline and Cephalexin. Ciprofloxacin is 100% resistant for Patuakhali but in case of Barguna 57% resistant and 43% intermediate. Streptomycin is 86% and 57% resistant, 14% and 29% intermediate, 0% and 14% sensitive for Patuakhali and Barguna respectively. Cephalexin is not sensitive for Barguna resistant for Patuakhali. It is 43% sensitive and 57% intermediate for Patuakhali where as 14% resistant and 86% intermediate for Barguna (Table 14).

Table 14: Region and breed basis antibiotic sensitivity and resistance pattern *Mycoplasma gallicepticum* isolates of layer birds

Type of poultry	Name of Antibiotic	Region								
		Patuakhali			Barguna			Vola		
		S %	I %	R %	S %	I %	R %	S %	I %	R %
Cased Layer	AMX	0	0	100	0	0	100			
	Cip	0	0	100	0	0	100			
	CH	0	10	90	0	30	70			
	CN	0	60	40	0	30	70			
	GEN	100	0	0	100	0	0			
	K	70	20	10	90	0	10			
	S	0	0	100	0	0	100			
	TE	0	0	100	0	0	100			
Sonali	AMX	0	0	100				0	0	100
	Cip	11	22	67				0	0	100
	CH	0	56	44				0	37	63
	CN	44	56	0				12	63	25
	GEN	100	0	0				25	12	63
	K	100	0	0				63	0	37
	S	0	0	100				0	0	100
	TE	0	0	100				0	0	100
Indigenous	AMX				0	0	100	0	0	100
	Cip				0	50	50	25	63	12
	CH				0	12	88	0	0	100
	CN				50	50	0	25	50	25
	GEN				100	0	0	100	0	0
	K				100	0	0	100	0	0
	S				0	88	12	0	12	88
	TE				0	0	100	0	0	100

Among the layer, all isolates of patuakhali and Bargunawere sensitive to Gentamycin whether resistant to Amoxicillin, Tetracycline, Ciprofloxacin and Streptomycin. Cephradin and Cephalexin are not sensitive. 90% and 70% isolates are resistant for Patuakhali and Barguna respectively whether 10% and 30% are intermediate to Cephradin. 40% and 60% isolates were resistant for Patuakhali and Barguna whether 60% and 30% are intermediate to Cephalexin. In case of Kanamycin 70% and 90% are sensitive, 20% and 0% are intermediate for Patuakhali and Barguna respectively and 10% are resistant for both (Table 14).

Among the sonali, all isolates of Patuakhali were sensitive to Gentamycin and Kanamycin whether, isolates of Vola are 25% and 63% sensitive, 63% and 37% are intermediate respectively and only 12% were intermediate to gentamycin. 100% are resistant to Amoxicillin, Tetracycline and Streptomycin. All isolates of Vola are resistant to Ciprofloxacin, but in caes of Patuakhali 11% are sensitive, 22% are intermediate and 67% are resistant. 56% and 44% isolates of Patuakhali were intermediate and resistant to Cephradin whether in case of Vola 37% and 63%. In case of Cephalexin, isolates of Patuakhali are 44% are sensitive and 56% were intermediate where as isolates of Vola 12% sensitive, 63% intermediate and 25% resistant (Table 14).

Among the indigenous breeds of chicken, all isolates were sensitive to Gentamycin and Kanamycin and resistant to Amoxicillin and Tetracycline. In case of Ciprofloxacin, isolates of Barguna are 50% are intermediate and 50% are resistant whether, isolates of Vola were 25% sensitive, 63% intermediate and 12% resistant. Isolates of Barguna are 88% intermediate and 12% resistant but isolates of Vola are 12% intermediate and 88% resistant to streptomycin. Cephradin is 100% resistant to the isolates of Vola where as 12% intermediate and 88% resistant to the isolates of Barguna. In case of Cephalexin, isolates of Barguna were 50% intermediate and 50% resistant but isolates of Vola are 25% sensitive, 50% intermediate and 25% sensitive (Table 14).

DISCUSSION

The present study was undertaken for the isolation, identification and molecular characterization of *Mycoplasma gallicepticum* from apparently healthy layer, sonali and indigenous layer of Patuakhali, Barguna and Vola districts of Bangladesh. For the characterization of the isolated *Mycoplasma gallicepticum* cultural examination, morphological studies, staining characteristics, biochemical tests and serological examination with agglutination test were performed. Toxin profile was done by inoculating different concentration of bacterial products in day old broiler chicks. The antibiogram study was performed with the *Mycoplasma gallicepticum* isolates to study their sensitivity and resistance pattern against the commonly used antibiotic discs.

A total number of 75 tracheal swab samples were collected using sterile cotton buds and transported in NB maintaining cool chain. For the isolation of *Mycoplasma gallicepticum*, several selective culture media were used simultaneously in this study. The media used in this study were selected considering the experience of the past researcher worked in various fields relevant to the present study by Hasina 2006, Nazir et al., 2005 and Buxton and Fraser, 1977.

The organisms produced circular, smooth, colorless colony on NA. Among them 45 were isolated by producing red-yellow colonies with fried egg shaped on heart infusion peptone agar which is very much characteristic to *Mycoplasma gallicepticum*. They also produced bright pink or red colonies on MA. The organisms produced hemolysis on BA with discoloration of the media around the growth of the organisms. On selective agar suspected isolates produced pinkish colony. Colony characteristics of *Mycoplasma gallicepticum* were similar to the findings of Kalin et al., 2012, Hasina 2006, Nazir et al. 2005, Derakhshantar and Ghanbarpour 2002, Sharada et al., 1999, Ali et al., 1998 and Buxton and Fraser 1977. In Geimsa staining the organism revealed Gram-posative, pink colour, small cocci shaped and they were as single or paired under microscope and motilewhich was supported by several authors Sharada et al., 1999, Freeman 1979, Buxton and Fraser, 1977 and Merchant and Packer, 1967.

All *Mycoplasma gallicepticum* isolates fermented the five basic sugars. Several previous studies also reported fermentation reactions of this organism with five basic sugars (Beutin et al., 1991; Shandhu and Clarke, 1996 and Mckec et al., 1995). But specific identification and differentiation *Mycoplasma gallicepticum*is somewhat difficult based on only cultural, morphological and biochemical examinations (Freeman, 1985). This is why Indole, MR-VP tests, Catalase and Oxidase test were done to characterize specifically. All the

isolates were MR Catalase, Oxidase and Indole tests positive but VP negative. Almost similar findings were reported by several researchers (Buxton and Fraser 1977; Honda et al., 1982).

Prevalence of *Mycoplasma gallicepticum* in this study was 45%. This was shared with the findings of Hashem et al., 2012 (54.55%); Sampa 2012 (52%) and Jakaria 2011 (78.86%). Slight variations in prevalence rate of *Mycoplasma gallicepticum* in this study were found with those studies. In those study samples were collected from limited number of breeds of chicken and area. But in our study samples were collected from layer, sonali and indigenous layer birds of Patuakhali, Barguna and Vola districts. Seasonal variation of sample collection also may affect the prevalence rate (Lambie et al., 2000). The prevalence rate of *Mycoplasma gallicepticum* in this study was 27% in layer, 23% in sonali and 22% in indigenous layer. Area basis prevalence was found at Patuakhali64%, Barguna 71% and Vola 65%. In case of inter area and breed basis prevalence rate of *Mycoplasma gallicepticum* was in broiler of Patuakhali70% and Barguna 47%; in layer of Patuakhali 67% and Barguna 100%; in sonali of Patuakhali 60% and Volat 80% and in indigenous layer of Barguna 80% and Vola 53%. In case of breed basis prevalence of *Mycoplasma gallicepticum* was the highest in layer. In case of area basis prevalence of *Mycoplasma gallicepticum* was the highest at Barguna and the lowest at Patuakhali. In case of inter area and breed basis prevalence rate of *Mycoplasma gallicepticum* was the highest in and also the lowest layer of Barguna. Such variation in prevalence of *Mycoplasma gallicepticum* might be due to farm practice, use of drugs and faulty transportation of samples.

In case of Toxin profile bacterial products (Bacteria, Toxin and Bacteria + Toxin) of multidrug sensitive and resistant *Mycoplasma gallicepticum* isolates of four breeds of three different areas were inoculated orally to a total of 120 day-old layer chicks. Among them bacteria was inoculated to 40, toxin to 40 and bacteria + toxin to 40 chicks. No clinical signs or mortality found in the chicks inoculated with bacteria. Only 2(5%) chicks inoculated with toxin shown some clinical signs with respiratory, loss of appetite and drowsiness but no mortality were found. 8 (20%) chicks inoculated with bacteria + toxin shown some clinical signs with coughing, loss of appetite and drowsiness but no mortality were found. All these observation indicated that the *Mycoplasma gallicepticum* isolates of chicken were non pathogenic, which disagreed with the result of Jakaria (2011).

The antibiotic sensitivity tests of all *Mycoplasma gallicepticum* isolates (45) were performed by disc diffusion method using eight different antibiotic discs. Most of the isolates were sensitive to Gentamycin (89.55%) and Kanamycin (86.55%). In case of other antibiotcs Cephalexin was 20.90% sensitive but Ciprofloxacin and Streptomycin shown very little percentage (4.48% and 1.49% respectively). On the contrary all *Mycoplasma gallicepticum* isolates were resistant to Amoxicillin and Tetracycline demonstrated. Streptomycin 80.60%, Cephradin 79.10% and Ciprofloxacin 74.62 % showed the resistance percentage. Lower rates of resistance were performed by Cephalexin (23.88%), Gentamycin (7.46%), Kanamycin (7.46%). Intermediately sensitive percentages of the isolates were as, Cephalexin 55.22%, Ciprofloxacin 20.90%, Cephradin 20.90%, Streptomycin 17.91%, Kanamycin 5.97% and Gentamycin 4.48%. Results of antibiotic sensitivity tests were shared with Islam (2008) and Samantha (2012) and Taslim (2006). There was little variation of antibiotic sensitivity and resistance pattern of this study with above mentioned.

In case of breed basis results of antibiotic sensitivity tests, Gentamycin was 100% sensitive to the *Mycoplasma gallicepticum* isolates of layer and indigenous layer, but in case of sonali layer 64.70%. Kanamycin was 100% sensitive to indigenous layer, but in case of sonali 82.35% and layer 80%.

Amoxicillin and Tetracycline demonstrated 100% resistance to all layer, sonali and indigenous layer. Ciprofloxacin was 100% resistant to layer, 82.35% to sonali, and 31.25% to indigenous. Chepradin was 93.75% to indigenous layer, 80% to layer and 52.94% to sonali. Streptomycin was 100% resistant to layer and sonali, but 50% to indigenous. Other resistance percentage is negligible. This result might be supported by previous study of Hashem et al., (2012) and Tanvir et al., (2011). So from this result, it can be concluded that *Mycoplasma gallicepticum* isolates of indigenous breeds of layer birda were most sensitive to the antibiotics and *Mycoplasma gallicepticum* isolates of sonali breeds were most resistant.

In case of area basis antibiotic sensitivity and resistance pattern of *Mycoplasma gallicepticum* isolates of chicken, Gentamycin was 100% sensitive to broiler, layer and sonali of Bogra and Kanamycin

to Broiler and sonali. Layer of Bogra was 70% sensitive to Kanamycin. 42.86% broiler and 44.44% sonali but no layer of Bogra is sensitive to Cephalexin, where as 50% indigenous of Gazipur and 25% indigenous and 12.5% sonali are sensitive to Cephalexin. 100% layer and sonali and 85.71% broiler of Bogra; 100% layer, 57.14% broiler and 12.5% indigenous of Gazipur; 100% sonali and 87.5% indigenous of Joypurhat are resistant to Streptomycin.

In case of Cephalexin, 190% layer and 44.44% indigenous layer; 70% layer and 88% indigenous layer of Barguna; 100% indigenous layer and 63% sonali are resistant .

100% layer and 67% sonali of Patuakhali; 100% layer, 57 and 50% indigenous of Barguna; 100% sonali and 12.5% indigenous layer of Vola are resistant to Ciprofloxacin. It can be concluded that *Mycoplasma gallicepticum* isolates from layer of Barguna and Patuakhali were more or less similar in sensitivity and resistance pattern. *Mycoplasma gallicepticum* isolates of layer from Barguna were little bit less sensitive than that of Patuakhali *Mycoplasma gallicepticum* isolates of sonali from Vola were more resistant than that of Patuakhali. *Mycoplasma gallicepticum* isolates of indigenous layer were more resistant than that of Barguna.

Over all observation of this study emphasizes that multiple drug resistance of *Mycoplasma gallicepticum* is developing day by day. Such high incidence of multidrug resistance may presumably be due to indiscriminate use of antibiotics at the present time, which may eventually supersede the drug sensitive microorganisms from antibiotic saturated environment (Islam et. al., 2008).The drug resistant bacteria can spread in the environment where man and animal acquire infection with bacteria carrying drug resistant plasmids (Joseph *et al.* 1979). The resistance may either be natural such as that in *Mycoplasma gallicepticum* or acquired possibly due to cross-resistance with lincosamides (Recklinghausen et al., 1989). In Bangladesh there is clear evidence of abuse of antibiotics, due to which emergence of multi-drug resistant *Mycoplasma gallicepticum* are increasing continuously (Hussain et al., 1982).

It may be noted that the drug sensitivity may be valuable as background information for future therapy for the effective control of the bacterial disease, otherwise indiscriminate use of the antibacterial drugs may lead to serious hazards of drug resistance. However, routine laboratory isolation and drug sensitivity test being impracticable, periodical check on the pattern of the drug sensitivity of the organisms remains all the more important.

Based on present study, it may be concluded that use of Gentamycin and Kanamycin will be of first choice of treatment against *Mycoplasma gallicepticum* infection in layer birds located at the study area. To a lesser extent Cephalexin may be used. It is to be noticed that ciprofloxacin which is the common choice of drug for the treatment of *Mycoplasma gallicepticum* become somewhat resistant.

CONCLUSION

Mycoplasma gallicepticum species were isolated and identified by cultural, morphological and biochemical examination. Confirmation of identification was done by Serum plate agglutination (SPA) test. Gentamycin and Kanamycin were the best choice of drug. To a lesser extent Cephalexin might be used. Pathogencity test of *Mycoplasma gallicepticum* was done by oral inoculation into day-old-chicks.

So more attention should be given at the age and season as mycoplasmosis is the most economically significant disease in poultry industry of Bangladesh which causes serious economic losses in poultry farm, reduce feed efficiency, decrease growth and reduce egg production. The present study is a preliminary work on the mycoplasmosis which was identified only on the basis of history, clinical signs, Serum plate agglutination (SPA) test and postmortem lesions. However, the result of this study will certainly help the future researchers to provide guidance in carrying out further detail study on mycoplasmosis of poultry in Bangladesh.

REFERENCES

Ali, M. Y., Rahman, M. T., Islam, M. A., Choudhury, K. A. and Rahman, M. A. 1998. Characteristics of *Mycoplasma gallicepticum* isolates of human and animal origin. *Progress. Agric* .9: 221-224.

Anisuzzaman, M. and Wahid M.A. 1988. A study on growth rate, feed efficacy and livability of Fayoumi chicken under Bangladesh condition. M. Sc thesis, Department of Poultry Science, BAU, Mymensingh, Bangladesh.

Buxton, A. and Fraser, G. 1977. Animal Microbiology. Vol. 1. *Mycoplasma gallicepticum*, Balckwell

Scientific Publications, Oxford, London, Edinburg, Melbourne. pp: 94-102

Charles L., Hofacre, S., Alison, C., Johnson, B., Barry, J. and Robrecht F. 2001. Effect of a commercial competitive Exclusion Culture on Reduction of Colonization of an Antibiotic-Resistant Pathogenic *Mycoplasma gallicepticum* in Day Old Broiler Chickens. *Avian Dis.* 45: 198-201.

Choudhury, T. I. M. F. R., Choudhury, M. U. A. and Rahman, M. 1967. Microflora of milk in a single herd. *Pakistan J. Vet. Sc.*1: 3.

Chulasiri, M. and Suthienkul, O. 1989. Antimicrobial resistance of *Mycoplasma gallicepticum* isolated from chickens. *Vet. Microbiol.* 21:189-194.

Clinical and laboratory standard institute (CLSI), 2007. Performance standard for antimicrobial susceptibility testing; seventeenth informational supplement. 27: 1-187.

Derakhshantar, A. and Ghanbarpour, R. 2002. A study on avian cellulitis in broiler chickens. *Veterivarski Archiv.* 72: 227-284.

Dho, M. and Lafont, J. P. 1984. Adhesive properties and iron uptake ability in *Mycoplasma gallicepticum* lethal and nonlethal for chicks. *Avian Dis.* 28: 1016-1025.

Freeman, B. A. 1979. Burrows Textbook of Microbiology. 22thedn. In: W. B. Saunders Company, Philadelphia, London, Toronto, Mexico City, Rio de Janerio, Sydney, Tokyo, pp. 464-475.

Hashem M. A., Elahi M. F., Mannan M. A., Kabir M. H. B., KashemM. A. and Pallab M. S. 2012. Isolation, identification and antibiogram of *Mycoplasma gallicepticum* from broiler at Chittagong districts in Bangladesh. Wayamba *J. Anim. Sci.* 1: 573-578.

Hasina, B. 2006. Enteropathotypic characterization of *Mycoplasma gallicepticum* isolated from diarrhoeic calves and there antibiogram study. MS Thesis, Department of Microbiology and Hygiene, BAU, Mymensingh.

Honda, T., Arita, M., Takela, Y. and Miwatani T. 1982.Further evaluation of the Biken Test (Modified Eleck Test) for deletion of enterotoxigenic *Escherichia coli* producing heat stable enterotoxin and application of the test to sampling of heat stable enterotoxin. *J. Clin. Microb.*16: 60-62.

Hussain, M. M., Glass, R. J. and Khan, M. R. 1982. Antibiotic used in a rural community in Bangladesh. *Int. J. Epidemiol.* 11: 402-405.

Islam, M. J., Sultana, S., Das, K. K., Sharmin, N. and Hasan, M. N. 2008. Isolation of plasmid- mediated multidrug resistant *Mycoplasma gallicepticum* from poultry. *Int. J. Sustain. Crop Prod*. 3:46-50.

Jakaria A.T. M. 2011.Prevalence and characteristics of *Mycoplasma gallicepticum* in poultry. MS Thesis, Department of Microbiology and Hygiene, BAU, Mymensingh.

Lambie, N., Ngeleka, M., Brown, G. and Ryan, J. 2000. Retrospective study on *Mycoplasma gallicepticum* infection in broilers subjected to post mortem examination and antibiotic resistance of isolates in Trinidad. *Avian Dis.* 44: 155-160.

Mckee M. L., Melton C. A., Moxley R. A., Fancis D. H and Brien O. A. D. 1995. Enterohaemorrhagic *Escherichia coli* 0157:H7 requires intimin to colonize the genotobiotic pig intestine and to adhere to HEP-2 cells. *Infect. Immun*. 63: 3739-3744.

Merchant, I. A. and Packer, R. A. 1967. Veterinary Bacteriology and Virology. 7th edn. The Iowa State University Press, Ames, Iowa, USA. pp.211-305.

Nataro, J. P. and Kaper, J. B. 1998. Diarrheagenic *Mycoplasma gallicepticum.Clin. Microbiol. Rev.*11: 142-201.

Nazir, K. H. M. N. H., Rahman, M. B., Nasiruddin, K. M., Akhtar, F., Khan, M. F. R. and Islam, M. S. 2005. Antibiotic sensitivity of *Mycoplasma gallicepticum* isolated from water and its relation with plasmid profile analysis. *Pak. J. Biol. Sci.* 8: 1610-1613.

Sammantha J. T. 2012. Studies on the prevalence of *Mycoplasma gallicepticum* infection in free- range chicken of Penampang, Sabah. B. Sc.(Ag) Thesis, School of Sustainable Agriculture, University of Malaysia Sabah, Malaysia.

Sampa Rani Roy 2012.Isolation and identification of bacterial flora from broiler and their antibiogram studies. MS Thesis, Department of Microbiology and Hygiene, BAU, Mymensingh.

Samsuddoha, M. and Sohel, M. H. 2003. Problems and prospects of poultry industry in Bangladesh: A study on some selected areas. *Chittagong Univ. J. Busi. Admin*. 19: 200

Sandhu K. S., Clarke R. C., MeFadden K., Brouwer A., Louie M., Wilson J, Lior H and Gyles C. L, 1996. Prevalence of the eaaA gene in verotoxigenic *Mycoplasma gallicepticum* strains from dairy cattle in southwest Ontario. *Epi. Infect*.116:1-7.

Sharda, R., Krishnappa, G., Raghavan, R., Sreevinas, G. and Upandra, H. A. 1999. Isolation and serotyping of *Mycoplasma gallicepticum* from different pathological conditions in poultry. *Indian. J. Poul. Sci.* 34: 366-369.

Tanvir, B., Mostafizur, R., Fazle, A. R., Rashed, N. and Majibur, M. R. 2011. Enterotoxin Profiling and Antibiogram of *Mycoplasma gallicepticum* isolated from poultry feces in Dhaka district of Bangladesh. *Stamford J. Microb*. 1:51-57.

Taslim H. M. 2006. Characterization, toxin profile and antibiogram of *Mycoplasma gallicepticum* isolated from chicken. MS Thesis, Department of Microbiology and Hygiene, BAU, Mymensingh.

Wooley, R. E., Spears, K. R., Brown, J., Nolan, L. K. and Fletcher, O. J. 1992. Relationship of complement resistance and selected virulence factors in pathogenic avian *Mycoplasma gallicepticum. Avian Dis.* 36: 348-352.