**CHAPTER-I**

**1. INTRODUCTION**

Poultry farming is recognized profitable business in Bangladesh and getting popularity as employment opportunities. Over 80% of the country’s people live in the rural sector and highly dependent on agricultural system that is finely attuned to a tropical monsoon climate. A major portion (44%) of the population of Bangladesh lives below absolute poverty line and the number of landless poor has been increasing by 3.4% per annum (BBS, 2000).About 47.5%people receive less than 1900 calorie per person per day as against the standard 2300 calories **(**HDI,UNDP 1996).According to the Bangladesh Bureau of Statistics (BBS), about 89% of the rural households rear poultry. Thus, poultry industries play an important role in poverty alleviation and economic development of Bangladesh. This reflection has got in the recent years due to the raising of commercial poultry farms to meet the demand of poultry meat and egg resulted from the establishment poultry belt in Dhaka, Chittagong, Gazipur, and Narshingdi district. The poultry farming has dramatically increased in recent years in Bangladesh but disease is one of the main constrains for its development. Avian Colibacillosis and Salmonellosis has been found to be major infectious diseases of all ages of birds. The majority of economic loss results from mortality and decrease in productivity of the affected birds (Otaki, 1995).

Microbes are widely spread out in nature. The presence of resistant bacteria in food and food producing animals has led to much more question and assumption about the transmission of these bacteria from animals to humans. Infection caused by those resistant organisms, usually lead to a high fatality rate than especially among immune-comprised individuals (Holmberg *et al.,* 1984). The microbiological food safety is an increasing public health concern worldwide. *Escherichia coli* are one of the common microbial floras of gastrointestinal tract of poultry and human being **(**Jawetz *et al.,* 1984). Although most isolates of *Escherichia coli* are nonpathogenic but they are considered as indicator of fecal contamination in food and about 10 to 15% of intestinal coliforms are opportunistic and pathogenic serotypes (Barnes *et al.,* 1997) and cause a variety of lesions in immune-compromised hosts as well as in poultry. Infection with bacteria genus *Salmonella* are responsible for a variety of acute and chronic disease in poultry reported n Bangladesh (Bhattacharjee *et al.,* 1996).Nevertheless feed have been responsible for the infection of poultry with multidrug-resistance nontyphoid *Salmonella* in several industrialize countries (Karuiki *et al.,* 2002). Many epidemiological studies and research have implicated foods of animal origin as major vehicles associated with illnesses caused by *Escherichia* *coli*, *Campylobacter*, *Salmonella* and *Yersinia spp*. (Cretikos *et al.,* 2008). Antibiotics have been used successfully in poultry for different purposes such as growth promotion, prophylaxis, or therapeutics. However, their use in animal production and human therapy has resulted in increased bacterial resistance to many (Castanon, 2007). Antimicrobial resistance, the ability of microorganisms (notably bacteria) to withstand antimicrobial agents, is an important and growing public health issue. *Escherichia coli* is the primary causative agent of cellulitis, septicemia, and airsacculitis in poultry and *Salmonella* is the causative agent of pullorum disease, fowl typhoid and fowl paratyphoid (Gomis *et al.,* 1997).Therefore, these are the most significant poultry bacterial pathogen.

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 (Moreno *et al.,* 2000). 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 (Neu, 1992). It was stated by well established evidence that antibiotics can lead to the emergence and dissemination of resistant *E. coli* 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 (Van de Bogaard *et al.,* 2001; Schroeder *et al.,* 2002). At butchery/ slaughter, resistant strains from the gut readily contaminate poultry carcasses which often cause contamination of poultry meats and eggs during lay with multi resistant *E. coli* (Turtura *et al.,* 1990).

Due to enormous exploitation of antibiotics in the field of veterinary medicine, an increased number of resistant bacterial strains were developed in recent years. The transmission of plasmid mediated resistance between different bacterial species and genera are now widely occurred (Davies, 1994). In different parts of the world, multi drug resistant strains of *E. coli* are ubiquitous in both human and animal isolates (Amara *et al.,* 1995) and multiple drug resistant, nonpathogenic *E. coli* found in the intestine is probably an important reservoir of resistance genes (Osterblad *et al.,* 2000) and momentarily drug-resistant *E. coli* of animal origin may colonize the human intestine (Marshall *et al.,* 1990). Acquired multi drug resistance to antimicrobial agents creates an extensive trouble in case of the management of intra and extra intestinal infections caused by *E. coli*, which is a major source of illness, death, and increased healthcare costs (Gupta *et al.,* 2001). Therefore, the present study was designed to isolate *E. coli* strains from of poultry and poultry environment of Chittagong, Bangladesh for assessing their susceptibility and resistance patterns to some selected antimicrobials. Some previous studies described the high gene load of resistance determinants in the bacterial community in chicken litter (Lu *et al.,* 2003; Nandi *et al.,* 2004). Since bacteria acquire most resistance genes through horizontal transfer, conjugative genetic elements such as plasmids and transposons are common vectors for the dissemination of antimicrobial resistance genes to the diverse microorganisms. To achieve our aim, different bacterial isolates were tested for their susceptibility to different antimicrobials. Considering the above facts, present study was targeted to fulfill the following aims and objectives.

1. To isolate the *E. coli* and *Salmonella* strain from poultry sample.

2. To detect the antibiotic resistance patterns of *E. coli* and *Salmonella*

**CHAPTER- II**

**2. REVIEW OF LITERATURE**

2.1. Colibacillosis

2.1.1. History

Among the first reports of infections in poultry caused by coliform organisms were those of Gross (1994) and Huq (2002). Later Wray (2001**)** reported the isolation of *E. coli* from ‘air sac disease’. Pathogenic sero-groups of *E. coli* are common in the environments in which poultry are raised and may cause air sacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis, cellulitis or yolk sac infection. Collectively, these diseases constitute a major economic loss. Colibacillosis refers to any localized or systemic infection caused entirely or partly by *E. coli*, including septicemia, granuloma, air sac disease, chronic respiratory disease, avian cellulitis, swollen head syndrome, peritonitis, salpingitis, synovitis, panophthalmitis, and omphalitis. Colibacillosis in mammals is most often a primary enteric disease, whereas Colibacillosis in poultry is typically a secondary localized or systemic disease occurring when host defense has been impaired or overwhelmed (Barnes and Gross, 1997). Collectively, infections caused by *E. coli* are responsible for significant economic losses to the poultry industry. For example, 43% of broiler carcasses condemned for disease at processing had lesions consistent with *E. coli* septicemia (Yogaratnam, 1995).

2.1.2. Epidemiology

2.1.2. a. Etiology

*E. coli* is a gram-negative, non-acid-fast, uniform staining, nonspore- forming bacillus, usually 2-3 \* 0.6μm. The organism may be variable in size and shape. Many strains are motile and have peritrichous flagella. In one study (Barnes and Gross, 1997),57% of 607 isolates were motile.

2.1.2. b. Environmental distribution

The most important reservoir of *E. coli* is the intestinal tract of animals, including poultry. In chickens, there are about 109 colony forming units of bacteria per gram of feces. Of these, 106 CFU are *E. coli*, 10-15% of which are pathogenic sero-groups (Gross, 1994) and probably infect most mammals and birds thus having a cosmopolitan distribution. At times, coliforms may be transmitted between poultry and humans (Ojeniyi, 1989).Egg transmission of pathogenic *E. coli* is common and can be responsible for high chicken mortality. Pathogenic coliforms are more frequent in the gut of the newly hatched chicks than in eggs from which they hatched (Barnes and Gross, 1997), suggesting rapid spread after hatching. The most important source of egg infection seems to be fecal contamination of the surface with subsequent penetration of the shell and membranes. Coliform bacteria can be found in litter and fecal matter. Dust in poultry houses may contain 105-106 *E. coli*/g. These bacteria persist for long periods, particularly when dry (Barnes and Gross, 1997). Feed is often contaminated with pathogenic coliforms, but these can be destroyed by hot pelleting process. Rodent droppings often contain pathogenic coliforms. Pathogenic serotypes can also be introduced into poultry flocks through contaminated well water (Nagi and Raggi, 1972).

2.1.2. c. Incidence of Colibacillosis in Bangladesh

According to a retrospective analysis of chicken diseases diagnosed at Central Disease Investigation Laboratory (CDIL), Dhaka, among the bacterial diseases the incidence of avian Colibacillosis was the highest (Bhattacharjee *et al.,* 1996).

2.1.3. Disease syndromes

2.1.3. a. Yolk sac infection

The incidence of yolk sac infection is the highest when eggshell contamination occurs late in incubation and many affected embryos will die. As few as bacteria of virulent O1: K1 organisms may result in death of all embryos, following inoculation into the yolk sac (Siccardi.1966; Gross, 1994).

2.1.3. b. Respiratory tract infection

Respiratory disease complex, involving a secondary infection with *E. coli* (Huq, 2002), usually occurs between 2 and 12 weeks of age, with most losses occurring between 4 and 9 weeks. This is one of the most common poultry diseases with losses at times exceeding 20%. Economic loss results from reduced growth and feed efficiency, increased mortality and increased condemnation at processing. Poultry frequently inhale pathogenic *E. coli* in dust derived from feces, but the normal host defense prevents respiratory tract infection. However, following infection with respiratory tract agents such as New Castle Disease virus (NDV), Infectious Bronchitis Virus (IBV) and *Mycoplasma gallisepticum* alone or in combination, certain *E. coli* are able to establish in the respiratory tract (Gross, 1994).Vaccine viruses (NDV and IBV) are as important as the more virulent field strains **(**Gross,1994).High level of environmental stress also increases the severity of the respiratory infection (Gross, 1994).Several host and environmental factors influence susceptibility of chicks to *E. coli*. Resistance to *E. coli* was the greatest in a strain termed LA chickens (Gross, 1984a).Resistance to *E. coli* increased as the level of environmental stress increased until protection was close to complete. Further increases in the severity of environmental stress resulted in increased susceptibility (Gross, 1984a).Under very low level of stress, birds became extremely susceptible. Socialization also resulted in increased resistance (Gross and Siegel, 1982).Exposure to ammonia and dust resulted in declination of the epithelium of the respiratory tract, which allowed coliforms to invade (Nagaraja *et al.,* 1984). Control of the disease by preventing the predisposing respiratory infections has been much more successful than treatment of the secondary *E. coli* infection. *Mycoplasma* *gallisepticum* has been eradicated from all commercial breeding stocks and is seldom seen under good management conditions. Most respiratory viruses now resemble the vaccine strains (Alexander *et al.,* 1987) and the severity of these viral infections can be reduced by raising birds under a relatively low level of environmental stress and by socializing the birds to their handlers. In some birds, respiratory tract infection is not controlled and the *E. coli* infection becomes bacteraemic. In most bacteraemic birds, infection spreads to the myocardium and later to the pericardial sac. Myocardial infection results in changes in the electrical conductivity of the myocardium resulting in major changes in the electrocardiogram (Gross, 1994).

2.1.3. c. Acute septicaemia of chickens

Acute *E. coli* septicemia is an infection of mature chickens characterized by a firm dark or greenish liver and congested pectoral muscles. Sometimes small necrotic foci can be seen on the liver. The crops are usually full and the birds are in good flesh. In some cases pericarditis and peritonitis are also present.

2.1.3. d. Salpingitis

When *E. coli* infects the left abdominal air sac, females may develop chronic salpingitis characterized by a large caseous mass in a dilated, thin/walled oviduct. The caseous mass contains necrotic heterophils and bacteria that persist for months. Size of the caseous mass may increase with time. Affected birds frequently die during the first 6 months post infection; those surviving rarely lay eggs. Salpingitis may also occur following entry of coliform bacteria from the cloaca in laying hens, ducks and geese (Bisgaard, 1995).

2.1.3. e. Peritonitis

Coliform infection of the peritoneal cavity occurs in laying hens and ischaracterized by acute mortality, fibrin, and free yolk. Infection occurs when bacteria through the oviduct grow rapidly in yolk material that has been deposited in the peritoneal cavity (Gross, 1994).

2.1.3. f. Swollen head syndrome

Swollen Head Syndrome (SHS) is characterized by an oedematous swelling, containing a diffuse cellulitis, over the eye of broilers, broiler breeders and in commercial layers. *E. coli* can be isolated from the lesions (O’Berien, 1985).Disease appears to require previous infection with a previously unknown coronavirus, and infection could be reproduced following a combined *E.coli* coronavirus infection.

2.1.3. g. Cellulitis

Cellulitis (sometimes known as necrotic dermatitis) of the lower abdominal wall below the vent and thighs of broilers does not result in mortality of clinical signs, but the presence of fibrinious plaques under the skin results insubstantial losses through condemnation or downgrading of carcasses (Vaillancourt *et al*., 1992).

2.1.3. h. Enteritis

A few reports have suggested that *E. coli* may be a cause of enteritis in poultry. The most universal presence of pathogenic sero-groups of *E. coli* in the intestinal tracts of poultry is not associated with any disease. Poultry withsevere septicaemic infections often have watery, yellowish droppings. These seem tobe associated with rapid reductions in bodyweight. Outbreaks of diarrhoeal diseaseassociated with enterotoxigenic *E. coli* occur rarely and have been reported from thePhilippines (Joya *et al.,* 1990). A heat-labile enterotoxin (LT) similar to LT fromhuman enterotoxigenic *E. coli* has been recovered from poultry strains (Tsuji *et al.,* 1994).A severe haemorrhagic typhlitis results from the oral inoculation of *E. coli* into *Eimeria brunette* infected chickens (Nagi and Mathey, 1972). Nakamura*et al.,* (1990)have reported dual infection with *E. coli* and *Eimeria tenella.*

2.1.4. Isolation and Identification of *E. coli*

Isolation of *E. coli* from heart, liver and spleen was first reported by Lignieres in 1894 (Palmer, 1923) between 1938 and 1965, coligranuloma and the role of *E. coli* in a variety of infections, including air sac disease, arthritis, planter abscesses, omphalitis, panopthalmitis, peritonitis and salpingitis were identified and described (Sojka, 1965).

2.1.4. a. Gross lesions

Gross (1994) and Samad (2005) categorized the various pathological manifestations as yolk sac infection, air sac disease, bacteraemia, salpingitis, peritonitis, swollen head syndrome, cellulitis, enteritis, synovitis and osteomyelitis. Except for cellulitis and yolk sac infection, these conditions represent different manifestations of infection with the same *E. coli* implicated in avian septicemic Colibacillosis.

2.1.4. b. Staining properties of *E. coli*

This organism is gram negative, uniform staining, non spore forming bacillus, may be variable in size and shape (Calnek, 1997).

2.1.4. c. Colony morphology of *E.* *coli*

After incubation for 24 hours at 37°C, On MacConkey agar: large pink coloured colonies. On Eosine Methylene Blue (EMB) agar: the colonies have a metallic sheen (Altwegg and Bockemiihi, 1998).

2.1.4. d. Biochemical character of *E. coli*

In vitro biochemical characterization of *E. coli* isolates revealed variable rates of carbohydrate fermentation and amino acid decarboxylation (Cloud *et al.,* 1995; Goswami *et al.,* *2002)* sero-typed *E. coli* isolates by biochemical and sugar fermentation test. Perimal Roy *et al.,* (2004) performed biochemical characterization of *E. coli* isolates by lactose fermentation tests and IMVIC methods.

.

2.2. Salmonellosis

2.2.1. History:

The genus *Salmonella* (of the family Enterobacteriaceae) named for the eminent United States Department of Agriculture veterinarian and bacteriologist Daniel E. Salmon, consist of more than 2300 serologically distinguishable variants (Gast, 1997). Towards the end of the 19th century, infectious enteritis causing heavy mortality in chicken was described in Europe and North America (Jordan and Pattison, 1996). Initially the causal agent was called *Bacillus gallinarum* and the name fowl typhoid was applied in 1902 (Shivaprashad, 1997). *Salmonella* *pullorum* was first isolated from chicks suffering from severe diarrhea and was described by Rettger and Stoneburn in 1909 (Marchant and Packer, 1983). The disease had been previously known as bacillary white diarrhoea (BWD), but as white diarrhea is not always a clinical feature, it becomes known pullorum disease (Jordan and Pattison, 1996).

2.2. 2. Epidemiology

2.2. 2. a. Etiology

The disease is caused by gram negative bacteria known as *Salmonella* pullorum. This organism belongs to a family known as enterobacteriaceae. Organism is motile and looks like slender rod measuring 0.3-0.5×1-2.5μm. It is nonliquefying, non-chromogenic, non-sporogenic facultative anaerobe (Snoeyenbos and Willims, 1994). It grows on beef agar or broth very readily. MacConkey agar can be very used for growth. The organism is non-lactose fermentater. The organism is resistant to heat and many chemicals. In suitable environment the organism contains a thermostable toxin. *S. gallinarum* is a short bacillus 1-2μm broad, which does not posses flagellae (Jordan and Pattison, 1996). Pullorum disease is caused by bacterium S.pullorum (Shivaprasad, 1997).In addition to *S. gallinarum, S. pullorum*, other Salmonallae such as *S. enteritidis, S. panama and S. Dublin* also belongs to the sero-group D1 (Le Minor, 1984). The various motile and non hosts adapted highly invasive serotypes such as *Salmonella* enteritidis and *Salmonella tytyphimurium* are commonly referred to as paratyphoid *Salmonella*e (Gast, 1997).

2.2. 2. b. Environmental Distribution

Salmonellosis is a serious systemic disease of domestic poultry which cause large scale economic losses through mortality, morbidity and reduction in egg production (Junior *et al.,* 2000). The disease occur sporadically and enzotically in most countries of the world including Bangladesh. It causes severe economic losses of the poultry with morbidity and mortality varying in chicken from 10-50% or more (Pomeroy, 1984). Salmonellosis is distributed in many countries of the world, and has economic significance (Barrow *et al.,* 1992).They are mainly distributed in Latin America, the Middle East, the Indian Subcontinent, Africa and perhaps other part of the world Shivaprasad, (1997). Salmonellosis has also been reported in many countries of South-East Asia including Bangladesh (Bhattacharjee *et al,*.1996 and Begum *et al*,. 1993), India (Ghosh, 1988; Kumar and Kaushik, 1988),Pakistan (Javed and Hameed, 1989; Muneer *et al*,. 1988) and Nepal (Jha *et al.,*  1994).Salmonellosis is common in both backyard chickens and in commercial poultry (Fricker, 1987).

2.2. 2. c. Incidence in Bangladesh

Fowltyphoid and pullorum disease are the most common disease in Bangladesh (Haque *et al.,*1997).According to a previous retrospective study the prevalence of Salmonellosis in Bangladesh is 9.28% (Bhattacharja *et al.,* 1996).

2.2. 3. Mode of Transmission:

The infection spreads in two ways (a) Vertical Transmission and (b) Horizontal Transmission. The vertical transmission takes place through the infected eggs. Extensive dissemination of infection may occur during hatching from infected embryos to non-infected chicks. The horizontal transmission takes place through contaminated utensils, contaminated water, contaminate feed, diseased pullets, dead embryos, dead chicks, infected eggs, cannibalism of infected birds, and egg eating, visitors’ rodents and Flies etc (Shivaprasad, 1997).

2.2. 4. Disease Syndromes

2.2. 4. a. Pullorum Disease

Bacillary white diarrhoea (BWD) is the synonym of pollorum disease. This is an acute systemic infection disease of chicks which is chronic in form in adult birds. The baby chicks sustain a heavy mortality within initial few weeks of life. Adults may remain as carrier.

2.2. 4. b. Fowl Typhoid

*Salmonella* gallinerum is the synonyms of fowl typhoid and also called as infectious leukaemia. It is an infectious septicemic disease of domestic fowls and Turkeys characterizes by acute manifestation having high mortality. Acute form is widely prevalent by chronic form is not uncommon in poultry farm.

2.2. 5. Isolation & Identification of *Salmonella*

*Salmonella* organisms were most frequently encountered in fowls (Simmons *et al.,* 1963).In India, 25 serotypes have been so far isolated from poultry (Khera, 1968).The caeca have long been considered the primary source of *Salmonella* in the chicken(Fanelli *et al.,* 1971).

2.2.5. a. Gross lesions

Grey nodules in one or more of the following sites: lungs, liver, gizzard wall, heart, intestinal wall, peritoneum etc. May there petechial haemorrhage or foci of necrosis in the liver (Barnes *et al*,. 1989) along with bronze discoloration (Samad, 2005). On necropsy, muscle degeneration or necrosis, hepatomegaly, spleno-megaly, airsacculitis, gastroenteritis and nephropathy. Numerous yellow necrotic foci are often present in organs (Altman *et al.,* 1997).

2.2. 5. b. Staining properties of *Salmonella*

This organisms are gram negative, slender rods, mostly occur singly but occasionally two or more can be found in smear preparation (Calnek, 1997).

2.2. 5. c. Colony morphology of *Salmonella*

On Nutrient Agar: The organisms produce smooth, glistering, opalescent colonies. On MacConkey Agar and Deoxychoclate Agar: appear colorless colonies (Jordan and Pattison, 1999). On S.S. Agar it produces smooth, blackish colonies (Samad, 2005).

2.2. 5. d. Biochemical Character of *Salmonella*

In TSI agar it produces acidic (Yellow) butt and alkaline slant (Red) with blackening due to production of H₂S gas (Waltman *et al.,* 1998)

2.3. Antibiotic Resistance in *E. coli, Salmonella* and Avian Zoonosis

The targeted selective toxicity of antimicrobial agents has ensured their widespread use to combat infection; however, it has paradoxically resulted in the emergence and dissemination of multi drug resistance zoonotic bacterial pathogens (Carattoli, 2001). Antibiotic resistance represents a significant challenge of global dimensions to human and veterinary medicines with prospect of therapeutic failure for life-saving treatments now a reality. Akinbowale *et al.,* (2006) reported that bacteria from the aquatic sources and environment were found resistant to different types of antibiotics to a great extent, even significant level of multi-drug resistance also observed. This indicating the highest possibility of transfer of resistance gene from aquaculture isolates to human pathogens, some assessment of risk of transfer of resistant organisms to humans via the food chain and the threats imposed by environmental contamination with antibiotic resistant bacteria.

Barton, (2004) reported that *E coli* strains showed widespread resistance to tetracycline and moderately common resistance (30-60%) to ampicillin and sulphadiazine. Resistances to more than one antibiotic were common. Barton also reported in 2000 that the development of antibiotic resistance in bacteria has been linked to the use of antibiotics in agriculture in overseas studies, particularly forintensively housed species such as pigs, poultry and feedlot cattle. Biswas *et al.,* (2001) reported that 100% of his poultry *E. coli* isolates were resistant to tetracycline but 72% isolates were found to susceptible to Gentamycin but 20% were found resistant to Gentamycin.

Alam *et al.,* (2006) reported about the *E. coli* from the aquatic sources in Bangladesh. He reported that Resistance was commonly observed against Penicillin-G (94%), Tetracycline (65%), Ampicillin (75%) and Trimethoprim-sulpfamethoxazole (49%). On the other hand, most of the strains were sensitive to Ciprofloxacin (76%), Chloramphenicol (70%), Ceftazidime (92%) and gentamicin 97%. Eighty-eight percent of the Tetracycline-resistant strains were also resistant to penicillin-G and Ampicillin. Sixty-nine percent of the strains were resistant to more than four drugs and 24% were resistant to more than seven drugs.

Jesus *et al.,* (1997) indicated increasing incidences of antibiotic-resistant *E. coli* strains isolated from chickens with Colibacillosis. However, the high percentage of *E. coli* strains that were resistant to Trimethoprim-sulfamethoxazole (67%) and to thenew fluoroquinolones (13 to 24%) in our study was surprising. Ellen K. Silbergeld in2007 reported that occupational exposure to antimicrobial-resistant *E. coli* from liveanimalcontact in the broiler chicken industry might be an important route of entry forantimicrobial-resistant *E. coli* into the community.Germon *et al.,* (2005)reported that the *ibeA* gene, which encodes a virulence factor of *E. coli* strains that can cause neonatal meningitis in humans were recently detected in avian pathogenic *E. coli* (APEC). Caya *et al.,* (1999)reported that the Virulence determinants common to both APEC and human isolates ExPEC (extraintestinal pathogenic *E. coli*) were previously identified, leading to the conclusion that APEC are potential human pathogens. Rahman *et al.,* (2009)reported that 150 *Salmonella* isolates were 100% sensitive to Gentamycin followed by Amoxicillin (90%), Colistin (70%), Co-trimoxazole (60%) and Furazolidone (40%) but the isolates were highly resistant to Norfloxacin, Flumequine, Ciprofloxacin and Enrofoxacin. The study demonstrated that the *Salmonella* gallinarum were more sensitive to Gentamycin than Amoxycillin or Colistin.

Molla *et al.,* (2003)reported that fifty-one (63.7%) of the 80 *Salmonella* strains were resistant to one or more antimicrobials of which 42 (52.5%) displayed multiple-drug resistance. Among the strains, 51.2% were resistant to sulfisoxazole, 46.2% to spectinomycin, 45% to amoxicillin-clavulanic acid and ampicillin, 41.2% to tetracycline and 30% to chloramphenicol. Less than 27.5% of the strains showed resistance to florfenicol, streptomycin, cotrimoxazole and to trimethoprim. *S.* *typhimurium* var. Copenhagen (100%), *S. anatum* (62.5%), *S. typhimurium* (33.3%) and *S. braenderup* (34.3%) showed multiple antimicrobial resistance to up to eight antimicrobials. None of the strains were resistant to amikacin, apramycin, gentamicin, kanamycin, neomycin, tobramycin, quinolones, cephalosporins and nitrofurantoin.They also indicated the potential importance of chickens as source of multiple antimicrobial-resistant *Salmonella* for human infections. Humphrey, (2000)mentioned that a wide range of food borne illness attributable to *Salmonella* enterica. Poppe, (2000)mentioned that Poultry is widely acknowledged to be a reservoir for *Salmonella* infections in humans due to the ability of *Salmonella* to proliferate in gastrointestinal tract of Chicken and subsequently survive on commercially processed Broiler carcasses and edible giblets.

Akond *et al.,* (2009) conducted an experiment on 50 identified strains of *E. coli* and were subjected to 13 antimicrobial agents to check their susceptibility. 88%, 82%, 80%, 76%, 70%, 68%, 64%, 58%, 52%, and 20% of the tested *Escherichia coli* strains from poultry sources were found resistant respectively to Penicillin, Ciprofloxacin, Riphampicin, Kanamycin, Streptomycin, Cefixine, Erythromycin, Ampicillin, Tetracycline, and Chloramphenicol and Neomycin. None of the strains showed resistance to Norfloxacin and Gentamicin. Sensitivity was recorded in case of 86%, 80%, 60%, 36%, 30%, and 26% of the strains to Norfloxacin, Gentamicin and Chloramphenicol, Neomycin, Tetracycline, Streptomycin and Ampicillin, respectively. Both, resistance and susceptibility were exhibited against Chloramphenicol, Ampicillin, Gentamicin, Neomycin, Tetracycline, Streptomycin and Norfloxacin. Multi drug resistance was recorded in case of 6-10 antibiotics for all strains tested.

Gregova *et al.,* (2012) investigate the antibiotic resistant *E. coli* strains isolated from bioaerosols and surface swabs in a slaughterhouse as a possible source of poultry meat contamination. The highest air coliforms contamination was during shackling, killing and evisceration of poultry. The strains showed resistance to ampicillin (89%), ceftiofur (62%) and cefquinome (22%), while resistance to ampicillin with sulbactam was only 6%. Resistance to streptomycin and gentamicin was detected in 43% *vs.* 14% isolates; to tetracycline 33%; to chloramphenicol and florfenicol in 10% vs. 18% isolates; to cotrimoxazol in 35% isolates; to enrofloxacin in 43 % isolates. Hemen *et al.,* (2012) conducted a study aimed at isolating and identifying *Shigella*, *Salmonella* and *Escherichia coli* bacteria associated with poultry litter. The antibiotic sensitivity patterns of the isolated bacteria tested against Septrin, Chloranphenicol, Sparfloxacin, Ciprofloxacin, Amoxacillin, Augmentin, Gentamycin, Pefloxacin, Tarivid and Streptomycin. *Shigella* and *Salmonella* were completely resistant to chloranphenicol, augmentin, pefloxacin, amoxicillin. *Shigella* were also resistant to all the antibiotics except Septrin and ciprofloxacin showed they are intermediate to the drugs. Percentage antibiotics susceptibility pattern of gram negative bacteria isolated from poultry litter showed all bacterial isolates (100%) were resistant to Chloranphenicol while most of the isolates were susceptible to Amoxacillin. All the bacterial isolates showed high level (10.2 MAR index) antibiotic resistance.

Barua *et al.,* (2012) suggested that antibiotic should not be used in the growth promotion of the poultry farm and the use of antibiotics by the respective users need to be monitored properly in order to avoid the emergence of antibiotic resistance in bacteria.

Begum *et al.,* (2010) mentioned 100% isolated strains *of Salmonella* were found to be sensitive Ceftriazone, Ciprofloxacin, Cephalexin, Gentamycin and Chlorampenicol. On the other hand, strains have shown resistance to Co-trimoxazole, Nalidixic acid, Ampicillin, Tetracyclin and Kanamycin. However, it was found that strains isolated from intestinal and environmental were more antibiotic resistant than egg isolated. Hayes *et al.,* (2004) mentioned that he prevalence of resistance among isolates of *E. faecalis was* comparatively higher among lincosamide, macrolide, and tetracycline anti-microbials, while isolates of *E. faecium* were observed to be more frequently resistant to fluoroquinolones and penicillins. Notably, 63% of the *E. faecium* isolates were resistant to the streptogramin quinupristin-dalfopris-tin, while high-level gentamicin resistance was observed only among the *E. faecalis* population, of which 7% of the isolates were resistant.

Islam *et al.,* (2008)stated that 50% isolates of *S. typhi*and 83.33% isolates of *S. paratyphi A* were multidrug resistant. All of the isolates of *S. typhi* were sensitive(100%) to Aztreonam Amikacin and Gentamycin and all of the isolates of *S. paratyphi A* were sensitive (100%)to Aztreonam, Amikacin, Cefaclor, Cefixime, Ceftazidime, Ceftriaxone, Gentamycin, Mecillinam. All of theisolates of *Salmonella typhi* and *Salmonella paratyphi A* were resistant to Nalidixic acid (100%). In additionisolates of *S. paratyphi A* were also resistant to Azithromycin, Netilmicin. Decreased susceptibility of *S. typhi* and *S. paratyphi A* was observed in case of ciprofloxacin 73.33% and 70% respectively.

**CHAPTER-III**

**3. METHODOLOGY OF THE STUDY**

3.1 Study area and duration

The study was conducted on layer poultry at Chittagong District, which is one of the most concentrated poultry areas of Bangladesh, during the period of September to December, 2012.

3.2 Diagnosis of Disease

Diagnosis of disease was made on the basis of post mortem examination and standard microbiological examination, using standard methods for bacterial identification described by OIE, (2000); Bains *et al.,* (1979); Mack and Bell, (1990).

3.3 Study Population

A total of 30 dead birds from different layer farms of Chittagong were subjected to postmortem during the study period at PRTC laboratory, Chittagong.

3.4 Post-mortem Examination

The post mortem examinations were performed using standard operation procedure described by PPIA, 2009.

3.5 Sample Collection

The liver sample was collected aseptically and used for microbiological test.

3.6 Isolation and Identification of Collected Samples

Isolation and identification of bacteria was done by using the method described by Collins and Lyne (1976). Culturing of various selective media and examination of colony characteristics and observation of the organisms under microscope was done to isolation and identification of *E. coli* and *Salmonella* organisms.

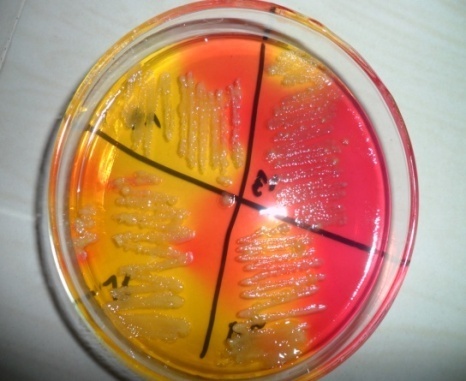
3.6.1 Culturing on Agar Media

For Suspected cases of Collibacilosis, inoculation from both broth sample and swab sample culturing were done at MacConkey Agar and Eusin Metheline blue (EMB) agar. After overnight incubation the bacterial growth was observed as pink colonies at McConkey and Metallic sheen colonies at EMB agar. Both lactose fermenting and non lactose fermenting colonies were found. *Salmonella pullorum* and *Salmonella gallinarum* both the organisms will grow on differential plating media such as MacConkey and SS Agar. It has been shown that *Salmonella pullorum* occasionally fails to grow on certain selective media such as Briliant Green or *Salmonella*-Shigella Agar but grows satisfactorily on Bismuth Sulfite and McConkey Agars (Carlson *et al.,* 1974). Confirmatory diagnostic was done by culturing on selective media such as Xylose- lysine- deoxycholate(XLD) Agar and Brilliant Greeen agar (BGA) Agar and observation of colony characteristics such as black centered pale pink colony and Red-pink-white opaque coloured colonies surrounded by brilliant red zones, respectively.

****

**Fig 2: Metallic sheen colony in EMB agar (*E. coli).***

**Fig1: Striking of culture on selective media.**

****

**Fig3 Pink colony on ManConkey agar *(E. coli****).*

**Fig4 Black centre colony on XLD agar (*Salmonella****).*

**Fig 5: Pale pink colony On BGA agar (*Salmonella****).*

3.9.2. Biochemical Tests

For confirmation of *E. coli*, biochemical tests were performed. Flat, pink colonies were suspected as the colonies of *E. coli*. More confirmation was made by biochemical tests of pink colored colonies. Various biochemical tests were done for identification of the isolates as described by Edward and Ewing (1972) and Cruickshank *et al.,* (1995). Notable performed bio-chemical tests were SIM (Sulfied Indole Motility Test), TSI (Triple Sugar Iron) Agar stab and Urea. Bio-chemical test at TSI agar stab was selected for *Salmonella.*Red slant and Yellow butt with blackening media of *Salmonella* at TSI agar stab was observed on biochemical test. These tests are enough to diagnose the *Salmonella* and relatively less expensive and less time consuming, than other expensive serological tests. So, these methods were selected for Salmonellosis.



Fig 7: Red slant and yellow butt with blackening media at TSI agar stab (*Salmonella*).

Fig 6: Indole test positive *(E. coli)*

3.9.2 Antibiotic Sensitivity analysis

The antibiotic sensitivity of the isolated strain at different concentration was performed by using standard paper disc diffusion method described by NCCLS, (2009). Antibiotics selected for susceptibility testing included a panel of antimicrobial agents of interest to the poultry industry and human public health authorities. From the range of antimicrobial drugs, 10 were selected on the basis of their range of activity against enterobacteria and on their use in local poultry farming. Veterinary antibiotics were chosen due to their use as therapeutic, prophylactic or growth promoting agents in poultry industry. Human antibiotics were selected on the basis of their use and /or importance in human medicine. The following antibiotics and disc potencies were used: GEN: Gentamicin (10μg), DO: Doxycycline (30μg), CIP:Ciprofloxacin (5μg), ENR: Enrofloxacin (5μg), AMC: Amoxycillin (10μg), N: Norfloxacin (10µg), CL : Colistin (10μg), TE: Tetracycline (30µg), Pf: Pefloxacin (10µg), K: Kanamycin (30µg) from HIMEDIA Ltd (Mombai, India). The antibiotic susceptibility tests were performed in Muller-Hinton agar by micro-disc diffusion techniques. By the standard method of inoculation, the top of a single and well-isolated colony was touched with a sterile loop and the growth was inoculated into 2ml of Mueller-Hinton broth. The broth culture was then allowed to incubate at 37°C for 4 hours to obtain the young culture. The turbidity of actively growing broth cultures was then adjusted to a 0.5 McFarland standard and then a sterile cotton swab was dipped into the adjusted suspension within 15 minutes and excess broth was purged by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then spread evenly over the entire surface of the plate of LB agar to obtain uniform inoculums. The plates were then allowed to dry for 3-5 minutes. Antibiotics impregnated discs were then applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. Even distribution of discs and minimum distance of 24 mm from center to center were ensured. Five discs were placed in each petridish. Within 15 minutes of the application of the discs, the plates were inverted and incubated at 37°C. After 16-18 hours of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate and resistant categories according to the interpretation table of the Becton Dickinson Microbiology Company, USA. Measurement of the growth inhibition zone permitted the classification of each isolates as susceptible, intermediate and resistant according to data provided by CLCI. The result of antibiotic sensitivity test was then recorded, analyzed and discussed.





Fig 8: **Comparing the dilution with**

**Fig 9: Placing Antibiotic disc on Muller Hinton agar**.

**McFarland nephalometer standard.**



**Fig 9: Measuring of growth inhibition zone diameter**.

Fig 8: **CS test showing Gentamycin sensitivity of *E. coli* isolates**.

**CHAPTER -IV**

**4. RESULT AND DISCUSSION**

4.1 Isolation of *E.coli* and *Salmonella*

A total of 13 individual colonies of *E.coli* and 8 individual colonies of *Salmonella* were isolated from poultry liver samples through different test. Nutrient broth sample were patched in the EMB and MacConkey agar plate for the isolation of *E. coli* from poultry sample. Characteristics Metallic sheen on the EMB agar plate and Pink colony on the MacConkey agar plates of the isolates assure the presence of *E. col*i. The isolated colonies were identified by conventional biochemical test. Isolation of *Salmonella*, sample from Nutrient broth was patched on the XLD and BGA agar plates. Characteristics black centre pink colony on the XLD agar plates and Pink white colony on the BGA agar plates of the isolates assure the presence of *Salmonella*. Experimental results are summarized in Table-1.

Table-1: Identification test result of *E. coli* and *Salmonella* sp.

|  |  |  |
| --- | --- | --- |
| Test | Test result |  |
|  | E.coli | *Salmonella* |
| Colony character  EMB | Metallic sheen colony | -- |
| MacConkey | Pink colony | -- |
| BGA | **--** | Pink white colony |
| XLD | **--** | Black centre pink colony |
|  |  |  |
| Biochemical test |  |  |
| Lactose fermentation test | **+** | **\_** |
| Indole test | **+** | **\_** |
| Presumptive test (TSI) |  |  |
| Slant | -- | Alkaline(red) |
| Butt  Gas | --  -- | Acidic(Yellow)  + |
|  |  |  |

+ Positive result, - Negative result, -- Not performed.

4.2 Antimicrobial resistance pattern analysis

Table-2(a): Antimicrobial resistance pattern against *E. coli*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **Antibiotic Disc** | | | | | | | | | |
| **GEN** | **CL** | **CIP** | **PF** | **DO** | **N** | **TE** | **K** | **ENR** | **AMX** |
| **EC 1** | S | R | R | R | S | S | R | R | R | R |
| **EC 2** | **S** | S | R | R | S | S | R | R | R | R |
| **EC 3** | S | R | R | R | S | S | R | R | R | R |
| **EC 4** | S | R | R | R | R | R | R | R | R | I |
| **EC 5** | S | S | R | R | S | R | R | R | R | R |
| **EC 6** | S | S | R | R | S | S | R | S | R | R |
| **EC 7** | S | R | R | R | S | R | R | S | R | R |
| **EC 8** | S | R | R | R | R | S | R | I | R | R |
| **EC 9** | S | R | R | R | R | S | R | I | R | R |
| **EC 10** | S | R | R | R | R | S | R | R | R | R |
| **EC 11** | S | S | R | R | R | S | R | R | R | R |
| **EC 12** | S | S | R | R | R | S | R | I | R | S |
| **EC 13** | S | S | R | R | R | S | R | I | R | R |

R= Resistance; I= Intermediate; S= Sensitive; GEN= Gentamycin; CL= Colistin; CIP= Ciprofloxacin; PF= Pefloxacin; DO= Doxucyclin; N=Neomycin; TE= Tetracycline; K= Kanamycin; ENR= Enrofloxacin; AMX=Amoxicillin

Table-2(b): Prevalence of antimicrobial resistance pattern against *E. coli* isolates

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Antibiotics** | **Isolates** | **Pattern** | | |  |  |  | | |
| **Resistance (%)** | **Intermediate (%)** | **Sensitive (%)** |  |  |  |
| Ciprofloxacin | 13 | 100 | 0 | 0 |  |  |  |  |
| Enrofloxacin | 13 | 100 | 0 | 0 |  |  |  |  |
| Pefloxacin | 13 | 100 | 0 | 0 |  |  |  |  |
| Tetracycline | 13 | 100 | 0 | 0 |  |  |  |  |
| Amoxicillin | 13 | 84.62 | 7.69 | 7.69 |  |  |  |  |
| Kanamycin | 13 | 69.24 | 15.38 | 15.38 |  |  |  |  |
| Colistin | 13 | 53.75 | 0 | 46.15 |  |  |  |  |
| Doxycycline | 13 | 53.75 | 0 | 46.15 |  |  |  |  |
| Neomycin | 13 | 23.08 | 0 | 76.92 |  |  |  |  |
| Gentamycin | 13 | 0 | 0 | 100 |  |  |  |  |

Gentamycin, Doxycycline, Tetracycline, Amoxicillin, Enrofloxacin, Colistin, Pefloxacin, Neomycin, kanamycin and Ciprofloxacin are the antibiotics chosen in the study as these antibiotics are still using in both veterinary and human medical practices. *E. coli* isolates were tested for resistance against 10 mentioned antibiotics. Resistances were observed against Doxycycline (53.75%), Tracycline (100%), Amoxicillin (84.62%), Enrofloxacin (100%), Ciprofloxacin (100%), Pefloxacin (100%), Colistin (63.75%) and Kanamycin (69.24%). All the isolates of present study exhibited multiple resistances to more antibiotics. A similar finding on multiple drug resistance of *E. coli* strains has been reported from Bangladesh and other parts of the world (Guerra *et al.,* 2003; Khan *et al.,* 2002). Due to indiscriminate exploitation of antimicrobial agents, such high incidence of multi drug resistance may apparently be occurred which may ultimately replace the drug sensitive microorganisms from antibiotic saturated environment (Van de Boogard and Stobberingh, 2000). High levels of sensitivity were found against Gentamycin (100%) and Neomycin (76.92%).Those above-mentioned isolates had considerable sensitivity to Colistin (46.15%). Present study was agreed with the previous study, where the poultry *E. coli* isolates were found resistant to tetracycline (Biswas *et. al.,* 2001). On the other hand, environmental *E. coli* isolates resistance was observed 65% against Tetracycline (Alam *et. al*., 2006). So, poultry isolates showing a variable resistance to Tetracycline in comparison with Environmental *E. coli* isolates. We suspected high level of resistance against Tetracycline because most of the farmers used commercially available tetracycline in the poultry feed regularly. The farmers used antibiotics largely for three purposes in poultry farms: therapeutic use to treat sick flock; prophylactic use to prevent infections in the flock; as growth promoters to improve feed utilization and production (Barton, 2000). Besides, isolates were sensitive 100% to Gentamycin. On the other hand, most of the environmental strains were 97% sensitive to Gentamicin (Alam *et al.,* 2006). Tricia *et al.,* (2006) reported 43% isolates of *E. coli* were resistant to ampicillin but no isolate was found resistant to gentamicin. So, the results of Gentamycin sensitivity in poultry isolates were mostly similar with Environmental *E. coli*. Gentamycin is still a choice of medication in both veterinary and human practice in Bangladesh. But to prevent the emergence of resistance in human pathogenic *E. coli,* Gentamycin use should be restricted from the view of public health concern. Eighty-eight percent (88%) of the tetracycline resistant strains were resistant to penicillin-G and ampicillin. Sixty-nine percent of the strains were resistant to more than four drugs and 24% were resistant to more than seven drugs (Alam *et al.,* 2006). Present study detected high frequency of resistance against Tetracycline (100%), Pefloxacin (100%) and Kanamycin (69.24%), which is similar with another study, where high frequency of resistance was found against Tetracycline, Kanamycin and Pefloxacin (Hemen *et al.,* 2004). Many strains were resistant to several antibiotics, but no pattern predominates (Brenda *et. al.,* 2005). Amoxicillin is a broad-spectrum antibiotic, but we have found highest (84.62%) resistance against this antibiotic. Data was not available about the high level of resistance against Amoxicillin of *E. coli* isolates in Bangladesh. It might be due to indiscriminate use of Amoxicillin in poultry sector of Bangladesh.

Graph-1: Antibiotic resistance pattern of *E. coli* (n=13)

We have found highest resistance pattern of *E. coli* isolates against Enrofloxacin, and Ciprofloxacin, which is dissimilar with study conducted on broiler chickens by Rahman *et al.,* (2004). It might be due to more use of Enrofloxacin and Ciprofloxacin in poultry industry of Bangladesh. The findings of this study indicating that there has been a significant increase in antimicrobial resistance in the *E. coli* affecting the chickens. It’s really a matter of concern about emergence of multi-drug resistance in avian isolates. Antibiotic resistant in human and animal pathogens is now an issue of global concern. While it is clear that most resistance problems in human pathogens originates from over-use and misuse of antibiotics in hospitals and community medical practices, in recent years there has been increasing recognition of the contribution of resistance bacteria in animals to the medical problem. None the less, evidence is mounting that antibiotic resistance enteric bacteria (for example, *E. coli, Salmonella, campylobacter and enterococci*) can transfer from animals to man via the food chain or by direct contact, leading to the establishment of a community reservoir of resistance genes (Van den Bogaard and Stobberingh, 1999). There are many reasons behind the emergence antibiotic resistance in poultry *E. coil*. In Bangladesh poultry litter are used in land for crop production and as well in pond for profitable fish production. Sometimes the dead poultry are dropped into pond where catfish are reared. In this way the *E. coli* from poultry that are resistant to antibiotics can be viable in the water sources. These waters are most of the times used for drinking and cooking purposes. In this way the avian *E. coli* can be transmitted in human.

Table-3 (a): Antimicrobial resistance pattern against *Salmonella*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **Antibiotic Disc** | | | | | | | | | |
| **GEN** | **CL** | **CIP** | **PF** | **DO** | **N** | **TE** | **K** | **ENR** | **AMX** |
| **Sa 1** | S | R | R | S | R | S | R | S | R | R |
| **Sa 2** | **S** | S | S | R | S | S | R | R | R | R |
| **Sa 3** | S | R | R | R | S | S | R | S | R | R |
| **Sa 4** | S | R | R | R | R | S | R | R | R | R |
| **Sa 5** | S | S | R | R | R | S | R | R | S | R |
| **Sa 6** | S | S | R | R | R | S | R | S | R | R |
| **Sa 7** | S | R | R | R | R | S | R | R | R | R |
| **Sa 8** | S | S | R | R | R | S | R | I.S | R | R |

R= Resistance; I= Intermediate; S= Sensitive; GEN= Gentamycin; CL= Colistin; CIP= Ciprofloxacin; PF= Pefloxacin; DO= Doxucyclin; N=Neomycin; TE= Tetracycline; K= Kanamycin; ENR= Enrofloxacin; AMX=Amoxicillin

Table-3(b): Prevalence of antimicrobial resistance pattern of *Salmonella* isolates

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibiotics** | **Isolates** | **Pattern** | | |
| **Resistant (%)** | **Intermediate (%)** | **Sensitive (%)** |
| Ciprofloxacin | 08 | 87.5 | 0 | 12.5 |
| Enrofloxacin | 08 | 87.5 | 0 | 12.5 |
| Pefloxacin | 08 | 87.5 | 0 | 12.5 |
| Tetracycline | 08 | 100 | 0 | 0 |
| Amoxicillin | 08 | 100 | 0 | 0 |
| Kanamycin | 08 | 50 | 12.5 | 37.5 |
| Colistin | 08 | 50 | 0 | 50 |
| Doxycycline | 08 | 50 | 25 | 25 |
| Neomycin | 08 | 0 | 0 | 100 |
| Gentamycin | 08 | 0 | 0 | 100 |

Resistances of *Salmonella* isolates was observed against Amoxicillin and Tetracycline to 100%, followed by Ciprofloxacin (87.5%), Enrofloxacin (87.5%), Pefloxacin (87.5%), Doxycyclin (50%), Colistin (50%) and Kanamycin (50%). Drug sensitivity pattern of *Salmonella* showed highly sensitive to Gentamycin and Neomycin. Gentamycin is exceptional aminoglycosidic antibiotic which is bacteriocidal for both gram positive and negative bacteria. *Salmonella* isolates are most sensitive to Gentamycin among all antibiotics. The present study was agreement with Rahman *et al.,* (2009). We have also found highest sensitivity against Neomycin, which was supported by Molla *et al.,* (2003). On the other hand, highest resistance was found against Amoxicillin and Tetracycline. Similar type of finding was observed by Hemen *et al.,* (2012). Besides high rates of resistance to Teracycline, Ampicillin, chloramphenicol and trimethoprim sulfamethoxazole have been reported from many areas of the world (Su *et al.,* 2004). Highest resistance against Amoxicillin and Tetracycline in case of both *E. coli* and *Salmonella* isolates is great threat to poultry industry. There is a disturbing general trend in *Salmonella* sero-vars being resistant to commonly used antimicrobials.Antimicrobial resistance among *Salmonella* isolatesis increasing worldwide and is likely due to the widespreaduse of antimicrobial agents for the empiric treatment and as growth enhancers in animal production (Bukitwetan *et al.,* 2007). The increasing rate of resistance to amipicillin, tetracycline and chlorampenical since 1996 among isolates from human, pigs, and chickens can be attributed to the emergence of multi resistance serovar Typhimurium (Van duijkeren, 2003).

Graph-2: Antibiotic resistance pattern of *Salmonella* (n=08)

Present study showed that, the *Salmonella* isolates had less sensitivity to Doxycycline (25%), which is supported by Shivhare *et al.,* (2001). The findings of our study showed that ,the high resistance against Ciprofloxacin, Enrofloxacin and Pefloxacin (87.5%) were supported by Hemen *et al.,* (2012). It might be due to more use of Enrofloxacin and Ciprofloxacin in poultry industry of Bangladesh. The variation in the sensitivity of antibiotic of the isolates of *Salmonella* from infected chicken may be due to indiscriminate or common use of these antibiotics as feed additives or growth promoters or as curative purpose as repoted by Jindal *et al.,* (1999). Out of the all tested antibiotic *Salmonella* isolates had moderate level of resistance to Colistin (50%) and Doxycycline (50%), this finding agrees with the report of drug resistance of *Salmonella* species isolated from poultry (Verma *et al.,* 1993; Bokanyi *et al.,* 1990).

Higher resistance of *Salmonella* isolates was due to rapid development of chromosomal mutation and irrational use of these drugs (Akond *et al.,* 2009**).** This implies that widespread use of antimicrobials in animals or humans may cause an increase in the frequency of occurrence of bacteria resistant to other antimicrobials as the R plasmid may encode resistance to additional antimicrobials (Poppe *et al.,* 2004).Due to indiscriminate exploitation of antimicrobial agents, such high incidence of multi drug resistance may apparently be occurred which may ultimately replace the drug sensitive microorganisms from antibiotic saturated environment (Van de Boogard and Stobberingh 2000). The high level of antimicrobial resistance of *Salmonella* isolates and multiple antimicrobial resistances of *Salmonella* serotypes in this study was probably due to indiscriminate and widespread uses of the commonly available antimicrobials both in the veterinary and public health practices. Multidrug-resistant strains of *Salmonella* are now encountered frequently worldwide and the rates of multidrug-resistance have increased considerably in recent years. Even worse, some variants of *Salmonella* have developed multidrug-resistance as an integral part of the genetic material of the organism, and are therefore likely to retain their drug-resistant genes even when antimicrobial drugs are no longer used, a situation where other resistant strains would typically lose their resistance (WHO, 2005).

**CHAPTER- V**

**5. CONCLUSION**

Present research findings showed that, the source of single and multiple antimicrobial-resistant *E. coli* and *Salmonella* isolates to frequently used antimicrobials including Pefloxacin, Doxycycline, Tetratracycline, Amoxicillin, Enrofloxacin, Ciprofloxacin and Kanamycin. Multidrug resistance *E. coli* and *Salmonella* has become a widespread pathogen in Bangladesh. More prudent use of antimicrobial agents in farm animals and more effective disease prevention on farm are necessary. Therefore, it is important to monitor the occurrence of resistance among bacteria from animals and food, as these bacteria are able to spread through food product to human. Finally, comprehensive study or research should be need to characterized the various serotypes of *E. coli* and *Salmonella* and identify the prevalence and pattern of antimicrobial resistance.

**REFERENCES**

Akinbowale O., Peng H., Barton M. D. (2006).Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. *J Appl Microbiol.,* 100: 1103-1113.

Akond M. A., Alam S., Hassan S. M. R., Shirin M. (2009). Antibiotic Resistance of *Escherichia Coli* Isolated From Poultry and Poultry Environment of Bangladesh. *Int. J. of Food Safety*, 11: 19-23.

Akhtar N., Hakim M. A. (2012). Isolation and Characterization of Multidrug Resistant *Escherichia coli* and *Salmonella spp* from the Wastes of Hospital Patients andPoultry. *J. of Phar. and Bio. Sci.,* 4: 29-36

Alam M., Nur-A-Hasan., Ahasan S., Pazhani G. P., Tamura K., Ramamurthy T., Gomes D. J., Rahman S. R., Islam A., Akhtar F., Shinoda S., Watanabe H., Faruque S. M., Nair B. (2006).Phenotypic and molecular characteristics of Escherichia coli isolated from Aquatic Environment of Bangladesh. *Microbiol. Immunol.,* 50 **(**5): 359-370.

Ali M. J. (1999).Current Status of Veterinary Biologics Production in Bangladeshand Their Quality Control Proceeding of BSVER Symposium held on July 28, 1999 at NIPSOM Auditorium, Mohakhali, Dhaka, Bangladesh.

Amara A., Ziani Z., Bouzoubaa k. (1995). Antibioresistance of Escherichia coli strains isolated in Morocco from chickens with colibacillosis. *Vet. Microbiol*., 43: 325-330.

Alexander D.J., Manvell R.J., Kemp P.A., Parsons G., Collins M. S., Brckman S., Russell P. H., Lister S. A., (1987).Use of monoclonal antibodies in thecharacterization of avian paramyxovirus type 1 (Newcastle disease virus)isolates submitted to an international reference laboratory. *Avian Pathology*, 16:553 – 565.

Barton M. D. (2004).Antibiotic susceptibility of veterinary isolates and antimicrobial Susceptibility Testing: Methods and Practices with an Australian Perspective.Australian Society for Microbiology, Sydney Australia (in press).

Barua A., Mahmud A. S. M., Khan M. S., Taznin T., Haque M. E., Islam F., Akhtar N., Hakim M. A. (2012). Isolation and Characterization of Multidrug Resistant *Escherichia coli* and *Salmonella spp* from the Wastes of Hospital Patients andPoultry. *J. of Phar. and Bio. Sci.,* 4: 29-36.

Barry A. L., Thornsberry C. (1985). Susceptibility Test, Diffussion test procedure. *J. Chem. Pathol*., 19: 492-500.

Barnes H.J., Gross W. B. (1997). Colibacillosis in Diseases of Poultry. 10th ed. B. W. Calnek, (Ed.), Mosby-Wolf Publication Ltd., London, UK, pp.131–139.

Bangladesh Bureau of Statistics (BBS) (2000).Agriculture sample Survey of Bangladesh-2005. Planning Division, Ministry of Planning, and Government of peoples Republic of Bangladesh.

Biswas P. K., Faruque R., Ahmed S., Dey V. C. ( 2001).Antibiotic Sensitivity Pattern of Pathogenic *Escherichia coli* isolated from Fayoumi chicken. *Bangladesh J. Microbiol.,* 18(2): 121-126.

Bauer A.W., Kirby W. M. M., Sheris J. C., Truck M. (1966).Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*., 145**:**225-230.

Brenda J., Allan Jan V., Hurk van de, Andrew A. (2005). Characterization of *Escherichia coli* Isolated from Cases of Avian Colibacillosis, Western Meeting of Poultry Clinicians and Pathologists, Veterinary Infectious Disease Organization, Alberta, Canada. www.canadianpoultry.ca

Barton M. D. (2000).Antibiotic use in Animal feed and its impact on human health. *Nutri. Res. Reviews,* 13: 279-299.

Barnes H. J., Gross W. B. (1997).Colibacillosis. In: Diseases of poultry, 10th ed. B. W.b calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University, Press, Ames, IA. pp. 131-141.

Bass L., Liebert C. A., Lee M. D., Summers A. O., White D. G., Thayer S. G., Maurer J. J. (1999).Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. *Antimicrob. Agents Chemother*., 43: 2925–2929.

Begum k., Reza T. A., Haque M., Hossain A., Hasan F. M. K., hasan S. N., Akter N., Ahmed A., Barua U. (2010). Isolation, Identification and antibiotic resistance pattern of *Salmonella Spp*. From chicken egg, intestine and environmental samples. *Bangl. Pharma*. *J.,* 13(1):23-27.

Bukitwetan P., Suryawidjaja J.E., Salim ChO., Hidayat A., Herwana E., Lesmana M. (2007). Serovar distribution and antibiotic susceptibility of nontyphoidal *Salmonella* isolated from pediatric patientsin Jakarta, Indonesia. *Southeast Asian J Trop Med Public Health*. 38(6):1088-1094

Bhattacharjee P. S., Kundu R. L., Biswas R. K., Mazumder J. U., Hossain E.,Miah A. H. (1996).A retrospective analysis of chicken diseases diagnosed at the Central Disease Investigation Laboratory, Dhaka. *BangladeshVet. J*., 30 (3 – 4): 105 – 113.

Bhattacharjee A., Majumder P. (2001).Fowl Typhoid outbreak in Broiler chick flocks in Tripura and its control. *Indian J. Anim. Sci****.,*** 71:1034-1035.

Bisgaard M. (1995).Salpingitis in web-footed birds: Prevalence, etiology and significance. *Avian Pathology*, 24: 443-452.

Bokanyi J. R. R. P., Stephens, Foster D. N. (1990).Isolation and Characterization of *Salmonella* from Broiler carcasses or parts. *Poultry Science*, 69**:** 592-598.

Cretikos M., Telfer B., McAnulty, J. (2008). Enteric disease outbreak reporting, New South Wales, Australia, 2000 to 2005. *NSW Public Health Bullettin*, 19(1-2), 3-7.

Castanon J. I. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science*, 86: 2466–2471.

Calnek B.W. (1997). Diseases of Poutry, 10th Edition, Iowa State University Press. Ames, Iowa, USA. pp: 83-89, 131, 151-153.

Cheville N. F., Arp. L. H. (1978).Comparative pathologic findings of *Escherichia coli* infection in birds*. J. Am. Vet. Med. Assoc*., 173:584-587.

Cruickshank R.,bDuguid J. H., Marimion B. P., Swain R. H. A. ( 1995).*In* Medical Microbiology, 12th end. Vol-II, Churchill Livingstone, London.

Carattoli A. (2001).Importance of integrons in diffusion resistnce. *Vet. Res*., 32:243-259.

Colins C. H., Lyne P. M. (1976).In Microbiological Methods. 4th edn. Laboratory Techniques Series. Buttenworths, London.

Cooke E. M., Breaden A. L., Shooter R. A., O’Farrell S. M. (1971).Antibiotic sensitivity of *Escherichia coli* isolated from animals, food, hospitalpatients, and normal people. *Lancet,* 2: 8-10.

Davies J., (1994). [Inactivation](http://medical-dictionary.thefreedictionary.com/inactivation) of antibiotics and the dissemination of resistance genes. *Science,* 264: 375-382.

Edward R. R., Ewing W. H. (1972).*In* Identification of Enterobacteriaceae. 3rd ed. Burgeross Pub. Co. Minneapolis, Minnesota.

Ellen K. Silbergeld. (2007).Poultry workers may spread antibiotic resistant *E. coli.* Environmental Health Perspectives. (www.foodproduction.com).

Franklin A. (1984).Antimicrobial drug resistance in procine enterotoxigenic *E.coli* of O group 149 and non-enterotoxigenic *E. coli* .*Vet. Micro***.,** 9:467 475.

Germon P., Yu-Hua Chen., Lina He., Jesus E., Blanco., Annie Bree., Catherine Schouler., Sheng-He Huang., Maryvonne Moulin-Schouleur. (2005).*ibeA* a virulence factor of avian pathogenic *Escherichia coli. Microb.*, 151: 1179-1186.

Gomis S. M., Goodhope R., Kumor L., Caddy N., Riddell C., Petter A. A., Allan J. J. (1997).Experimental reproduction of *Escherichia coli*, cellulitis andsepticemia in broiler chickens. *Avian Dis*., 41:234–240.

Gupta K., Hooton T. M., Stamm W. E. (2001). Increasing antimicrobial resistance and the management of uncomplicated communityacquired urinary tract infections. *Ann. Int. Med*., 135: 41-50.

Gross W.G. (1994).Diseases due to Escherichia coli in poultry. In Gyles C.L., ed. *E.coli* in domestic animals and humans. Wallingford: CAB International; 237- 259.

Gregova G., Kmetova M., Kmet V., Venglovsky J., Feher A. (2012). Antibiotic resistance of *Escherichia coli* isolated from a poultry slaughterhouse. *Ann. Agric. Environ. Med.*, 19(1): 75-77.

Gross W.B. (1984a).Combined effects of deoxycorticosterone and furaltadone on *Escherichia coli*. *American J. Vet. Res.,* 45: 963 – 966..

Guerra B., Junker E., Schroeter A., Malorny B., Lehmann S., Helmuth R. (2003). Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and poultry. *J. Antimicrob. Chemother*., 52 (3): 489-92.

Hemen J. T., Johnson J. T., Ambo E. E., Ekam V. S, Odey M. O., Fila W. A. (2012). Multi-Antibiotic Resistance of Some Gram Negative Bacterial Isolates from Poultry Litters of Selected Farms in Benue State*. Int. J. Sci. Tech*., 2(8):543-547.

Holmberg S. D., Osterholm M. T., Senger K. A., Cohen M. L. (1984). Drug Resistance Salmonella from animals feed antimicrobial. *New Eng. J. Med.,* 331:617-22.

Hui A. K., Das R. (2001).Studies on Isolation, serotyping and antibiotic sensitivity of *Salmonella* isolated from ducks. *Indian Vet. J*., 78:1058-1059.

Huq M. R. (2002**).** Longitudinal Study of the Causes of Mortality of Chickens in Parent Stock Flocks of the Department of Livestock Services (DLS) of Bangladesh with a Special Emphasis on *Esherichia coli* Infection. (M. Sc. Thesis**).**

Islam M. J., Das K. K., Sharmin N., Hasan M. N., Azad A. K. (2008). Antimicrobial Susceptibility of *Salmonella* Serovars Isolated from Blood. *J. innov. dev. Strategy,* 2(2): 22-27).

Jesus E., Blanco M., Mora A., Blanco J. (1997).Prevalence of Bacterial Resistance to Quinolones and Other Antimicrobialsamong Avian *Escherichia coli* Strains Isolatedfrom Septicemic and HealthyChickens in Spain. *J. Cli. Microbiol.,* **35** (8): 2184–2185

Jindal N., Raja N., Kumar S., Narang G., Mahajan N. K. (1999).*Salmonella gallinarum* and *Salmonella enteritidis* infection in poultry in some parts of Haryana*. Ind. Vet. J*., 76:563-564.

Joya J. E., Tsuji T., Jacaline A.V., Arita M., Tsukamoto T., Honda T., Miwatani, T. (1990).Demonstration of enteropathogenic *Escherichia coli* indiarrheic broiler chicks. *European J. Epi.*, 6: 88 – 90.

Joshua R., Hayes., Linda L.,English., Lewis E., Carr., David D., Wagner., Sam W. Joseph. (2004), Multiple-Antibiotic Resistance of *Enterococcus* spp. Isolated from Commercial Poultry Production Environments. *Appl. Environ. Mnicrobiol,* 70(10): 6005–6011.

Jawetz E., Melnick J., Adelberg E. A. (1984). Review of Medical Microbiology. 16th ed. Los Altos, California: *Long Medical Publication*, pp. 122-144.

Kamal A. H. M., Hossain M. I., Baki B. A. (1988).Pathological investigation on the mortility of chickens in BangladeshAgriculcurul University Poultry Farm. *Bangladesh Vet. J.,* 9:20-25.

Kariuki S., Revathi G., Gakuya F., Yamo V., Muyodi J., Hart C. A. (2002). Lack of clonal relationship between non-typhoid *Salmonella* strain types human and those isolated fromanimals living in close contact. *FFMS Immunol. Med. Microbial*., 33:165-171.

Khan A., Das S. C., Ramamurthy T., Sikander A. (2002). Antibiotic Resistance, Virulence Gene, and Molecular profile of Shiga Toxin-Producing Escherichia coli Isolates from Diverse Source in Calcutta, *India. J. Clin. Microbiol.,* 40(6): 2009-2015

Lu J., Sanchez S., Hofacre C., Maurer J. J., Harmon B. G., Lee M. D. (2003).Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. *Appl. Environ. Microbiol.,* 69:901– 908.

Lee M. D., Sanchez S., Zimmer M., Idris U., Berrang M. E., McDermott P. F. (2002).Class 1 integron-associated tobramycin-gentamicinresistance in Campylobacter jejuni isolated from the broiler chicken houseenvironment. *Antimicrob. Agents Chemother*., 46:3660 – 3664.

Lance B. P., Jay P., Graham., Leila G., Lackey., Roess A., Vailes R., Silbergeld E. (2007).Elevated Risk of Carrying Gentamicin-ResistantEscherichia coli among U.S. *Poultry Workers, Environ Health Perspect*., 115(12): 1738-1742.

Marshall B. D., Petrowski., Levy S. B. (1990). Inter- and [intraspecies](http://www.thefreedictionary.com/intraspecies) spread of Escherichia coli in a farm environmental in the absence of antibiotic usage. *Proc. Natl. Acad. Sci.,* 87: 6609-6613.

Molla B., Mesfin A., Alemayehu D. (2003).Multiple antimicrobial-resistant *Salmonella* serotypes isolated from chicken carcasses and giblets in Debre Zeit and Addis Ababa, Ethiopia. *Ethiop. J. Health Dev****.,*** 17(2): 131-149.

Moreno M.A., Dominguez L., Teshoger T., Herrero I. A., Porrero M. E. (2000). Antibiotic resistances monitoring: The Spanish Programme*. Int. J. Antimicrob. Agents*., 14: 285-290.

Nandi S., Maurer J. J., Hofacre C. L., Summers A. O. (2004).Gram positive bacteria, major reservoir of class 1 antibiotic resistance integrons in poultry litter. *Proc. Natl. Acad. Sci. USA,* 101:7118–7122.

Nijsten R., London N., Van den Bogaard A., Stobberingh E. (1993).Antibiotic resistance of *Enterobacteriaceae* isolated from faecal flora of fattening pigs.

NCCLS (2009**).** Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A6. Wayne, Pa: National Committee for Clinical Laboratory Standards.

Nagi M. S., Raggi L.G. (1972).Importance to air sac disease of water supplies contaminated with pathogenic *Escherichia coli*. *Avian Diseases*, **16**: 718 –723.

Nagi M. S., Mathey W. J. (1972).Interaction of *Escherichia coli* and *Eimeria brunetti* in chickens. *Avian Diseases***,** 16**:** 864-873.

Nagaraja K. V., Emery D. A., Jordan K. A., Sivananda V., Newman J. A., Pomeroy B. S. (1984).Effect of ammonia on the quantitative clearance of *Escherichia coli* from lungs, air sacs, and livers of turkeys aerosol vaccinated against *Escherichia coli*. *American J. of Vet. Res.*, 45:392-395.

Neu H. C., (1992). The crisis in antibiotic resistance. *Science,* 257: 1064-1073.

Osterblad M., Hakanen A., Manninen R., Leistevuo T., Peltonen R., Meurman O., Huovinen P., Kotilainen P. (2000). A between-species comparison of antimicrobial resistance in enterobacteria in fecal flora. *Antimicrob. Agents Chemother.,* 44: 1479-1484

Otaki Y. (1995).Poultry disease control programme in Japan. *Asian livestock*, 20:65-67.

O’Berin. (1985).Swollen head syndrome. *Vet. Record*., 117: 619 - 620.

Peighambari S. M., Vaillancourt J. P., Wilson R. A., Gyles C. L. (1995).Characteristics of *Escherichia coli* isolates from avian cellulitis. *Avian**Dis*., 39:116–124.

Poppe C.(2000). *Salmonella* infections in the domestic fowl. pp.107-132,in:C. . Wray and A. Wray (eds). *Salmonella in Domestic Animals*. New York, NY:CAB International.

Rahman M. (2003). “Growth of Poultry Industry In Bangladesh Poverty Alleviation and Employment Opportunity” in 3rd International Poultry Show and Seminar, held on February 28-March-2, 2003, Organizes by World’s Poultry Science Association, Bangladesh Branch, Dhaka, pp.1-7.

Rahman M. M., Hossain M. K., Aziz F. B., Akter M. R. (2009).Comparative Study of Some Antibacterial Drugs Available in Market Against Field Isolates of *Salmonella gallinerum* of Poultry. *Int. J. Ani. Fish. Sci.,* 2(1):122-125.

Samad M. A. (2005). Poultry Science and Medicine. LEP Publication, Mymensing, Bangladesh. pp: 504-528.

Shivaprasad H. L. (2000).Fowl typhoid and pullorum Disease. Revue-Scientifiquet-Technique- Office-International-des-Epizootics. 19:405-24.

Shivaprasad H. L., Calnek B. W., Barnes H. J., Bearb C.W., McDoughald L. R., Saif, Y. M. (1997). Diseases of Poultry,10th ed. Iowa StateUniversity Press. Ames, IA.pp:82-96.

Shivhre S., Sharda R., Reddy A. G., Sharma R. K., Sharma V.(2001). Antibiotic sensitivity of *Salmonella typhimurium* isolates from domestic poultry. *Ind. Vet. J.,* 77:998-999.

Su LH, Chiu CH, Chu C, Ou JT. (2004) Antimicrobial resistance in nontyphoid *Salmonella* serovars: a global challenge. *Clin Infect Dis.,* 39(4):546-51.

Tricia D. M., McLaughlin W., Brown P. D. 2006). Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans. BMC. *Vet. Res*. 2: 7.

Turtura G. C., Massa S., Chazvinizadeh H. (1990). Antibiotic resistance among coliform bacteria isolated from carcasses of commercially slaughtered chickens. *Int. J. Food Microbiol.,* 11: 351-354.

Tsuji A., Ito K., Hoshi H., Hayakawa T. (1994).A severe outbreak of haemorrhagic colitis and haemolytic uraemic syndrome associated with Escherichia coli O157:H7 in Japan. *Eur J Pediatr.,* 153:650-5.

United States Department of Health and Human Services. (2001).Code of federal regulations, title 21, vol. 6, part 520,revised on April 1. Oral dosage form new animal drugs: Streptomycin sulphate oral solution. Document 21CFR520.2185a. U.S.Government Printing Office, Washington, DC.

United States Department of Health and Human Services. (2003).Code of federal regulations, title 21, vol. 68, part 520, revised April 1. Oral dosage form new animal drugs: Oxytetracycline hydrochloride soluble powder. Document 21CFR520.1660d.U.S. Government Printing Office, Washington, DC.

Van den Bogaard A. E., Stobberingh E. E. (1999**).** Antibiotic Use in Animals. *Drugs*, 58: 589-607

Van de Boogard A. E., Stobberingh E. E. (2000). Epidemiology of resistance to antibiotics links between animals and humans. *Int. J. Antimicrob. Agents*, 14: 327-335.

Vaillancourt J. P., Elfadil A., Bissailon J. R. (1992).Cellulitis in poultry, *Canada Poultry man.*, 79: 34 – 37.

Verma J. C., Gupta B. R., Ghosh S. S. (1993).Studies on *Salmonella* Virchow: in vitro sensitivity. *Indian Vet. J*., 70**:**572-573.

WHO. 2005. Fact Sheet Nº139, Revised April 2005.

Wray C., Davies R. H. (2001).Enterobacteriacae In: Poultry Diseases, 5th ed. F. Jordan, M. Pattison, D. Alexander and T. Faragher, eds. W. B. Saunders, pp: 95-130.

Waltman W. D., Gast R. K., Mallinson E. T. (1998).Salmonellosis, cited in A Laboratory Manual for the Isolation and Identification of Avian Pathogens. American Association of Avian Pathologist, 4th ed. Printed in Florida,USA. pp: 4-13.

Yogaratnam V. (1995).Analysis of the causes of high rates of carcass rejection at a poultry processing plant. *Veterinary Record*, 137: 215 – 217.

**APPENDIX-I**

**Questionnaire for Farm Information**

**A. Basic Information**

**Date:**

1. Name of the farm: ……………………………………………….

2. Name of the owner: ……………………………………………...

3. Address of the farm: …………………………………………….

4. Farm size and composition

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Age | Total number | Strain | Morbidity | Mortality |
|  |  |  |  |  |

**B. Housing Pattern and Management:**

1. Housing System: Intensive/Semi-Intensive

2. Floor Type: Litter/Concrete/Slat/Cage

3. Ventilation status of house: poor/moderate/good/nothing

4. Drainage status: poor/moderate/good/nothing

5. Frequency of cleaning of the house: Once a week/twice per week/daily/other schedule

6. Bio-security: Visitor access/Restricted/Not restricted

7. Foot Bath: Present/Absent

8. Tress around the farm: Present/Absent

**C. Feeding Management:**

1. Feeding System: Scavenging/Supplement/Both

2. If supplement then: Homemade/ Commercial balanced feed

3. If homemade; kinds of feed:

a) CHO (Rice, Rice polish, Wheat Bran, Rice gruel and other)

b) Protein (Fish, Snail Weed, Cake and other)

c) Minerals and others

4. Frequency of feeding: Once a day/Twice a day/Thrice a day

**D. Preventive measures:**

1. Regular practice of vaccination: yes/no

2. Types of vaccine use

**i**

**APPENDIX-II**

**Diagnostic Information Sheet**

**Date: ……………. Case No.:…….**

1. Name of the farm: ………………………………………………………………………….

2. Name of the Owner: ………………………………………………………………………

3. Address of the farm: …………………………………………………………………….

**4. Post Mortem Examination of birds:**

**Nares/Nose:** (inflammation/serous exudation/mucoid & catarrhal exudation)

**Trachea**: (congestion/thickening/caseous exudates/serous exudate/oedema/granular

appearance (nodular growth)

**Lungs:** (Pneumonic change/Yellow gray nodule/Gray

hepatization/Congestion/Granulomatous lesion/Whitish spot in the lung/Nodule in the lung in case of BT/Aspergillosis)

**Proventiculous:** (Pin point hemorrhage on the apex of the gland/Thickening & firm/Petechial haemorrhage at the junction of the proventiculous & gizzard)

**Gizzard:** (Pin point nodules/haemorrhage under horney layer)

**Small Intestine:** (Viscid mucous/ Petechial haemorrhage/Necrotic foci with deposition of cheesy mass in the lumen/Reddish black area around the intestine/White spot in intestinal wall/Wall thickened, distended & flabby/Purulent or diptheretic inflammation/ Haemorrhage & ulceration or bran like deposition/Necrotic mucosa with cracked surface/Congestion in the wall of small intestine/Intestine full with fluid & gasses)

**Large Intestine:** (Necrotic foci/Normal)

**Caeca:** (Necrotic foci/Filled with yellow casts/Wound or ulcer in caecal tonsil/filled with caseo-necrotic casts/Presence of caecal plug/Dilated caeca containing clotted or unclotted blood/ petechy on the wall/detouched caecal core/haemorrhage/necrosis)

**Liver:** (Swollen on enlarge or hepato-megali/dark/transparent to cloudy deposition on the surface looking like itching of cake/focal area of coagulative necrosis/petechiation/cooked appearance & friable/pin head size white or grayish necrotic foci/congestion/pin point nodules/impression of pale color(ischemic)/various color/bronge color/coarse granular appearance/fragile/whitish spot on the surface/fatty change)

**Spleen:** (enlargement or splenomegali/necrotic foci/various color impression /haemorrhage/ fragile)

**ii**

**Pericardium:** (Sac filled with light yellow fibrinous exudates/thickened/fibropurulent exudates/sero-fibrinous gelatinous materials on the pericardium/pericardial adhesion/hydropericardium/sac filled with turbid yellow fluid/fibrin attached to the surface of the heart/inflammatory change/serous exudates on pericardium)

**Heart:** (mishappened/necrotic foci & gray nodules/necrotic changes/pale heart/single or multiple number tumour in myocardium)

**Bursa of Febricious:** (Atopic/swollen/edematous/yellowish/haemmoragic)

**Ovary & follicle:** (appear as flaccid mass/congestion/yolk material in abdominal cavity/egg peritonitis due to ruptured in peritoneum/shunken ova/irregular (misshaped ova/ova attached to the ovary by a stalk/Cystic ova/Degeneration of ovarian follicle/ovary cauliform like appearance)

**Muscles:** (Haemorrahage in various muscles/Congestion in pectoral muscles/Skeletal muscles are dark in color/tinny whitish streaks to nodular tumours in muscles/congestion, haemorrhage in pectoral, thigh & leg muscles)

**4. Tentative Diagnosis:**

**6. Microbiological Examination:**

Staining property

Colony Morphology:

On MacConkey agar:

On Muller Hinton Agar

Biochemical Test:

**7. Confirmative Diagnosis:**

**8. Antibiotic Sensitivity Test:**

**iii**

**APPENDIX-III**

**Composition of MacConkey Agar:**

**Ingredients g/dl**

Peptone 17

Prteose 3

Bile Salts no. 31.5

Lactose 10

NaCl 5

Glucose 13.5

Neutral Red 0.03

Cristal Violate 0.01

**Composition of Muller Hinton Agar:**

**Ingredients g/dl**

Beef Infusion form 300

Casein acid hydrolysate 17.5

Starch 1.5

Agar 17.0

**Composition of Brilliant Green Agar**

**Ingredients gm/litre**

Proteose peptone 10.0

Yeast extract 3.0

Lactose 10.0

Sucrose 10

Sodium chloride 5.0

Phenol red 0.08

Brilliant green 0.125

pH 6.9 ± 0.2 @ 25°C

iv