International Journal of Animal Biology
Vol. 3, No. 2, 2017, pp. 5-11
http://www.aiscience.org/journal/ijab
ISSN: 2381-7658 (Print); ISSN: 2381-7666 (Online)

Isolation, Identification and Antibiogram Study of *Salmonella* spp. from Poultry Farm Environment

Parbati Paul[1], Sonia Akther[2], Md Zulfekar Ali[3, *], Hasna Banu[4], Md. Shahidur Rahman Khan[5], Mst. Minara Khatun[5]

[1]Department of Livestock Services (DLS), Ministry of Fisheries and Livestock Khamarbari, Dhaka, Bangladesh
[2]Goat and Sheep Production Research Division, Bangladesh Livestock Research Institute (BLRI), Dhaka, Bangladesh
[3]Animal Health Research Division, Bangladesh Livestock Research Institute (BLRI), Dhaka, Bangladesh
[4]ACI Animal Health, ACI Limited, Dhaka, Bangladesh
[5]Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh

Abstract

Wide spread use of antimicrobials in poultry farming here is a concern of multi-drug microbial resistance development that can potentially be transmitted to human pathogens even from non-pathogenic carrier strains. Attempt was made to assess drug susceptibility in *Salmonella* from poultry sources of Bangladesh. *Salmonella* isolated from poultry sources were thoroughly characterized by standard cultural and biochemical tests from which 5 were tested for susceptibility to 10 antibiotics following disk diffusion method. Out of total 60 samples 32 (53.33%) were found positive for *Salmonella*. Highest number of *Salsmonella* was isolated from egg surface washing (90%), followed by cloacal swab (80%), intestinal content (60%), hand wash of poultry handler (50%) and in fecal materials(40%) but no isolate was found on environment (air) samples. 100% of the tested *Salmonella* isolates from poultry sources were found to be resistant to Erythromycin, Tetracycline and Nalidaxic Acid and 80% strains showed resistance to Ciprofloxacin whereas, Ampicillin, Amoxicillin, Sulphamethoxazole and Kanamycin showed 60% resistance. No isolate showed resistance to Gentamicin. Sensitivity was recorded in case of 40% strains to Chloramphenicol, Ampicillin, Amoxicillin, Sulphamethoxazole, Kanamycin and 20% isolate against Ciprofloxacin. Both, resistance and susceptibility were exhibited against Chloramphenicol, Ampicillin, Amoxicillin, Sulphamethoxazole, Kanamycin, and Ciprofloxacin. Further study is required on the role of poultry borne bacteria as vectors in transmitting drug resistance. Attention is to be paid for personnel hygiene in processing and handling of poultry and poultry products and excess use or abuse of antibiotics should be reduced or stopped by the judicious application of antibiotics for the safety of public health in Bangladesh.

Keywords

Salmonella spp., Poultry, Isolation, Antibiogram, Environment

Received: August 8, 2017 / Accepted: October 11, 2017 / Published online: November 1, 2017

1. Introduction

Poultry rearing is considered superior to the other agricultural sector in Bangladesh because of the quick economic return in a relatively short period of time. Poultry is emerging as an important agribusiness started practically during eighties (1980) [1]. The present population of poultry is estimated to be 232.62 million including 221.39 million of chicken and 41.23 million of ducks [2]. The annual rate of ascending agribusiness is chicken 6.2% and duck 2.38% while in commercial bird it is 15% [2]. But this sector facing various

* Corresponding author
E-mail address: zulfekar@blri.gov.bd (Md Z. Ali), zulfekarvet@gmail.com (Md Z. Ali)

problems related to production and health management. Outbreaks of several devastating diseases constitute major constraints causing economic loss and discouraging poultry rearing in this country [3].

Salmonella is a leading cause of food borne illness worldwide, with an estimated millions cases each year in the world. Salmonella infections are typically due to consumption of food products of animal origin. Several lines of evidence indicate that antibiotic-resistance among human Salmonella infections results from the use of antimicrobial agents in food animals.

Salmonellae are Gram negative, small rod shaped, non spore forming, non capsulated, aerobic and facultative anaerobic organisms and classified under the family *Enterobacteriaceae* [4]. The genus Salmonella includes a large group of serologically and biologically related bacilli and as a rule they are motile by means of peritrichous flagella with the exception of *Salmonella pullorum* and *Salmonella gallinarum* [5]. More than 2300 serotypes exist based on 67 "O" antigen (for non-motile species) and the numerous "H" antigens (for motile spp.) recognized thus far [5]. Salmonellae are classified into three main groups based on their association with human and animal hosts. The first group is specific for human host, second group consists of serotypes which are adapted to specific animal hosts and the third group consists of un-adapted serotypes that cause diseases in human and a variety of animal species. Transmission of this organism from one host to another is primarily through the air. The bacteria shred from an infected bird in the nasal and or ocular secretions, fecal material, and feather dust. The organism remains stable outside the host body and dries as a dusty substance. This dust or aerosol contaminates the air that is then inhaled by another possible host [6]. Antibiotics are extensively used as growth promoters in poultry production or to control infectious disease. Anti-microbial exercise and/or especially abuse are considered to be the most vital selecting force to antimicrobial resistance of bacteria [7]. Moreover, antibiotic treatment is considered the most important issue that promotes the emergence, selection and spreading of antibiotic-resistant microorganisms in both veterinary and human medicine [8]. It was stated by well established evidence that antibiotics can lead to the emergence and dissemination of resistant *Salmonella* which can then be passed into people via food or direct contact with infected animals. These resistant microbes may function as a potential source in the transportation of antimicrobial resistance to human pathogens [6]. Side by side, antimicrobial resistant strains of *Salmonella* spp. are now widespread all over the world. In developed countries it is becoming more and more accepted that a majority of resistant strains are of zoonotic origin and have acquired their resistance in an animal host before being transmitted to humans through the food chain [9]. This resistance occurs due to the indiscriminate and continuous use of sub therapeutic doses of antibiotics. This study was designed to select the appropriate antibiotics against *Salmonella* determining the resistance and sensitivity patterns of the isolates with the view to make people more prudent in the use of antibiotics. The apparently healthy chickens available in the farms and live bird markets are important sources of human Salmonellosis. Therefore, the present study was designed to isolate *Salmonella* strains from six different sources of poultry and poultry environment at Mymensingh, Bangladesh for assessing their susceptibility and resistance patterns to some selected antimicrobials.

2. Materials and Methods

This study was carried out in the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh throughout the period of June to November, 2012.

Collection of samples: A total of 60 samples were collected from cloacal swabs of chicken, intestinal fluid of chicken, egg surface, faecal material of chicken, air and hand wash of poultry handlers from different poultry markets in Mymensingh, Bangladesh.

Transportation of sample: After collection, all the samples were transported to the laboratory immediately. Sampling was conducted in different market and farm of Mymensingh such as BAU poultry farm, Boira poultry farm, Boira market and KR Live bird market, Shasmor market and Natun bazar. After collecting aseptically, the samples were transported to the Department of Microbiology and Hygiene, BAU, Mymensingh for isolation and characterization of *Salmonella*

Isolation of bacteria: After collection, the samples were grown in the freshly prepared nutrient broth at 37°C for 24 hours. The overnight bacterial broths were streaked on SS agars which were incubated at 37°C for 24 hours. Black colonies were further streaked on SS agar until single colonies were obtained. Black cantered colonies were further inoculated on Nutrient agar, Blood agar, EMB agar plates and incubated at 37°C for 24 hours and examined for the growth of characteristic colonies of *Salmonella* on these media.

Gram's staining

The representative *Salmonella* colonies were characterized morphologically using Gram's staining method according to the method described [10].

Motilitytest

The motility test was performed to differentiate motile

bacteria from non-motile one [11]. One drop of cultured broth was placed on the clean cover-slip and was placed inversely over the concave depression of the hanging drop slide to make hanging drop preparation. Vaseline was used around the edge of the concave depression of the hanging drop slide for better attachment of the cover-slip to prevent air current and evaporation of the fluid. The hanging drop slide was then examined carefully under 100x objective of a compound light microscope using immersion oil. The motile and non-motile organisms were identified by observing motility in contrasting with swinging movement of bacteria.

Biochemical tests:

Isolated organisms showing characteristic colony morphology of *Salmonella* on SS, BA, and EMB agar were subjected to biochemical tests such as; sugar fermentation test, Indole and MR-VP test. Standard methods were followed to conduct these tests [12].

Antibiogram study

Total 5 isolates were tested for antimicrobial drug susceptibility against 10 commonly used antibiotics belonging to different groups by disc diffusion method or Kirby-Bauer method [13]. Briefly, by using a sterile inoculating loop a single bacterial colony was taken from the surface of SS agar was removed and emulsified in physiological saline on the test tube. Antimicrobial discs (Oxoid Ltd. Hampshire, England) were plated onto MH agar using sterile forceps. Within 30 minutes after applying the discs, the plates were incubated at 37°C for 24 hours. After incubation, the plates were examined and the diameters of the inhibition zones were measured in mm from the edge of the disc to the edge of the zone using ammeter ruler. Susceptibility and resistance were determined according to the interpretation criteria described in the Medical Laboratory Manual for Tropical Countries [11]. Antimicrobial agents with their disc concentrations are presented in Table 1.

Table 1. Antimicrobial agents with their disc concentrations.

Antimicrobial agents	Symbol	Disc concentration
Erythromycin	E	15 µg
Ampicillin	AMP	10 µg
Amoxicillin	AML	10 µg
Chloramphenicol	C	30 µg
Ciprofloxacin	CIP	5 µg
Tetracycline	TE	30 µg
Kanamycin	K	30 µg
Gentamicin	CN	10 µg
Sulphamethoxazole	SXT	25 µg
Nalidaxic Acid	NA	30 µg

3. Results

The swab samples inoculated into nutrient broth with revealed the growth of bacteria after 24 hours of incubation at 37°C. The growth of bacteria was indicated by the presence of turbidity (Figure 1). Cultural characteristics on different solid media are shown in Figure 2 to 3.

Identification of Salmonella spp.

Gram's staining characteristics:

The thin smears prepared with the colony from SS agar, MC agar and BG agar for Gram's staining revealed Gram-negative, pink colored, small rod shaped appearance, arranged in single or paired under the microscopic examination (Figure 6).

Motility profile

All the isolates were found to be motile when examined using hanging drop slide under microscope. All of the isolates fermented dextrose, maltose and mannitol with the production of acid and gas but did not ferment lactose and sucrose. Acid production was indicated by the color change from reddish to yellow and gas production was noted by the presence of gas bubbles in the inverted Durham's tubes. The results of sugar fermentation tests are shown in Figure 7. All the isolates were Methyl-red positive, Voges-Proskauer and Indole negative. Results of these tests are shown Figure 8. The prevalence of *Salmonella* was studied in cloacal swabs, hand wash of poultry handler, egg washing, fecal materials, intestinal content, and poultry environment, A total of 60 samples were collected in which cloacal swabs (n=10), hand wash of poultry handler(n=10), egg washing(n=10), fecal materials(n=10), intestinal content (n=10), and poultry environment(n=10). The prevalence differed among sources and locations, 8 samples were found positive out of 10 in cloacal swabs (80%), 5 found positive results among 10 samples in hand washing of poultry handler (50%), 9 were positive out of 10 in egg washing (90%), in case of fecal materials (40%), intestinal content (60%), and poultry environment (air 0%) positive results were respectively 4, 6, 0. The overall 53.33% prevalence of *salmonella* was recorded in poultry and poultry environment which are shown in Figure 9 and 10.

The overall prevalence of *Salmonella* in poultry and poultry environment was recorded as 80%, 46.67%, 30%, 70%, and 30% at Shasmor market, KR live bird market, BAU poultry farm, Boira poultry farm and Natun Bazar respectively shown in Table 2.

Furthermore, the different in prevalence in respect of different locations was also recorded among the sources. The highest prevalence of S*almonella* was found in case of egg washing 80% and 100% in Shasmor market and Boira Poultry Farm respectively. In cloacal swabs the prevalence of *Salmonella* was 100% and 60% in Shasmor market and KR

live bird market respectively. In case of Intestinal content the prevalence was 40% and 80% in BAU Poultry farm and k R live bird market. On the other hand, in feces the prevalence was recorded 20% and 60% in BAU poultry farm and Natun Bazar, whereas 60% and 40% in Shasmor market and Boira poultry farm was recorded respectively in case of hand wash of poultry handler.

Antibiogram profile of Salmonella Isolated in poultry and poultry environment:

Antibiotic sensitivity test of 5 isolates revealed different sensitivity and resistant patterns according to different sources out of ten antibiotics Ampicillin (AMP), Amoxicillin (AML), sulphamethoxazole (SXT), Chloramphenicol (C), Kanamycin (K), were sensitive against the 40% isolated bacteria, followed by Ciprofloxacin (CIP), which was sensitive against 20% isolate. On the other hand Gentamycin (CN) was intermediate against 100% isolates whereas, Chloramphenicol (C) against 20% isolate. The highest resistance was found with Erythromycin (E), Tetracycline(TE) and Nalidaxic Acid (NA) were resistance against 100% isolated *Salmonella* while Ampicillin (AMP), Amoxicillin (AML), sulphamethoxazole (SXT), Kanamycin (K), were resistant against 40% bacteria. Only Chloramphenicol (C) was resistance against 40% isolated Salmonella.

Figure 1. Growth of bacteria in nutrient broth indicated by turbidity.

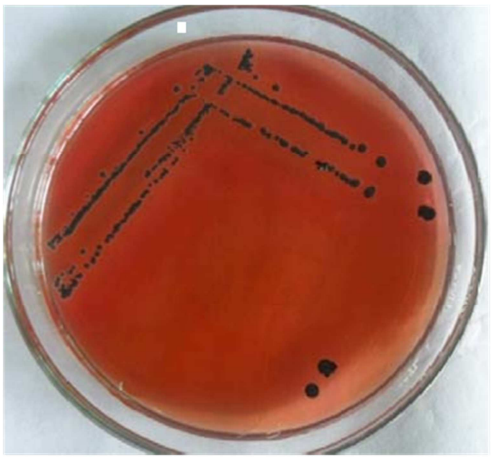

Figure 2. Smooth, black centered colonies on Salmonella - Shigella agar.

Figure 3. Non hemolytic and grey colonies in Blood agar.

Figure 4. Translucent, opaque and smooth colonies on Nutrient agar.

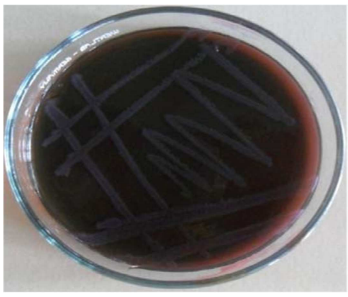

Figure 5. Pink, circular colonies in EMB.

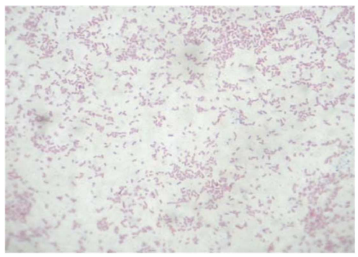

Figure 6. Gram-negative, pink colored, small rod shaped bacteria under a light microscope (100x).

Figure 7. Results of sugar fermentation tests of *Salmonella* using five basic sugars such as Dextrose (1), Maltose (2), Lactose (3), = (4) Mannitol (5) and control (6).

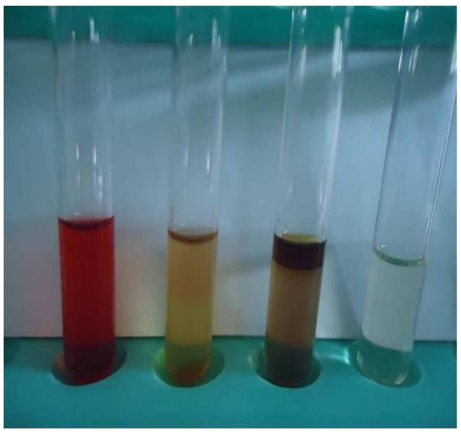

Figure 8. Results of Indole, VP-MR tests.

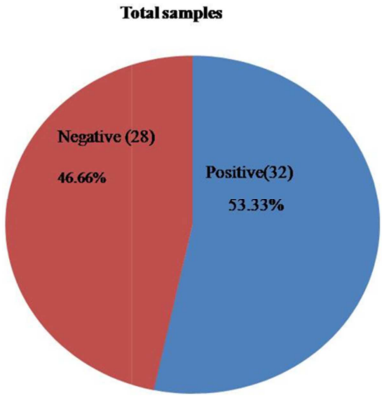

Figure 9. The results of prevalence were expressed by percentage.

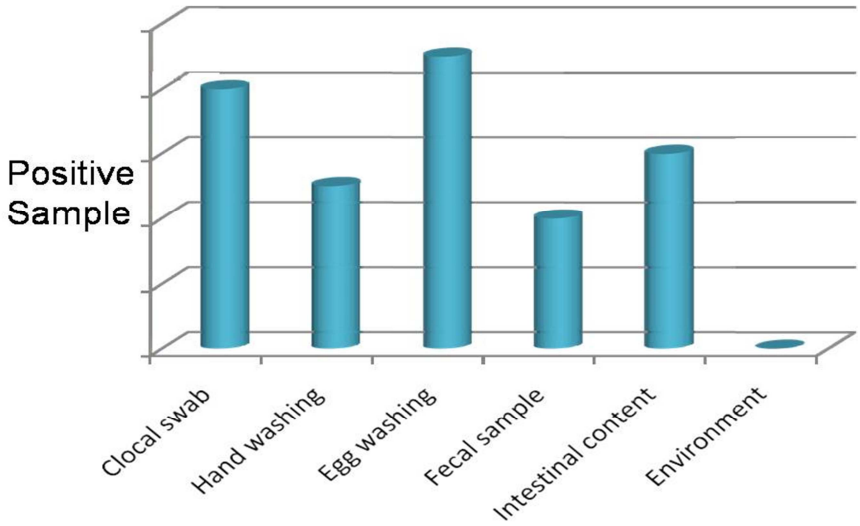

Figure 10. Prevalence rate of *Salmonella* in poultry and poultry environment according to different sources.

Table 2. Prevalence of *Salmonella* in poultry and poultry environment according to locations.

Sl. No.	Locations	No ofSamples	Positive for *Salmonella*	Prevalence(%)
1	Shasmor market	15	12	80%
2	K R live bird market	15	7	46.67
3	BAU Poultry farm	10	3	30%
4	Boira Poultry Farm	10	7	70%
5	Natun Bazar	10	3	30%
Overall		60	32	53.33%

4. Discussion

The aim of the present research work was the isolation, characterization and determination of antibiotics resistance of *Salmonella* spp. Isolated from poultry and poultry environment from different areas in and around Bangladesh Agricultural University Campus, Mymensingh, Bangladesh. The isolates were confirmed as *Salmonella* by cultural and staining characteristics, motility, and biochemical test. Furthermore, this study has shown the differences in prevalence of *Salmonella* spp. in different sources and locations. Finally, the antibiotic sensitivity and resistance patterns of the isolates of different sources were identified in this study. Characterization of the isolates based on the multiple antimicrobial resistance (MAR) was an important part of the study since the results indicated that chickens play important role as reservoirs of multi-drug resistant *Salmonella*.

Specific enrichment media and biochemical tests were used for the isolation and identification of *Salmonella* which was previously suggested by a number of researchers [14]. In this study the colony characteristics of *Salmonella* serovars observed on SS agar, EMB agar and BA showed similarity with the findings of other authors [15].

In Gram's staining, the morphological characteristics of the isolated *Salmonella* exhibited Gram negative, small rod shaped, single or paired in arrangement under microscope

which was supported by other researchers [16].

In motility test, all the isolates showed swinging movement which correlated with the results of Merchant and Packer [10].

In sugar fermentation test, all the isolated *salmonellae* fermented dextrose, maltose and mannitol and produce acid and gas but did not ferment sucrose and lactose which satisfy the statement of Merchant [10]. Again, all the isolates were positive to methyl red test and negative to indole and VP test which strongly supports the observation of [10].

In this study the highest prevalence of *salmonella* was found in case of egg washing followed by cloacal swabs, intestinal content, hand wash of poultry handler and in feces. The egg surface was contaminated with *Salmonella* probably from poultry feeds and/ with feces during lay in unhygienic condition or also from infected poultry. Among the animal protein ingredients, a major ingredient of poultry feeds, locally processed cheap fish wastes were found to be important causes for bacterial contamination of poultry feeds [17]. *Salmonella* was reported as a common microflora in raw feeding materials and poultry feeds [18]. Present study showed a high percentage of egg surface samples 90% contained *Salmonella*. In case of *salmonella* in egg surface the prevalence rate reported by Harsha*et al.* (2011)[19] were slightly lower than our experiment. The prevalence of *Salmonella* was slightly higher as compared to the prevalence rate reported by other researcher [20]. However, prevalence rate in our study are in agreement with the prevalence rate Rahman *et al.* (2011). This study recorded differences of prevalence among samples which satisfied the finding of another researcher [21]. *Salmonella* in hand washing of poultry handler were 50% positive in our study which in agreement with the prevalence rate[22]. The prevalence of *salmonella* in intestinal content found in this study satisfies the data of other studies [23].

The pre-stuffed chickens in poultry shops, poultry and poultry products like eggs and plastic-wrapped poultry meat in various super shops get contaminated easily by *Salmonella* for the careless unhygienic handling process and ready-to-eat foods become cross contaminated with *Salmonella* as well as other pathogenic bacteria from poultry handlers with poor personal hygiene and from other raw poultry products [24].

Total 5 isolates were investigated for susceptibility and resistance patterns by disc diffusion method using 10 commonly used antibiotics belonging to different groups. Among the variety of antibiotics tested, the highest resistance was found with Tetracycline, Nalidaxic acid and Erythromycin followed by ciprofloxacin and after that Kanamycin, Amoxicillin, Ampicillin and Sulphamethoxazole and lower in chloramphenicol. These findings were more or less similar to other researchers [1]. On the other hand, none of the isolates showed resistance to Gentamicin In addition, the isolates were intermediately sensitive to Gentamicin (100%) and Ciprofloxacin (20%) which satisfy partly the findings of other researchers [25].

Among the 5 isolates tested, no one showed susceptibility to all antibiotics notably, salmonella isolated from egg washing were higher than any other source, and probably the source may be contaminated feed or bedding materials. Antibiotics resistance pattern of *salmonella* isolate between cloacal swabs and intestinal content were similar. Again, important point to note is that all the isolates originated from broilers showing resistance to more than five antibiotics. Results of the study indicate that isolates from broilers are more likely to be multi-drug resistant in and continuous useof sub therapeutic doses of antibiotics during rearing in commercial production system. Therefore, more sensible use of antibiotics can be strongly suggested for the veterinarians since drugres is tance could be a major public health oncernas fluoroquinolones are important antimicrobial compounds in the treatment of Salmonellosis in humans [25] [26] [27].

Data of this study suggest that, poultry and poultry products at farms and live bird markets are the major reservoirs of *Salmonella* spp. that might be potential cause of food poisoning if proper hygienic measure is not undertaken during rearing, handling and processing of poultry and poultry by products.

5. Conclusion

A total of 60 samples were collected from chickens of different sites of poultry and poultry environment, of them, total 32 samples were found to be positive (53.33%) for *Salmonella* spp. The prevalence of *Salmonella* in egg washing, cloacal swabs intestinal content, feces, hand wash of poultry handler and in air was 90%, 80%, 60%, 40%, 50% and o% respectively. The prevalence of *Salmonella* also varied among the locations which were 80%, 46.67%, 30%, 70%, and 30% at Shasmor market, KR live bird market, BAU Poultryfarm, Boira Poultry Farm and Natun Bazar, respectively.

Antibiotic sensitivity and resistance patterns of isolated *Salmonella* spp. were studied by disc diffusion method using 10 commonly prescribed antibiotics. *Salmonella showed* resistance to various antimicrobial agents at variable degree. It was observed that the most sensitive antibiotics were Ampicillin, Amoxicillin Sulphamethoxazole and Kanamycin, followed by Gentamicin and Ciprofloxacin. Conversely, Tetracycline, Erythromycin and Nalidaxic acid, Chloramphenicol and were found to be most resistant drugs. These findings would certainly help the veterinarians to select the correct antibiotics against *Salmonella* infections.

Overall, the prevalence of *Salmonella* in chickens and their drug resistance is obviously significant. Moreover, the disease caused by *Salmonella* has a great public health importance. Therefore, poultry sector should be provided with immediate attention by the government to maintain strict hygienic measurement in farm and live bird markets all over the country.

References

[1] Haque ATM (2011). Investigation on Salmonellosis of geese population in sadarupazilla of Mymensingh district. MS thesis, submitted to the Department of Microbiology and Hygiene, Mymensingh-2202.

[2] Department of Livestock Services (DLS), 2008. Annual report of Department of Livestock Services, Khamarbari Road, Farmgate, Dhaka.

[3] Ali MZ, Rahman MM, Sultana S. Seroprevalence of Mycoplasma gallisepticum antibody by ELISA and serum plate agglutination test of laying chicken. Veterinary world. 2015 Jan; 8(1):9.

[4] OIE (2006). Manual of standards for diagnostic tests and vaccines. Office International Des Epizooties. Salmonellosis. Chapter X.4. http://www.oie.int/.

[5] Blood DC, Henderson AJ, Radosintits AJH and Gay CC (2003). A text book of the diseases of animals. 9thedn pp: 809-829.

[6] Ali MZ, Sultana S. Determination of humoral immune response in chickens against formalin-inactivated alum-precipitated fowl cholera vaccine. International Journal of Animal Biology. Vol. 1, No. 4, 2015, pp. 114-117.

[7] Moreno MA, Domínguez L, Teshager T, Herrero IA, Porrero MC, de Ávila LD, de Badajoz LD, de Canarias LD, de Ciudad LD, de Salamanca LD, de Valladolid LD. Antibiotic resistance monitoring: the Spanish programme. International journal of antimicrobial agents. 2000 May 31;14(4):285-90.

[8] Witte W. Medical consequences of antibiotic use in agriculture. Science. 1998 Feb 13; 279(5353):996-7.

[9] Molbak K, Gerner-Smidt P, Wegener HC. Increasing quinolone resistance in Salmonella enterica serotype Enteritidis. Emerging infectious diseases. 2002 May;8(5):514.

[10] Merchant IA and Packer RA (1967). Veterinary bacteriology and virology, 7th edn. The Iowa University Press, Ames, Iowa, USA, pp: 286-306.

[11] Cheesbrough M (1984). Medical laboratory Mannual for Tropical Countries. In: Antimicrobial Sensitivity Testing. 10thedn, Tropical Health Technology. 14 Bevills Close, Doddington, Cambridgeshire. pp: 196-205.

[12] Cowan ST (1985). Cowan and Steel's Manual for identification of medical bacteria. 2nd edition. *Cambridge University press, Cambridge, London*. pp: 138-139.

[13] Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology. 1966 Apr; 45(4):493.

[14] Mdegela RH, Yongolo MG, Minga UM, Olsen JE. Molecular epidemiology of Salmonella gallinarum in chickens in Tanzania. Avian pathology. 2000 Oct 1;29(5):457-63.

[15] Cherry B, Burns A, Johnson GS, Pfeiffer H, Dumas N, Barrett D, McDonough PL, Eidson M. Salmonella Typhimurium outbreak associated with veterinary clinic. Emerging infectious diseases. 2004 Dec;10(12):2249.

[16] Gene O. The isolation, identification and serotyping of Salmonella isolated from domestic poultry in Kars district. KafkasUnivarsikesiVeterinerFakultesi, Dergisi. 2002;8:23-30.

[17] Okoli C, Ndujihe G, Ogbuewu I. Frequency of isolation of salmonella from commercial poultry feeds and their anti-microbial resistance profiles, Imo State, Nigeria. Online Journal of Health and Allied Sciences. 2006;5(2).

[18] da Costa PM, Oliveira M, Bica A, Vaz-Pires P, Bernardo F. Antimicrobial resistance in Enterococcus spp. and Escherichia coli isolated from poultry feed and feed ingredients. Veterinary microbiology. 2007 Feb 25;120(1):122-31.

[19] Harsha HT, Reshmi R, Varghese R, Divya PS, Rahiman KM, Hatha AM. Prevalence and antibiotic resistance of Salmonella from the eggs of commercial samples. Journal of Microbiology and Infectious Diseases. 2011;1(03).

[20] Akbarmehr J. Isolation of Salmonella spp. from poultry (ostrich, pigeon, and chicken) and detection of their hilA gene by PCR method. African Journal of Microbiology Research. 2010 Dec 18;4(24):2678-81.

[21] Kobayashi H, Kanazaki M, Shimizu Y, Nakajima H, KHATUN MM, Hata E, Kubo M. Salmonella isolates from cloacal swabs and footpads of wild birds in the immediate environment of Tokyo Bay. Journal of veterinary medical science. 2007;69(3):309-11.

[22] Rahman MM, Hossain MK, Akhter MR, Hasan SM, Rahman MM. Characterization and antibiogram study of salmonella serovars isolated from duck, quail and pigeon in Dinajpur district of Bangladesh. International Journal of Sustainable Agricultural Technology. 2011;7(2):23-9.

[23] Dallal SMM, Taremi M, Gachkar L, Modarressi S, Sanaei M, Bakhtiari R, Zali MR. Characterization of antibiotic resistant patterns of Salmonella serotypes isolated from beef and chicken samples in Tehran. Jundishapur Journal of Microbiology. 2009; 2(4):124-31.

[24] Akond MA, Shirin M, Alam S, Hassan SM, Rahman MM, Hoq M. Frequency of drug resistant Salmonella spp. isolated from poultry samples in Bangladesh. Stamford Journal of Microbiology. 2013 Jun 4; 2(1):15-9.

[25] Khan MF, Rahman MB, Khan MS, Nazir KH, Rahman M. Antibiogram and plasmid profile analysis of isolated poultry Salmonella of Bangladesh. Pakistan Journal of Biological Sciences. 2005;8:1614-9.

[26] Sultana S, Rahman MM, Ali MZ. Evaluation of gentian violet and copper sulphate as fungi inhibitor in broiler diet. International Journal of Animal Biology. Vol. 1, No. 4, 2015, pp. 146-149.

[27] Sultana S, Rashid SM, Islam MN, Ali MH, Islam MM, Azam MG. Pathological investigation of avian aspergillosis in commercial broiler chicken at chittagong district. International Journal of Innovation and Applied Studies. 2015 Jan 1;10(1): 75-85.