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ORIGINAL ARTICLE

Seroprevalence and antibiotic sensitivity of *Salmonella* spp. in commercial layer chicken of Pirojpur district

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Abstract

Background: Salmonellosis is one of the most important diseases of poultry that seriously impedes the development of the poultry industry. The study was investigated to determine the seroprevalence and antibiotic sensitivity of Salmonella spp. in commercial layer chicken at study area.

Methods: The study was conducted from June 2020 to July 2020. For seroprevalence study, a total of 200 serum samples were randomly collected from 20 layer farms at Nesarabad Upazila of Pirojpur district, Bangladesh. The *Salmonella* spp. were isolated and identified by conventional methods like culture, Gram's staining and biochemical test. The risk factors for salmonellosis in layer birds were identified by multivariable logistic regression analysis.

Results: The overall seroprevalence of salmonellosis in layer chicken was 58% (95% confidence interval [CI]: 50.8; 64.8). The seroprevalence was significantly higher (71.25%) in > 50 weeks age (Odd ratio [OR]=4.5; 95% CI: 1.94; 10.3) than that of > 15-30 weeks age (37.5%). In addition, the seroprevalence of salmonella infection was also significantly higher (74%) in medium (OR=2.3 ;95% CI: 1.1; 4.7) and large flocks (OR=4.7; 95% CI: 1.9; 11.3) compared to small flocks. The *Salmonella* spp. was found to be distributed in 68.75% liver, 25% spleen, and 18.75% intestinal swab. Antibiogram study showed that 63.64% of *Salmonella* spp. was multidrug resistance (MDR). Salmonella isolates were most resistant to oxytetracycline (90.91%) followed by amoxicillin (81.82%) and sulfamethoxazole-trimethoprim (63.64%); but the isolates were most sensitive to ceftriaxone (100%) followed by gentamicin (81.82%), ciprofloxacin (72.73%) and streptomycin (63.64%).

Conclusions: The seroprevalence of salmonellosis in the layer bird of the study area seems to be very high. Aged layer birds of medium and large flocks should be targeted for future control and surveillance program. Antibiogram guided treatment may help prevent treatment failure and development of antimicrobial resistance

Keywords: Multidrug resistance, age, flock size, risk factors

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Introduction

Commercial poultry farming plays a pivotal role in the socio economic system in the country. It has been growing rapidly in Bangladesh since early 1990 by using improved genetics, manufactured feeds and management of poultry. In the 90s total investment in this sector was only BDT 15 thousand million, but now the investment in this industry is above Taka 300 billion (Hamid *et al*., 2017). Although commercial poultry increasing day by day, there are different causes that impede the development of poultry sector. The common disease that hamper the poultry sectors in Bangladesh are New Castle disease, infectious bursal disease, salmonellosis, mycoplasmosis, infectious bronchitis, fowl cholera, avian influenza etc. Among the diseases salmonellosis is common in poultry. The disease is important in commercial poultry that hindered the development of the poultry industry in Bangladesh and as a source of human food-borne zoonotic diseases (Mahmud *et al*., 2011; Waltman *et al*., 2008)

The *Salmonella* serovar *Gallinarum* may be divided into biovars *Gallinarum* and *Pullorum*, which are respectively responsible for the fowl typhoid and the pullorum disease of chickens and are widely distributed throughout the world, especially in developing countries (Barbour *et al*., 2015). Pullorum disease caused by *S. pullorum* is mainly occurring at the first 2-3 weeks of age, but it can also affect adults (Shivaprashad and Barrow, 2008). Infected eggs are known to be intimately connected with the epidemiology of fowl typhoid and pullorum disease in chicken, particularly in terms of transmission from one generation to the next (Wigley *et al*., 2001). Poultry and poultry products are frequently mentioned as potential risk factor for human salmonellosis (Rahman *et al*., 2018). Vaccines form local isolates commercially still not available in the market for effective preventive measure. So, the control of the disease mainly relies on the use of antimicrobial drugs that leads to indiscriminate use of these drugs in poultry industry and making antibiotic resistance and limits the therapeutic possibilities the diseases. Antibiotics have been used in livestock and poultry to cure infections and enhance feed efficiency as well as to control and prevent infections (Tollefson and Miller, 2000). Poultry products are one of the most widely consumed foods worldwide, yet many critical antibiotics are utilized in their production in many countries endangering product safety (through antimicrobial residues) and increasing the risk of microbial resistance and dissemination in poultry settings (Agyare *et al*., 2018). Antimicrobial resistance (AMR) is a growing public health concern, especially with the introduction of multidrug-resistant (MDR) bacteria.

The prevalence and antibiogram profile of Salmonella infection had been widely reported all over the world including Bangladesh (Akter *et al*., 2007; Hossain *et al*., 2010 and Barua *et al*. 2012). Few investigations were conducted on the prevalence of salmonella in commercial poultry in South Bengal of Bangladesh (Sayeed *et al*., 2020). Nesarabad area of Pirojpur district is a commercial poultry rearing zone of South Bengal of Bangladesh. However, there was no published report on the detection of *Salmonella* spp. on this poultry zone before. So, isolation, identification and antibiogram studies are necessary for the prevention and control measures of salmonellosis in layer chickens. Therefore, the present study was designed to estimate the prevalence of salmonellosis in commercial layer chicken and assessof the antimicrobial resistance patterns of the *Salmonella* spp. in study area.

Materials and methods

Study area and duration

This study was conducted during the period from July 2019 to June 2020 in Nesarabad (Swarupkathi) upazila of Pirojpur district in different commercial layer farm (Figure 1). Samples were collected from the birds of selected layer farm and brought to the Department of Medicine Surgery & Obstetrics for laboratory

analysis. The birds were divided into three age groups as group A (15 to 30 weeks), group B (31 - 50 weeks old) and group C (above 50 weeks old). Birds were also divided according to flock size like small (≤ 1000 birds), medium (1001-2000 birds), large (2001-3000 birds).

Sample collection

A total of 200 blood samples (2 ml) were collected from 20 selected flocks from the wing vein of individual chickens. Blood was collected aseptically in sterile vial with sterile (3 ml) syringe. Then the samples were allowed to clotting in the syringe and kept for 1-2 hours at room temperature, after clotting, sera were separated, and poured in sterile vials, labeled individually and stored at -20°C until used.
In specimen, a total of 16 liver, 16 spleen and 16 intestinal swabs of dead chickens were immediately brought to the Department of Medicine, Surgery & Obstetrics, FANSVM, Patuakhali Science & Technology University, Babugonj, Barishal for bacteriological examination maintaining cool chain in ice box.

Rapid serum plate agglutination test

The rapid serum plate agglutination test was preformed according to the procedure described by OIE (2000) with crystal violet stained Salmonella antigen (Nobilis® SP antigen). For this test, 0.02 ml of antigen and 0.02 ml of chicken sera were placed side by side with a micropipette on a glass plate and was mixed thoroughly by stirring with stirrer stick followed by rocking. The results were observed within 2 minutes. In positive cases granules were formed slowly which was seen during rocking. In the absence of antibody, no such granules were formed. All rapid serum plate agglutination test results were recorded.

Isolation and identification of *Salmonella* spp.

Isolation and identification of *Salmonella* spp. was carried out using standard method. Homogenized preparation of visceral samples (liver, spleen and intestinal swab) were inoculated to nutrient broth and incubated at 37°C for 24 hours. Turbid nutrient broth was inoculated separately through inoculating loop onto selective SS agar media under aseptic condition and then incubated at 37°C for 24 hours (Johnson, 2004). The *Salmonella* spp. were isolated and identified by culture, Gram's staining and biochemical test. The cultural characteristics or colonial morphology of the *Salmonella* spp. grown on the SS agar media was recorded. Gram's staining was performed to study the morphology of the bacteria. Samples were sub cultured several times to get pure culture. In biochemical test, Triple Sugar Iron (TSI) phosphate agar was used to confirm the *Salmonella spp.* where acid production was indicated by the color change from red to black.

Figure 1. Location of study area

Antibiotic sensitivity test

Antibiotic susceptibility test of *Salmonella* isolates against eight commonly used antimicrobial agents by disc diffusion methods, as stated by the guidelines of Clinical and Laboratory Standard Institute (Humphries *et al*., 2018). Sensitivity and resistance of the isolates were determined against amoxicillin, oxytetracycline, gentamicin, ciprofloxacin, ceftriaxone, streptomycin, oxacillin and sulfamethoxazole-trimethoprim. The antimicrobial discs were placed onto the surface of Muller Hinton agar using sterile forceps, keeping a distance of about 24mm apart. The diameters of the zone of inhibition of each plate were measured using a meter ruler and recorded separately. Any isolate resistant to at least three classes or more of antimicrobials were considered as multidrug resistant (MDR) (Magiorakos *et al*., 2012).

Statistical analysis

All of the field and laboratory data were entered in the Microsoft Office Excel-2007 and transferred to R 4.1.3 (The R Foundation for Statistical Computing, 2022) for analysis. The risk factors for salmonellosis were identified using multivariable logistic regression analysis based on the previously described method (Sah et al., 2018)

Results and discussion

Postmortem examination of dead birds

Postmortem lesions were observed after skinning of dead birds and recorded the pathognomic lesions of salmonella infection in different organs such as-greenish to bronze color enlarged liver with small necrotic foci and/or congestion,engorgement of kidneys and spleen and enteritis of anterior small intestine (Calnek,1991)

Overall seroprevalence of salmonella infection in selected layer farms

The overall seroprevalence of salmonellosis in the layer farms in the study area was (116/200) 58% (95% confidence interval: 50.8; 64.8) (Figure3). Nath *et al*., (2015) also reported similar prevalence (60%) in commercial layer farm of Chittagong district, Bangladesh. Other studies also reported similar seroprevalence of *Salmonella* spp. in layer chicken (Sikder *et al*.,2005;Ashenafi *et al*., 2003;Sundar *et al*., 2007; Jalil and Islam, 2011). Morever, Islam *et al*. (2006) reported 43.4% seroprevalence of salmonellosis in layer chicken in Dhaka and Gazipur regions of Bangladesh. Bhattacharya *et al*. (2001) reported 37.7% seroprevalence in layer farms in India. However, some authors from different districts of Bangladesh and Ehiopia reported lower prevalence of salmonellosis than our finding (Alam *et al*., 2003;Sikder *et al*.,2005;Akter *et al*.,2007; Hossain *et al*.,2010;Barua *et al*.,2012; Kindu and Addis, 2013) . The variation might be due to differences in environmental, managemental and geographical location of the farm.

Prevalence of salmonella infection in different age groups of birds

The age-and floc size wise distribution of the salmonellosis seroprevalence of layer chicken were shown in Table 1. The estimated seroprevalence of salmonella infections were 37.5%, 55% and 71.25% in the age of 15-30 weeks, 31-50 weeks and > 50 weeks. The seroprevalence was significantly higher (71.25%) in > 50 weeks age (Odd ratio [OR]=4.5; 95% CI: 1.94; 10.3) than that of > 15-30 weeks age (37.5%) (Table 2). Sabuj *et al*. (2019) recorded highest seroprevalence of 68% at above 55 weeks age followed by 20% at 15-24 weeks age birds at Cox's Bazar, Bangladesh. Similarly Jalil and Islam (2011) recorded higher seroprevalence (76.6%) in layer chicken at 56 weeks of age than those of other age groups in Bangladesh. Hossain *et al*. (2010) also found the highest seroprevalence of salmonella infection (37.6%) at 64 weeks of age which supports the present findings. In addition, Sikder *et al*. (2005) reported 30.8% prevalence at 39 weeks of age and 13.3% at 32 weeks of age. Several authors reported increased seroprevalence of salmonellosis with increasing age of the birds (Islam *et al*., 2006; Akter *et al*., 2007;Barua *et al*., 2012; Nath*et al*., 2015). In contrast, Rahman *et al*. (2011) reported the higher prevalence of salmonellosis in grower chicken (52.6%) than layer chicken (38.4%). In present study, the increased seroprevalence of salmonellosis in adult chicken might be due to concurrent infection in the commercial poultry farms and lower body immunity to natural infection (Rahman *et al*., 2004).

The seroprevalence of *Salmonella* spp. in layer chicken were demonstrated on the basis of flock size where the highest occurrence in larger flock (74%) followed by medium Flock (59%) and small flock (40%) shown in Table 2. The seroprevalence of salmonella infection was also significantly higher (74%) in medium (OR=2.3;95% CI: 1.1; 4.7) and large flocks (OR=4.7; 95% CI: 1.9; 11.3) compared to small flocks (Table 2). According to present investigation, highest seroprevalence of *Salmonella* spp. (74%) in layer chickens were observed in large flocks followed by medium flocks (59%) and in small flocks (40%). Similar result was also observed by Sabuj *et al*. (2019) who recorded 54.28% seroprevalence in large flocks (≥2000) and 20% in small flocks (500-1000) in Cox's Bazar district. Hossain *et al*. (2010) reported higher seroprevalence (34.2%) of salmonella infection in large flocks (≥5001 birds) in comparison to small (≤1000 birds) flocks (21.3%) in Rajshahi district. Similar observation was also made by Jalil and Islam (2011) at Khulna district of Bangladesh. Mdegela *et al*. (2000) reported higher prevalence of salmonella infection in commercial flock (18.4%) than in scavenging chickens (6.3%) in Tanzania. The variation of infection prevalence in larger flock might be due to difficulties in management, faulty biosecurity, unhygienic environment, horizontal transmission of the bacteria at different geographical location.

Distribution of *Salmonella* spp. in dead layer chicken

The distribution of *Salmonella* spp. was 68.75% in liver, 25% in spleen, and 18.75% in intestinal swab of dead layer birds (Table 3, Figure 2,3). Ahmed *et al*. (2008) also reported 64% distribution of *Salmonella* spp. in liver of layer chicken of Mymensingh district in Bangladesh. Hossain *et al*. (2010) reported 15% and 5% distribution of *Salmonella* spp. in liver and intestine of layer flocks in Mymensingh, Bangladesh, respectively. Akter *et al*. (2007) reported 7.5% distribution of *Salmonella* spp. in liver of layer chicken. Islam *et al*., (2006) showed 35.1% distribution of *Salmonella* spp. in liver of layer chicken in Mymensingh, Bangladesh. In another study, Goldstein *et al*. (2001) reported that *Salmonella* spp. was distributed 41.7% in liver, 33.3% in spleen and 8.3% in cecum. The variation of occurrence of *Salmonella* spp. in different organ might be due to sample types, procedure of sample collection and method of isolation and identification of *Salmonella* spp.

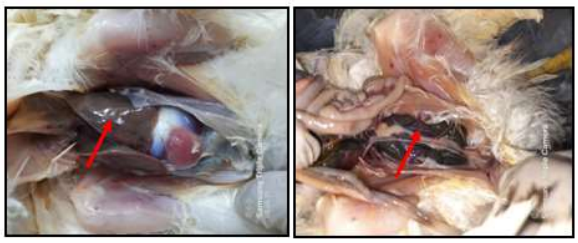

Figure 2: Postmortem lesion of dead chicken showing bronze color liver (left red arrow) and enlarged kidney (right red arrow)

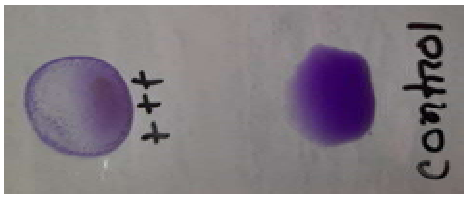

Figure 3: Rapid serum plate agglutination test of *Salmonella* spp.

Table 1. Seroprevalence of Salmonellainfection in different age groups and flock size of layer chicken

Variable	Category	No. of flocks	Tested	Positive				Prevalence (%)	P Value
				+++	++	+	Total		
Age (Week)	15-30	4	40	-	5	10	15	37.5	0.001
	31-50	8	80	7	15	22	44	55.0	
	Above 50	8	80	11	18	28	57	71.2	
Flock size	Small ≤1000	5	50				20	40	
	Medium 1001-2000	10	100				59	59	
	Large 2000-3000	5	50				37	74	0.002
Total	20		200				116	58	

N.B: +++ = High load-, ++ = Medium load-, + = Low load of *Salmonella* spp. in serum.

Table 2: Risk factors for salmonellosis in the layer birds of Pirojpur district

Variables	**Category**	Estimate	SE	Odds ratio (95% Confidence Interval)	P-value
Age (weeks)	15-30	Reference	-	-	-
	31-50	0.73	0.41	2.1 (0.93; 4.6)	0.07
	Above 50	1.49	0.42	4.4 (1.9; 10.3)	<0.001
Flock size	Small ≤1000	Reference	-	-	-
	Medium 1001-2000	0.83	0.36	2.30 (1.1; 4.7)	0.02
	Large 2000-3000	1.54	0.45	4.7 (1.9; 11.3)	<0.001

Table 3. Distribution of *Salmonella* spp. in liver, spleen and intestinal swabs of layer chicken.

Types of samples	No. of sample tested	No. of cases of salmonella positive	Distribution (%)
Liver	16	11	68.75
Spleen	16	4	25.00
Intestinal swab	16	3	18.75

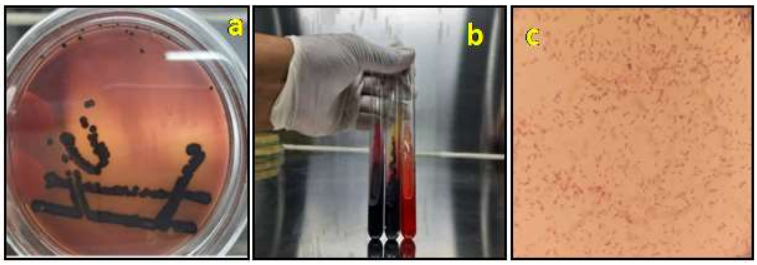

Figure 4: a) *Salmonella* spp. on SS agar showing black smoothsmall round colonies; **b)** *Salmonella* spp. on TSI agar showing black precipitate in both butt and slant (Right one is control); **c)** Gram's staining of *Salmonella* spp. showing Gram negative, short rod shapedarranged in single and paired.

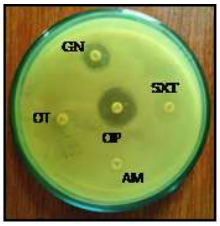

Figure 5: Antibiotic susceptibility test of*Salmonella* spp. showing sensitive to CIP = Ciprofloxacin and GN = Gentamicin and resistant to OT = Oxytetracycline, AM = Amoxicillin, SXT = Sulphamethaxole & Trimethoprim.

Table 4. Antimicrobial susceptibility test result in *Salmonella* spp.

Antibiotic disk	Concentration	No. of isolates %		
	(µg /disc)	Sensitive	Intermediate	Resistant
Amoxicillin	10	2(18.18)	0(0)	9(81.82)
Oxacillin	1	8(72.73)	3(27.27)	0(0)
Oxytetracycline	30	1 (9.09)	0(0)	10(90.91)
Ciprofloxacin	5	8(72.73)	0(0)	3(27.27)
Ceftriaxone	30	11(100)	0(0)	0(0)
Gentamicin	10	9(81.82)	1(9.09)	1(9.09)
Streptomycin	10	7(63.64)	4(36.36)	0
Sulphamethaxole & Trimethoprim	1.25	3(27.27)	1(9.09)	7(63.64)

Antibiogram study of *Salmonella* spp.

Based on the susceptibility to antibiotics, the *Salmonella* spp. were categorized into three groups- sensitive, intermediate and resistant. A total of eight antibiotics were used in this study; all the isolates of *Salmonella* spp. were highly (90.91%) resistant to oxytetracycline followed by amoxicillin (81.82%) and sulfamethoxazole-trimethoprim (63.64%). *Salmonella* spp. were highly sensitive to ceftriaxone (100%) followed by gentamicin (81.82%) and ciprofloxacin (72.73%) (Table 4; Figure 5). It has been reported that huge amount of antibiotics is used in commercial poultry farm to check morbidity and mortality as well as growth promoter. Extensive use of antibiotics in livestock has led to increased antibiotic resistance in various bacterial strains (Mölstad *et al*., 2017). *Salmonella* spp.isone of the MDR bacteria, showing resistance to ampicillin, streptomycin, chloramphenicol, sulfonamides and tetracycline (Guilfoile and Alcamo, 2007).Other findings have been documented that *Salmonella* spp. were resistant to penicillin (100%) and nalidixic acid (100%) followed by sulfamethoxazole-trimethoprim (55%), ampicillin(40%)andamoxicillin(25%) (Sarkar *et al*., 2021). The present study reported that *Salmonella* isolates were most sensitive to ceftriaxone (100%) followed by gentamicin (81.82%), ciprofloxacin (72.73%) and streptomycin (63.64%). The findings are similar with other studies in different parts of the world (Nesa *et al*., 2011; Obi & Ike, 2015; Fallah *et al*., 2013).

In present study, 11 *Salmonella* isolates were undergone for antibiogram study of which 7 were resistant to three or more classes of antibiotics. So, the MDR of *Salmonella*spp. in this study was 63.64 % (7/11). Similar observations were also made by other authors

(Zhu *et al.,*2017; Nghiem*et al.*,2017; Ahmed *et al*.,2014). But the prevalence of MDR varied from 50%-100% (Hasan *et al*., 2014; Aslam *et al*., 2012; Ifeanyichukwu *et al*., 2016) in commercial layer chicken including egg. Due to low cost and availability of oxytetracycline (Chopra and Roberts, 2001) and sulphonamide drugs are commonly used in commercial poultry farm for long time which may be responsible for MDR *Salmonella* spp. However, the emerging and dissemination of antimicrobial resistant salmonella in food animals has major public health implications, especially for large- scale suppliers who export their products both regionally and internationally (Zishiri *et al*., 2016). Higher MDR might be due to indiscriminate use of antibiotics in inappropriate dose. Therefore, rational use of antibiotics should be followed in all aspects of management as well as treatment purpose through registered veterinarian which would be reduced the MDR *Salmonella* spp. Retail chicken meat including liver, spleen could constitute a source of human exposure to MDR salmonella and further research should forces on impact of these MDR source on the human isolates (Nghiem et al., 2017).

Conclusions

The seroprevalence of salmonellosis in the layer bird of the study area seems to be very high. Aged layer birds of medium and large flocks should be targeted for future control and surveillance program. Antibiogram guided treatment may help prevent treatment failure and development of antimicrobial resistance.

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References

1. Agyare C, Boamah VE, Zumbi CN, Osei FB. Antibiotic use in poultry production and its effects on bacterial resistance. Antimicrobial resistance-A global threat. 2018; 1-20.
2. Ahmed AK, Islam MT, Haider MG, Hossain MM. Seroprevalence and pathology of naturally infected Salmonellosis in poultry with isolation and identification of causal agents. Journal of the Bangladesh Agricultural University. 2008; 6(2):327-34.
3. Akter MR, Choudhury KA, Rahman MM, Islam MS. Seroprevalence of salmonellosis in layer chickens with isolation, identification and antibiogram study of their causal agents. Bangladesh Journal of Veterinary Medicine. 2007; 5(1&2):39-42.
4. Alam J, Koike I, Giasuddin M, Rahman MM. Seroprevalence of poultry diseases in native chickens in Bangladesh. In9th Annual Scientific Conference of the Bangladesh Society for Veterinary Education and Research 2003; pp.6-7.
5. Ashenafi H, Shetu Y, Oldemeskel M. Identification of major infections of local chickens of Central Ethiopia. Bulletin of Animal health and Production in Africa. 2003; 51(2):95-101.
6. Aslam M, Checkley S, Avery B, Chalmers G, Bohaychuk V, Gensler G, Reid-Smith R, Boerlin P. Phenotypic and genetic characterization of antimicrobial resistance in Salmonella serovars isolated from retail meats in Alberta, Canada. Food Microbiology. 2012; 32(1):110-7.
7. Barbour EK, Ayyash DB, Alturkistni W, Alyahiby A, Yaghmoor S, Iyer A, Yousef J, Kumosani T, Harakeh S. Impact of sporadic reporting of poultry Salmonella serovars from selected developing countries. The Journal of Infection in Developing Countries. 2015; 9(01):001-7.
8. Barua H, Biswas PK, Olsen KE, Christensen JP. Prevalence and characterization of motile Salmonella in commercial layer poultry farms in Bangladesh. PloS one. 2012; 7(4):e35914.
9. Bhattacharya A, Majumder P. Fowl typhoid outbreak in broiler chick flocks in Tripura

and its control. Indian journal of animal sciences. 2001; 71(11):1034-5.
10. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and molecular biology reviews. 2001; 65(2):232-60.
11. Clanek BW, Barnes HJ, Beard CW, Mcdougald LR, Saif YM. Diseases of Poultry. 10th edn. Iowa State University Press, Ames, USA. 1991; pp. 81-130.
12. Fallah SH, Asgharpour F, Naderian Z, Moulana Z. Isolation and determination of antibiotic resistance patterns in nontyphoid Salmonella spp isolated from chicken. International Journal of Enteric Pathogens. 2013; 1(1):5-9416.
13. Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, Grady M, Liebert C, Summers AO, White DG, Maurer JJ. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrobial agents and chemotherapy. 2001; 45(3):723-6.
14. Guilfoile P, Alcamo IE. Antibiotic-resistant bacteria. Infobase Publishing; 2007.
15. Hamid MA, Rahman MA, Ahmed S, Hossain KM. Status of poultry industry in Bangladesh and the role of private sector for its development. Asian Journal of Poultry Science. 2017; 11(1):1-3.
16. Hassan MM, Amin KB, Ahaduzzaman M, Alam M, Faruk MS, Uddin I. Antimicrobial resistance pattern against E. coli and Salmonella in layer poultry. Res. J. Vet. Pract. 2014; 2(2):30-5.
17. Hossain KM, Hossain MT, Yamato I. Seroprevalence of Salmonella and Mycoplasma gallisepticum infection in chickens in Rajshahi and surrounding districts of Bangladesh. International Journal of Biology. 2010; 2(2):74-80.
18. Humphries RM, Ambler J, Mitchell SL, Castanheira M, Dingle T, Hindler JA, Koeth L, Sei K. CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. Journal of clinical microbiology. 2018; 56(4):e01934-17.
19. Ifeanyichukwu I, Chika E, Ogonna A, Chidinma I, Monique A, Ikechukwu M, Stanley E, Emmanuel N, Ngozi A, Agabus N. Prevalence and antibiogram of Salmonella species isolated from poultry products in Ebonyi State, Nigeria. Journal of Advanced Veterinary and Animal Research. 2016; 3(4):353-9.
20. Islam MM, Haider MG, Chowdhury EH, Kamruzzaman M, Hossain MM. Seroprevalence and pathological study of Salmonella infections in layer chickens and isolation and identification of causal agents. Bangladesh Journal of Veterinary Medicine. 2006; 4(2):79-85.
21. Jalil MA, Islam MT. Serological survey of Salmonella infection in non-vaccinated commercial layer birds in Khulna District of Bangladesh. Bangladesh Journal of Veterinary Medicine. 2011; 9(1):27-31.
22. Johnson TR. Laboratory Experiments in Microbiology, 12th Edition. 2004.
23. Kindu A, Addis M. A survey on Salmonella infection among chicken flocks in Jimma town, Ethiopia. African Journal of Microbiology Research. 2013; 7(14):1239-45.
24. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection. 2012; 18(3):268-81.
25. Mahmud MS, Bari ML, Hossain MA. Prevalence of Salmonella serovars and antimicrobial resistance profiles in poultry of Savar area, Bangladesh. Foodborne

pathogens and disease. 2011; 8(10): 1111–1118.
26. Mölstad S, Löfmark S, Carlin K, Erntell M, Aspevall O, Blad L, Hanberger H, Hedin K, Hellman J, Norman C, Skoog G. Lessons learnt during 20 years of the Swedish strategic programme against antibiotic resistance. Bulletin of the World Health Organization. 2017; 95(11):764.
27. Nath SK, Akter S, Dutta A, Sen AB, Chakrabarty R, Gupta MD. Prevalence and Antibiogram of Salmonella in Hisex Brown Strain at Commercial Poultry Farm in Chittagong. Int. J. Curr. Res. Biol. Med. 2015; 2(3):14-9.
28. Nesa MK, Khan MS, Alam M. Isolation, identification and characterization of salmonella serovars from diarrhoeic stool samples of human. Bangladesh Journal of Veterinary Medicine. 2011; 9(1):85-93.
29. Nghiem MN, Nguyen VT, Nguyen TT, Nguyen TD, Vo TT. Antimicrobial resistance gene expression associated with multidrug resistant Salmonella spp. isolated from retail meat in Hanoi, Vietnam. Int Microbiol. 2017; 20(2):85-93.
30. Obi OJ, Ike AC. Prevalence and antibiogram profile of salmonellae in intensively reared and backyard chickens in Nsukka Area, Nigeria. Nigerian Journal of Biotechnology. 2015; 30:18-25.
31. OIE (Office International Des Epizooties). Manual of standards for diagnostics test and vaccines. OIE Guide-2, Paris, France. 2000.
32. Rahman MA, Rahman AK, Islam MA, Alam MM. Detection of multi–drug resistant Salmonella from milk and meat in Bangladesh. Bangladesh Journal of Veterinary Medicine. 2018; 16(1):115-20.
33. Rahman MA, Samad MA, Rahman MB, Kabir SM. Bacterio-pathological studies on salmonellosis, colibacillosis and pasteurellosis in natural and experimental infections in chickens. Bangladesh Journal of Veterinary Medicine. 2004; 2(1):1-8.
34. Rahman MR, Shahinuzzaman AB, Saha AK, Sufian MA, Rahman MH, Hossain MM. Prevalence of Salmonella infection in naturally infected layer of birds in Bangladesh. Bangladesh Veterinarian. 2011; 28(1):8-18.
35. Sabuj AAM, Rahman M, Barua N, Haque ZF, Pondit A, Islam K, Hassan MM. Seroprevalence of Salmonella infection in commercial layer chickens in Cox's Bazar district, Bangladesh. American journal of microbiological Research. 2019; 7(1) 19-23.
36. Sarker BR, Ghosh S, Chowdhury S, Dutta A, Chandra Deb L, Krishna Sarker B, Sultana T, Mozaffor Hossain KM. Prevalence and antimicrobial susceptibility profiles of non-typhoidal Salmonella isolated from chickens in Rajshahi, Bangladesh. Veterinary Medicine and Science. 2021; 7(3):820-30.
37. Sayeed A, Islam B, Nahar N, Bari MS, Sultana S, Arfin S, Haldar PK, Islam A. Epidemiology of livestock and poultry diseases in Jhenaidah district of Bangladesh. Advances in Animal and Veterinary Sciences.2020; 8: 804-812.
38. Sah RP, Talukder MH, Rahman AA, Alam MZ, Ward MP. Seroprevalence of *Toxoplasma gondii* infection in ruminants in selected districts in Bangladesh. Veterinary Parasitology: Regional Studies and Reports. 2018; 11:1-5.
39. Shivaprasad HL. Fowl typhoid and pullorum disease. Revue scientifique et technique (International Office of Epizootics). 2000; 19(2):405-24.
40. Sikder AJ, Islam MA, Rahman MM, Rahman MB. Seroprevalence of Salmonella and Mycoplasma gallisepticum infection in the six model breeder poultry farms at Patuakhali district in Bangladesh. International journal of poultry science. 2005; 4(11):905-10.
41. Sikder AJ, Islam MA, Rahman MM, Rahman MB. Seroprevalence of Salmonella and Mycoplasma gallisepticum infection in the six model breeder poultry farms at Patuakhali district in Bangladesh. International journal of poultry science. 2005;4(11):905-10.

42. Skov MN, Angen O, Chriel M, Olsen JE, Bisgaard M. Risk factors associated with Salmonella enterica serovar typhimurium infection in Danish broiler flocks. Poultry science. 1999; 78(6):848-54.
43. Sundar J, Rai RB, Kundu A, Senani S, Chatterjee RN, Jeyakumar S. Seroprevalence of poultry diseases in Andaman and Nicobar Islands. Indian veterinary journal. 2007; 84(1):95-6.
44. Tollefson L, Miller MA. Antibiotic use in food animals: controlling the human health impact. Journal of AOAC international. 2000; 83(2):245-54.
45. Wigley P, Berchieri Jr A, Page KL, Smith AL, Barrow PA. Salmonella enterica serovar Pullorum persists in splenic macrophages and in the reproductive tract during persistent, disease-free carriage in chickens. Infection and immunity. 2001; 69(12):7873-9.
46. Zhu Y, Lai H, Zou L, Yin S, Wang C, Han X, Xia X, Hu K, He L, Zhou K, Chen S. Antimicrobial resistance and resistance genes in Salmonella strains isolated from broiler chickens along the slaughtering process in China. International Journal of Food Microbiology. 2017; 259:43-51.
47. Zishiri OT, Mkhize N, Mukaratirwa S. Prevalence of virulence and antimicrobial resistance genes in Salmonella spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. Onderstepoort Journal of Veterinary Research. 2016; 83(1):1-1.