**CHAPTER – I**

**INTRODUCTION**

Bangladesh is highly deficient in animal origin protein. The World Health Organization (WHO) recommends 56 kg of meat and 365 eggs per head per annum but, in Bangladesh consumption of meat is only 14.57 kg with the egg consumption being only 31 per head per annum (WHO, 2003). To overcome this enormous shortage of meat and eggs in the country poultry can play a vital role. This is because that the population of poultry has been seen to be growing steadily (from 198.0 million in 2002-03 to 278.76 million in 2010-11) and more rapidly compared to other livestock species for example, cows (from 22.5 million in 2002-30 to 23.12 million in 2010-11). It is likely that production of poultry has increased further in the last two years. This is thought to be due to that poultry requires less investment to start with the farming and persons from low income group may also start the business on a small scale. Poultry farming also offers opportunities for part-time employment particularly for village women, children and elderly persons on the farm operation (Bangladesh Economic Review, 2009 and 2011). Despite its tremendous growth during the last decade the poultry sector is constrained with several impediments and one of the major constraints to the expansion of poultry industry in Bangladesh is the presence of various infectious diseases which also include several bacterial diseases. Ali (1994) has reported about 30% mortality of chickens in Bangladesh every year due to outbreaks of several diseases. Mortality of poultry may even reach up to 94% in severe outbreak of Colibacillosis a bacterial disease (McPeake *et al.,* 2005 and Biswas *et al.,* 2006). According to the annual report 2011 of Bangladesh Rural Advancement Commission (BRAC) the mortality rate of poultry is as high as 25% due to a combination of improper feeding practices, diseases and poor distribution of vaccines. Biggs (1982) computed the total economic loss in poultry due to diseases (including vaccines and condemnation) to be about 20 percent gross value of the production (GVP) which is three times of cost of losses from mortality. He also stated that the economic importance of poultry diseases may differ between countries and geographical areas. Among the bacterial diseases Salmonellosis alone has been reported to cause more than 10% mortality in poultry and reduces egg production and hatchability for up to 20-30% (Fehervari, 1994; Haque *et al*., 1997).

Several workers (Giasuddin *et al*., 2002; Islam *et al.,* 2003; Rahman *et al*., 2004; Rahman *et al.,* 2007; Kamaruddin *et al,* 2007; Uddin et al., 2010) have studied the prevalence of infectious diseases of poultry in Bangladesh including bacterial diseases. According to them the important bacterial diseases of poultry to be named in Bangladesh are Salmonellosis, Omphalitis, Collibacillosis, Fowl cholera, Nectrotic enteritis, Infectious coryza, Gangrenous dermatitis, Mycoplasmosis and Staphylococcosis. Again, all those studies cited above have been found to be conducted encompassing the poultry zones from Dhaka, Gazipur, Narsingdi, Mymensingh, Tangail, and Sylhet but, so far our knowledge goes no work has been done on the prevalence of poultry diseases in the poultry zones of Chittagong and surrounding areas.

Furthermore, all the papers consulted above to review the prevalence of poultry diseases indicates that diagnosis of the diseases were made mostly on the basis of conventional methods of clinical symptoms, postmortem findings, bacteriological culture, biochemical tests and serological findings. However, recently from Bangladesh Tonu *et al.,* 2011 reported using PCR tests for further confirmation of pathogenic *E. coli* in poultry affected with colibacillosis. Candrian *et al.,* (1991) first adopted a PCR assay specific for *Escherichia coli* allowing rapid and unambiguous identification of the organism. Molecular techniques have several advantages compared with the conventional bacteriological culture methods for detection of organisms; the major advantage is that it is rapid and sensitive compared with culture methods. The most serious drawback of the conventional method of bacteriological culture seems to be due to that in most of the cases only a small fraction of bacteria can be recovered from a sample as up to 99% of the bacteria in many environments fail to grow under artificial conditions (Amann *et al.,* 1995). Amplification of DNA using PCR has been increasingly used to identify several bacterial species from food and clinical samples with the advantage that PCR is not dependent on the utilization of a substrate or the expression of antigens. PCR is also required to confirm bacterial infections when the disease is clinically suspected and situations in which co-infections are suspected (Wray *et al.,* 2001). The use of PCR for rapid diagnosis of causative agents of the diseases of poultry is very much anticipated for launching an effective disease control program in the poultry farms of Bangladesh.

Antibiotic resistance has been recognized as a global health problem for many decades. The problem has now been recognized as one of the top health challenges that the world will be facing in the 21st century (FDA, 2000 and CDC 2010). Some of its causes are widely accepted, for example, the overuse and inappropriate use of antibiotics for nonbacterial infections such as colds and other viral infections and inadequate antibiotic stewardship in the clinical arena (Levy, 2002). Microbial resistance to antibiotic is also a decade old problem in veterinary medicine (Smith *et al.*, 1957; Howells *et al*., 1975 and Gorbach, 2001). There also exists a relationship of drug-resistant bacteria in people to antibiotic use in food animals (Witte,2000 ;Sorensen *et al*., 2001 ;Donabedin *et al*.,and Lee,2003; Perreten *et al.,* 2005; de Boer *et al.,*2009 ) and some of it is probably due to the use of antibiotics as growth promoters in animal feed (Johnson *et al,* 2006). Bangladesh is also not out of this problem. Antibiotics are extensively used as growth promoters in poultry production or to control infectious disease. Anti-microbial abuse is considered to be the most vital selecting force to antimicrobial resistance of bacteria (Okeke *et al.,* 1999 and 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 and Witte, 1998). A good number of scientists from Bangladesh worked on the antibiotic resistant organisms in food animals (Khan *et al.,* 2005; Akter *et al*., 2007; Purkaysstha *et al.,* 2010; Hashem *et al.,* 2012). They have only studied whether the organisms isolated were sensitive or resistant to some selected antibiotics but other factors that might or might not have any effect were not studied.

Considering the above facts that (i) there is a lack of information on the prevalence of bacterial diseases of poultry with their risk factors from the poultry zones of Chittagong and surrounding areas, (ii) that there is a need to develop fast and reliable diagnostic techniques for the diagnosis of poultry diseases and (iii) whether the antibiotic resistance of the causal agents of the diseases have any relation with other environmental factors or not the present study was designed to fulfill the following objectives:

**1.1 Objectives of the study**

* **T**o determine the prevalence of bacterial diseases of poultry in the commercial farms of Chittagong in Bangladesh.
* To confirm the diseases using modern molecular technique the PCR.
* To determine the antibiotic sensitivity of the organisms to help suggesting effective treatment.

**CHAPTER – II**

**REVIEW OF LITERATURE**

**Importance of poultry in Bangladesh**

Livestock plays a crucial role in the economic and social fabric of Bangladesh the country that has long been enjoying the contribution of livestock and poultry for meeting the demand of nutrition, livelihood, customs and culture. The livestock and poultry density is very high in Bangladesh and has tangible impact on the economy as well as public health. The population of livestock and poultry has been growing steadily over the decade with the growth of poultry being rapid compared to other livestock species (Table- 1), which is because poultry requires less investment to start the farming and person from low income group may also start the business on a small scale. Poultry farming offers opportunities for part-time employment particularly for village women, children or elderly person on the farm operation (Bangladesh Economic Review, 2011).

**Table- 1:** Livestock and poultry population (million) in Bangladesh (2004-2011) add with this table the data of 2002-03 and 2003-04 from Bangladesh Economic Review, 2009.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Species | Year | | | | | | |
| 2004-05 | 2005-06 | 2006-07 | 2007-08 | 2008-09 | 2009-10 | 2010-11 |
| Chicken | 183.45 | 194.82 | 206.89 | 212.47 | 221.39 | 228.03 | 234.64 |
| Duck | 37.28 | 38.17 | 39.08 | 39.84 | 41.23 | 42.67 | 44.12 |
| Cattle | 22.67 | 22.80 | 22.87 | 22.90 | 22.98 | 23.05 | 23.12 |
|  |  |  |  |  |  |  |  |

Source: Bangladesh Economic Review, 2011

Poultry constitutes 30% of animal protein supply in the country and will increase to 40% before 2015 (IFPRI, 2000).As an important sub sector of livestock production, the poultry industry in Bangladesh plays a vital role in economic growth and simultaneously creates numerous employment opportunities. The poultry industry, as a fundamental part of animal production, is committed to supply the nation with a cheap source of good quality nutritious animal protein in terms of meat and eggs (Akter and Uddin, 2009). Farm produced meat and eggs are gaining popularity throughout the country. Hossain (1999) stated that farm produced broilers, spent hens and cockerels constitute about 55% of the total chicken meat, while farm produced eggs are 82% of the total eggs marketed in Dhaka.

In spite of tremendous growth of poultry the shortage of eggs and meat is still very high in our country as the supply against the demand is not high enough. The World Health Organization (WHO) recommends 56 kg of meat and 365 eggs per head per annum but, in Bangladesh consumption of meat is only 14.57 kg with the egg consumption being only 31 per head per annum (WHO,2003). To fulfill the high demand of meat and eggs in the country the poultry sector has to be given proper attention through minimizing the constraints that are hindering the expansion of poultry industries. One of the major constraints to the expansion of poultry industry in Bangladesh is thought to be the presence of various infectious diseases which also include several bacterial diseases. Ali (1994) has reported about 30% mortality of chickens in Bangladesh every year due to outbreaks of several diseases. Mortality of poultry may even reach up to 94% in severe outbreak of Colibacillosis a bacterial disease (McPeake *et al*., 2005; Biswas *et al.*, 2006). Among the bacterial diseases Salmonellosis alone has been reported to cause more than 10% mortality in poultry and reduces egg production and hatchability for up to 20-30% (Fehervari, 1994; Haque *et al*., 1997).

**2.1 Prevalence of bacterial diseases of poultry in Bangladesh**

Several workers (Giasuddin *et al*., 2002; Islam *et al*., 2003; Rahman *et al*., 2004; Rahman *et al*., 2007; Kamaruddin *et al*, 2007; Ahmed *et al*., 2009; Hasan *et al*., 2010; Uddin *et al*., 2010; Islam *et al*., 2012) have studied the prevalence of infectious diseases of poultry in Bangladesh that also included bacterial diseases. According to them the important bacterial diseases of poultry prevalent in Bangladesh include Collibacillosis, Salmonellosis, Omphalitis, Fowl cholera, Nectrotic enteritis, Infectious coryza, Gangrenous dermatitis, Mycoplasmosis and Staphylococcosis.

Giasuddin *et al*., (2002) conducted postmortem and serological investigation of poultry diseases in the different farms of Bangladesh. A total of 1653 either dead or sick birds were examined. According to them the incidence of Aflatoxicosis was highest (27.59%) followed by nutritional deficiency (12.40%), Infectious bursal disease (11.80%), Chronic respiratory disease (8.11%), Newcastle disease (7.50%), Salmonellosis (5.56%), Collibacillosis (4.42%) and Fowl cholera (3.08%). Another 5.32% cases remained undiagnosed due to lack of proper diagnostic facilities. In sero-evaluation, commercial and native birds were found 93 and 97%, 80 and 55%, 56 and 12%, 60 and 73%, 67 and 61%, 22 and 3% sero-positive for Newcastle disease, Infectious bursal disease, Pullorum disease, *M. gallisepticum*, *M. synoviae* and Chicken infectious anemia respectively.

A pathological investigation was conducted by Islam *et al.,* (2003) on the occurrence of poultry diseases in Sylhet region of Bangladesh during the period from November 2001 to October 2002. Out of a total of 1352 birds investigated they recorded Infectious Bursal disease (IBD) to occur at the rate of 24.26% followed by Newcastle Disease (ND) 6.73%, Infectious Bronchitis 0.29%, Omphalitis 2.81%, Fowl Cholera 0.44%, Salmonellosis 6.73%, Colibacillosis 5.17%, Necrotic enteritis 0.44%, Aspergillosis 17.53%, Infectious Coryza 0.37%, Chronic respiratory disease (CRD)/Mycoplasmosis 5.32%, Coccidiosis 9.46% and deficiency disorders/stress condition 1.03%. In general, the highest number of cases were recorded in the age group of 8-21 days (42.60%), followed by 22-35 days age group (26.62%), 0-7 days age group (26.10%), 36-60 days age group (1.03%) and over 60 days age group (3.62%) of Poultry. Distribution and proportionate incidence of poultry disease revealed the diseases to occur mostly in rainy season (56.36%), followed by summer (28.11%) and the least in winter season (15.53%).

Rahman *et al*., 2004 when investigating 1751 dead chickens at the BRAC Poultry Disease Diagnostic Centre, Gazipur found 39.81% (n=697) cases with seven types of different bacterial diseases of which Salmonellosis (n=385), Colibacillosis (n=147) and Fowl cholera (n=114) were found at a significantly higher rate of prevalence followed by Nectrotic enteritis (n=24), Gangrenous dermatitis (n=17), Staphylococcosis (n=6) Infectious coryza (n=4). According to them age wise prevalence of avian Salmonellosis showed highest infection rate in adult layers (53.25%) in comparison to brooding (14.55%), growing (16.10%) and pullet (16.10%) chickens. The avian Colibacillosis was found widely prevalent in all age group of chickens (9.52 to 36.73%) with especially high prevalence rate in adult layer birds (36.73%). Fowl cholera was recorded in chickens more than two weeks of age with significantly (P<0.01) highest occurrence rate in adult chickens. Seasonal influence showed significantly (p<0.01) highest proportionate prevalence of Salmonellosis during summer (48.05%) in comparison to rainy (28.31%) and winter (23.66%) seasons. Colibacillosis was recorded more or less uniformly in all the three seasons of the year with significantly (P <0.01) higher rate during summer (40.82%) broiler chickens. From pathogenicity study the incubation period of this three bacterial diseases were recorded as 96 hours and clinical signs appeared on 4th day of season. Similarly, the prevalence of fowl cholera was also found significantly (P<0.01) highest during summer (49.12%) in comparison to rainy (26.32%) and winter (24.56%) seasons. The isolated causative agents of avian Salmonellosis (*Salmonella pullorum*), avian Colibacillosis (*Escherichia coli*) and avain Pasteurellosis (*Pasteurella multocida*) were characterized by bacteriological methods which were also subjected to pathogenically study in 52-day old incubation and observed that *S. pullorum*, *E.coli* and *P. multocida* resulted 100% morbidity in chickens.

A 17-month (from January 2002 to May 2003) longitudinal study was undertaken by Biswas *et al*., (2005) to elucidate the epidemiology of important endemic and epidemic diseases affecting semi-scavenging chickens reared in the Participatory Livestock Development Project area in Bangladesh. During the study period 1227 birds, which belonged to different age, breed and sex categories, were found dead as a result of disease occurrence. From every dead bird organ samples such as the liver, heart, spleen, brain lung, trachea and bursa of Fabricius were collected. The incidence rate of mortality was 0.01976 per bird-months at risk. Of the total deaths 58.44% had single or mixed type of infections. Newcastle disease had the highest proportional mortality rate (15.81%). The proportional mortality caused by Fowlpox, Fowl cholera, Salmonellosis, Colibacillosis, Aspergillosis, Infectious bursal disease, mixed infections and undiagnosed cases were 8.96%, 6.76%, 7.09%, 6.93%, 0.33%, 2.04%, 10.51% and 41.56%, respectively. Newcastle disease affected a significant higher proportion (18.81%) of birds older than 60 days of age (P=0.00). Younger birds (age < or = 60 days) had a higher proportional mortality due to Fowlpox and Infectious bursal disease than older birds (P=0.00). Sonali (male Rhode Island Red x female Fayoumi) birds reared under the semi-scavenging system had a higher infection rate with Newcastle disease virus compared with indigenous and Fayoumi birds (P=0.00). Fowlpox was more prevalent in Fayoumi birds compared with Sonali. Surprisingly, Newcastle disease was more common in the vaccinated birds rather than the unvaccinated birds.

Rahman *et al*., (2007) conducted a study to determine the incidence of bacterial diseases of poultry in various age groups of birds from different poultry farms in some selected areas of Bangladesh (Dhaka, Gazipur, Narsingdi, Mymensingh and Tangail). A total of 8169 dead chickens (2960 chicks, 1083 grower and 4126 adults) were examined. In chicks Salmonellosis was found at a rate of 53.90%, followed by Omphalitis 28.42%, Colibacillosis 13.36%, Mycoplasmosis 2.55%, Necrotic enteritis 1.18% and Infectious coryza 0.59%. In grower group of birds the diseases Salmonellosis, Colibacillosis, Infectious coryza and Necrotic enteritis were detected at the rate of 55.96, 11.93, 29.91 and 2.20%, respectively. In adult chicken Salmonellosis was found at a rate of 53.32% followed by Mycoplasmosis 39.09%, Infectious coryza 6.11% and Necrotic enteritis 1.48%. They did not find any Colibacillosis infection in adult chicken.

Khaton *et al*., (2008) (n=25) from 30 dead birds were collected in sterile nutrient broth, with histopathological studied the prevalence of colibacillosis in layer chickens from May to September 2007. Sixty five cloacal swabs from apparently healthy birds and 55 swabs of liver (n=15), lung (n=15) and intestine samples. Bacteria were isolated and identified. Tissue samples were studied under light microscope. *Escherichia coli* (*E. coli)* was isolated from 83% of cloacal swabs of apparently healthy chickens and 87% of samples from dead birds. Affected birds had cloudy thickened air sacs, pericarditis, and congestion in the liver, lung and spleen. On histopathological examination focal necrosis in liver and infiltration of heterophils, lymphocytes and macrophages in liver and lung was found. Thickening of pericardium was found due to infiltration of reticulo endothelial (RE) cells. In duodenum, severe infiltration of leukocytes mainly heterophils, lymphocytes and macrophages was found in the sub-mucosa.

Ahmed *et al*., (2009) conducted a study to determine the occurrence of infectious diseases in broiler chickens at Kapasia in Gazipur district during the period from 16October to 16December 2008. Detection was made on the basis of history, clinical findings and post-mortem lesions. A total of 199 broiler chickens were examined during the study where Colibacillosis 104 (52.26%), Mycoplasmosis 25 (12.56%), Salmonellosis 02 (1.01%), Omphalitis 23 (11.56%), Coccidiosis 09 (4.52%), Gumboro 22 (11.06%), Mycotoxicosis 11 (5.53%) and mixed infection of Gumboro & Coccidiosis 03 (1.51%) were recorded. The concluded the Collibacillosis to be the major problem in broiler production for which the poultry farmers can not earn their profit in full.

*Hasan et al*., (2010) studied with a view to compare clinical and laboratory diagnoses of various bacterial diseases of poultry during the period from March 2009 to February 2010. A total of 135 sick and dead chickens (47 broilers and 88 layers) were collected from 12 different poultry farms (4 broilers and 8 layers) of Mymensingh and Gazipur districts which were subjected to clinical followed by laboratory diagnosis. Clinical diagnosis was made on the basis of clinical history, clinical signs and postmortem findings of the affected birds whereas; confirmatory diagnosis was made by using cultural examination, Gram’s staining and different biochemical tests. In this study, out of 47 broilers, 16 (34%) chickens were clinically diagnosed as Colibacillosis, 11 (23.40%) as Salmonellosis and 2 (4.25%) as Fowl cholera. In the same way, out of 88 layer chickens, 28 (31.82%) were diagnosed as Colibacillosis, 16 (18.18%) as Salmonellosis and 11 (12.50%) as Fowl cholera. In laboratory, out of 47 suspected broiler chickens, 12 (25.53%) chickens were diagnosed as Colibacillosis, 7 (14.89%) as Salmonellosis and 0 (0%) as Fowl cholera. Correspondingly of the 88 layer chickens 22 (25%) were diagnosed as Colibacillosis, 11 (13.64%) as Salmonellosis and 8 (9.09%) as fowl cholera. So the findings concluded that clinical diagnosis is not always accurate like laboratory diagnosis because in most cases clinical history, clinical signs and post-mortem lesions of different bacterial diseases including mixed infections are almost similar to other related diseases and it is recommended to confirm laboratory diagnosis before treatment of the diseases.

Hossain *et al*., (2010) carried out a serological survey on the prevalence of antibodies against *Salmonella* and *Mycoplasma gallisepticum* (MG) in layer chickens in Rajshahi and surrounding districts of Bangladesh. A total of 605 sera samples were examined by rapid plate agglutination (RPA) test using commercial *Salmonella* and MG antigens to determine the *Salmonella* and MG specific antibody. Out of 605 sera samples 14.1% showed single *Salmonella,* 45.1% showed single MG and 11.2% showed their concurrent infection. Prevalence of *Salmonella* was recorded the highest (37.6%) in adult compared to young (16.7%). On the contrary, MG and concurrent infections were recorded the highest (71.7% and 13.3%) in young compared to adult (50.4% and 10.4%). The prevalence of *Salmonella*, MG and concurrent infections were recorded the highest (34.3%, 68.6% and 17.1%) in large flocks compared to small flocks (21.3%, 50.0% and 8.8%). The prevalence of *Salmonella* infection was the highest (30.4%) in summer followed by winter (23.7%), rainy (25.0%) and autumn (23.3%). The prevalence of MG infection was the highest (61.6%) in winter followed by autumn (56.9%), rainy (55.0%) and summer (49.6%). The concurrent infection of *Salmonella* with MG however, was found to be highest (12.1%) in winter followed by summer (11.9%), rainy (10.8%) and autumn (10.0%).

A study was conducted by Uddin *et al*., (2010) to determine the prevalence of poultry diseases in some selected areas of Narsingdi district of Bangladesh. The diseases were diagnosed based on clinical history, signs and symptoms in sick birds and postmortem lesions in dead birds followed by isolation and identification of causal agents. A total 1263 birds were examined. Among the diseases diagnosed Infectious Bursal Disease (IBD) was found at the rate of 24.96% followed by Chronic Respiratory Disease (CRD)/ Mycoplasmosis at 9.87%, Newcastle Disease (ND) at 8.92%, Aspergillosis at 7.98%, Salmonellosis at 7.68%, Coccidiosis at 7.32%, Colibacillosis at 5.70%, Ascites at 5.45%, Omphalitis at 2.64%, Deficiency Disorders/Stress at 1.34%, Necrotic Enteritis at 0.40%, Infectious Coryza at 0.32%, Fowl Cholera at 0.24%, and Infectious Bronchitis at 0.24%. In general, the highest number of cases were recorded in the age group of 8-20 days which was 42.64%, followed by 35.76% in 21-35 days age group, 16.12% in 0-7 days age group, 1.52% in 36-60 days age group and 3.96% in >60 days age group of poultry. Distribution and proportionate incidence of poultry diseases revealed the fact that the diseases occur mostly in rainy season (47.09%) followed by summer (27.53%) and the least in winter season (25.38%).

Mahmud *et al*., (2011) conducted a study to determine the seroprevalence of Salmonella in poultry and their drug-resistant patterns, variability in infectivity and mortality rate of birds, and predilection of some serovars to cause zoonoses. The average seroprevalance of Salmonella in three different age groups was found to be 37.9%. A total of 503 samples were examined over a period of 1 year from five different poultry farms of a semiurban area of Savar, Dhaka, Bangladesh. The prevalence of Salmonella was recorded to be 21.1%. Salmonella was found high in dead birds (31.2%) than live birds (18.1%). Salmonella infection was higher (23.6%) in summer than in winter (12.9%) season. Among the 106 isolates, 46 belong to serogroup B (43%) and 60 isolates to serogroup D (57%). The highest Salmonella infection was recorded as 47.9% in the 30–35 week-old birds. A total of 106 Salmonella isolates were used for antimicrobial susceptibility test against 10 common antibiotics and 17 multiple drug resistance patterns were found. Among the isolates, 69 (65%) harbored plasmids 1–4 with size variation between >1.63 and >40 kb and rest 37 (35%) isolates were plasmid free but showed resistance against 5–10 antibiotics. The results of the present investigation suggested that multiple drug resistance is common among the Salmonella isolates of poultry and some of these isolates may have zoonotic implications.

Tonu *et al*., (2011) detected the pathogenic *Escherichia coli* (*E. coli*) through pathological study of the colibacillosis affected birds. These isolated *E. coli* were further confirmed by PCR using specific primer. For this purpose, a total of 20 swabs (10 from lung and 10 from intestine of 10 dead birds) were collected in sterile nutrient broth. The histopathological samples were collected in 10% buffered neutral formalin. The used methods were histopathology, isolation and identification of *E. coli* by conventional methods and as well as by PCR method. A total of 10 isolates of *E. coli* from 20 swabs of lung and intestine was characterized by conventional routine methods of bacteriology. DNA of 8 isolates out of 10 isolated *E. coli* organisms was amplified by PCR using ECO-f and ECO-r primer targeting 16S ribosomal DNA and found 585bp amplicon which is specific for *E. coli* with enteroinvasive type confirmed by histopathological lesions in duodenum. They suggested further investigation focusing on serotyping and detection of genes of *E. coli* which are responsible for pathogenicity of the organism.

Hashem *et al*., (2012) conducted a study to isolate and identify *Escherichia coli* from dead broiler chickens from different poultry farms in Chittagong, Bangladesh, from July, 2010 to June, 2011. They also determined the antibiogram of the isolates. A total of 275 dead broiler chickens were examined and 150 (54.55%) were diagnosed as infected with *E. coli.* The *E. coli* were isolated and identified by cultural and biochemical characteristics. The isolated *E. coli* were highly sensitive to colistin sulphate followed by ciprofloxacin, amoxicillin, ampicillin, oxytetracyclin, and resistant to cotrimoxazole, gentamycin and penicillin. They concluded from the results of the study that the high resistance of *E. coli* to antibiotics constitutes a threat to poultry industry in Bangladesh.

Islam *et al*., (2012) conducted a study with an aim to explore the occurrence of different diseases among commercial chickens. A total of 232 commercial chickens were divided into different age groups and the whole year was divided into three seasons. The occurrence of parasitic diseases was the highest among layers (81.9%) and broilers (95.2%). Occurrence of bacterial diseases (p<0.01) and parasitic diseases (p<0.05) was significantly lower in summer than rainy and winter seasons. Viral diseases were observed among 25.2% layers and occurrence was significantly (p<0.01) higher in pullet stage (40.9%) compared to laying stage (8.2%). Occurrence of non-infectious (p<0.01) disease, cannibalism (p<0.05) and egg bound disease (p<0.05) was significantly higher in laying stage compared to pullet stage. Bacterial disease occurrence was significantly (p<0.01) lower in 8-21 days age group compared to 0-7 days age group of broilers. Occurrence of viral diseases was significantly (p<0.01) higher among broilers of 22-35 days age group compared to 0-7 days age group.

Hossain *et al*., (2013) conducted an experiment during July 2011 to May 2012 to investigate the prevalence of bacteria as well as pathological lesions in the nasal passages of dead chickens. Thirty nasal passage swabs from 30 dead birds (20 from SK Veterinary Diagnostic Centre (SKVDC) and 10 from the Department of Pathology, BAU were collected in sterile nutrient broth. The histopathological samples (n=6) were collected in 10% neutral buffered formalin. The isolation and identification of bacteria were performed by culturing in different media, staining and biochemical tests. The collected tissues were fixed, processed, sectioned, stained and studied with light microscope. The prevalence of bacteria was recorded as 30.43% *Escherichia coli,* 47.83% *Staphylococcus* sp*.,*13.04% *Pasteurella* sp*.,* and 8.69% *Klebsiella* sp. in nasal passages (n=30) of dead chickens. Two cases of mixed infection with *E. coli* and *Pasteurella* and one case with *Klebsiella* and *E. coli* isolates were identified. Six tissue samples of grossly identifiable lesions such as congested and mucus filled nasal passages from 6 dead chickens were processed for histopathology. Microscopically, the section of nasal passages in general showed congested mucosa with excessive infiltration of heterophils and lymphocytes. The lining epithelia of nasal passage revealed acanthosis, hyperkeratosis and disruption of nasal epithelia. There was also hyperplasia of different mucous glands of nasal passages. *Klebsiella* sp*.* affected nasal tissues showed comparatively severe lesions than that of other bacterial infection in chickens.

Rowshan *et al*., (2013) conducted a study for isolation, identification and characterization of Haemophilus paragallinarum from layer chicken in Bangladesh. A total of 122 samples (Nasal / tracheal swab, visceral organs like liver, lung, heart) were collected from Rangpur (Paragon Poultry Farm ), Thakurgaon (North Agro Poultry Farm) and Dinajpur (Nizam Poultry Farm)  districts of Bangladesh  during the period from March 2011 to February 2012.  The samples were collected from suspected birds based on age, sex, breed, temporal and spatial differences for the isolation and identification of Haemophilus paragallinarum by morphology, staining, cultural and biochemical properties. The overall prevalence of Haemophilus paragallinarum was detemined as 47.54 %. The prevalence was very high in laying hen (52.8%) and growing birds (42.8) compared to the prelaying stage (16.6%). Prevalence of the disease in Dinajpur, Rangpur and Thakurgaon was found to be 86.67%, 25% and 34.21% respectively. The isolates were resistant to norfloxacin and tylosin. It was evident that amoxycillin and gentamicin can be of better value in the treatment of infectious coryza in layer chickens in Bangladesh.

**2.2 Bacterial diseases of Poultry; clinical and laboratory diagnosis:**

The bacterial diseases of poultry encountered commonly in Bangladesh have been reviewed here putting emphasis on Colibacillosis and Staphylococcosis.

**2.2.1 *Salmonella* infection (paratyphoid and pullorum disease)**

Salmonellosis is one of the most important bacterial diseases in poultry causing heavy economic losses through mortality and reduced meat and egg production (Haider *et al*., 2004). Avian salmonella infection occurs in poultry either in acute or chronic form by one or more member of the genus *Salmonella*, under the family *Enterobacteriaceae* (Hofstad *et al*., 1984). There are mainly two types of non-motile avian *Salmonella spp*. namely *Salmonella gallinarum* and *Salmonella pullorum,* are responsible for fowl typhoid (FT) and pullorum disease (PD) of poultry respectively. *S. gallinarum* and *S. pullorum* are short non flagellated, non spore forming, non capsulated, gram negative plump rods (Cheesbrough, 1984), capable of producing septicemic disease in most domestic and wild birds all over the world. Mortality in chickens has been reported 0 to 100% by PD and 10 to 93% by FT. Increased mortality, anorexia, sudden drop in egg production and white or yellow diarrhea are the characteristic clinical signs of the diseases. The gross lesions in chicks include unabsorbed yolk sac and turbid yellow color fluids in the peritoneal cavity and in the adults there found peritonitis; discrete, small, white necrotic foci in the liver and enteritis.

(i)Paratyphoid (Salmonellosis)

*Salmonella* infections may affect all domestic poultry, although adult birds often do not show any symptoms. *Salmonella* bacteria may also contaminate hatching eggs, which results in diarrhoea, depression and death in young chicks. Paratyphoid is highly infectious and can be transmitted by mice, rats, other birds and/or through contaminated feed.

(ii)Pullorum disease (Bacillary white diarrhoea)

Pullorum disease is usually a symptomless disease of adult chicken. The disease is transmitted to young chicks through the eggs that results in fatal diarrhoea within the first four weeks of life. Adult birds may suffer from infection of the ovaries or septicaemia. This disease can be eliminated with good hatchery hygiene. To avoid this disease, chicks should be collected only from a reputable hatchery .It is reported that the pullorum disease is found at the rate of more than 51% in the commercial and government poultry farms of India or Bangladesh (Kamaruddin *et al.,*2007).

**2.2.2 Infectious coryza**

Infectious coryza is caused by a small bacterium known as *Haemophilus* paragallinarum that infects mainly the growing and laying fowls under conditions of poor hygiene, ventilation and nutrition (Clark and Godfrey, 1961). According to (Beach and Sclam, 1936).Symptoms of the disease include discharge from the eyes and nostrils, a swollen wattle, infection mainly in lower respiratory tract, causing rales and difficult breathing, drop in egg production and sometimes diarrhoea. They also told that, it is a chronic disease, which can result in mortalities when complicated with other infectious agents Such as fowl pox infectious bronchitis.

**2.2.3 Fowl Cholera**

Fowl Cholera (FC), which is also known as Avian Cholera, Avian Pasteurellosis or Avian Hemorrhagic Septicemia is an infectious disease affecting domesticated and wild birds (Rimler and Glisson, 1997). It is caused by *Pasteurella multocida*, a gram negative, non-motile, non-spore forming rod occurring singly or in pairs, and occasionally in chains or filaments (Rimler and Glisson, 1997). FC can affect birds of any age, but it rarely occurs in commercial poultry of less than 8 weeks of age (Rimler, 1994). Mortality in chicken and ducks accounted in Bangladesh due to FC is around 25% to 35% (Choudhury *et al*., 1985; Kamaruddin *et al*., 2007). Death of birds may be the first sign of FC while other signs are depression, diarrhea, ruffled feathers, increased respiratory rate, and cyanosis (Calnek *et al*.,1997). Commonly observed lesions are swollen liver with focal necrotic area and hemorrhage at the base of the heart, increased pericardial and peritoneal fluids (Calnek *et al.,* 1997).

**2.2.4 *Clostridial* infection (Necrotic enteritis and Botulism)**

(i)Necrotic enteritis

*Clostridial*infections may affect all domestic poultry, although adult broiler birds often show symptoms. The causal microorganism is *Clostridium perfringens* (type A and C). The disease is worldwide in farms where poultry is reared in litter. Prevalence rate of the disease in Bangladesh has been reported to be around 2% (Kamaruddin *et al*, 2007).

(ii)Botulism (Limberneck, Western duck sickness)

This is a toxaemic disease characterized by locomotive paralysis. The disease is seen usually in duck, chicken, turkeys and wild birds. The causal microorganism is *Clostridium botulinun* (type A, B, C alpha, C beta, D, E, F, and G) .

**2.2.5 Colibacillosis**

*Escherichia coli* is a common pathogen for commercial poultry causing Colibacillosis all over the world. It is a major cause of respiratory and septicemic diseases in broiler chicken causing mortality less than 5% with morbidity over 50% but in layer it affects the reproductive tract resulting in failure of egg production and infertility (Barens and Gross, 1997). It may cause about 28% death in Sonali variety of birds in Bangladesh (Biswas *et al.,* 2006). The incidence rate of the disease has been reported to be about 22% in Bangladesh (Kamaruddin *et al*., 2007). *E*. *coli* infections cause many clinical manifestations such as airsacculitis, pericarditis, septicemia, and death of the birds (Hofstad *et al*., 1984). The infection has also been extended to various parts and organs such as skin, joints, eyes, head, blood, heart, yolk sac, peritoneum, etc. (Stebbins *et al*., 1992).In India the disease was first isolated and recorded from yolk sac infection (Pathak *et al*., 1960).

**2.2.5.1 Epidemiological factors for avian colibacillosis:**

**Host**

Avian Colibacillosis is the commonest pathogen of all avian species. All domestic and wild birds usually suffer. But it does not affect mammals including human being (Calnek *et al*., 1997). In Bangladesh Avian Colibacillosis was found by Rahman *et al*., 2004 to be widely prevalent in all age groups of chickens (9.52 to 36.73%) with especially high prevalence rate in adult layer birds (36.73%) Khaton *et al*., 2008 however, reported the disease to occur at a rate of 60% in commercial broiler and layer birds.

**Habitat**

*Escherichia* *coli*are normal inhabitants of intestinal tracts and can be found in chicken feces, litter, dust and rodent droppings (Barnes, 2008). In fact, *Escherichia* *sp* can be isolated from healthy birds and it has been suggested that these bacteria may be part of the normal intestinal flora of both human and warm blooded animal (Ruch, 1959). *Escherichia coli*thus, has been considered as an indicator organism for faecal contamination and is an important parameter in food and water hygiene.*Escherichia* *coli* is recovered frequently from a secondary infection of tissues damaged by viral infections (Barnes, 2008 and Beckman, 2006).

**Mode of transmission**

Horizontal dissemination is the most likely method of transmission (Dho-Moulin *et al*., 1999) via the environment, more specifically from other birds, faeces, water and feed however, Barnes *et al*., (1997) suggested that rodents may be carriers of Avian pathogenic *E. coli* (APEC) and hence a source of contamination for the birds. An unfavourable housing climate, like an excess of ammonia or dust, renders the respiratory system more susceptible to APEC infections through deciliation of the upper respiratory tract (Barnes *et al*., 1997). Airsacculitis is observed at all ages in this case the bird is infected by inhalation of dust contaminated with faecal material, which may contain 106 CFU of *E. coli* per gram (Harry, 1964). This aerogenic route of infection is considered as the main origin of systemic Colibacillosis or Colisepticemia (Pourbakhsh *et al*., 1997; Dho-Moulin, 1999).

**2.2.5.2 Clinical sign and pathogenesis:**

Avian Colibacillosis causes a variety of disease manifestations in poultry including yolk sac infection, Omphalitis, respiratory tract infection, Swollen head syndrome, Septicemia, Polyserositis, Coligranuloma, Enteritis, Cellulitis and Salpingitis. Colibacillosis of poultry is characterized in its acute form by septicemia resulting in death and in its subacute form by peri-carditis, airsacculitis and peri-hepatitis (Calnek *et al*., 1997).

Avian Colibacillosis has been noticed to be a major infectious disease of birds of all ages. This disease has an important economic impact on poultry production worldwide. APEC are responsible for a considerable number of various diseases at different ages. Neonatal infection of chicks can occur horizontally from the environment or vertically from the hen. A laying hen suffering from *E. coli*-induced oophoritis or salpingitis may infect the internal eggs before shell formation. Faecal contamination of the eggshell is possible during the passage of the egg through the cloaca and after laying. The latter possibility is considered as the main route of infection for the eggs (Barnnes *et al*., 1997). He also proved that before hatching, APEC causes yolk sac infections and embryo mortality. The chick can also be infected during or shortly after hatching. In these cases, retained infected yolk, omphalitis, septicemia and mortality of the young chicks up to an age of three weeks is seen. It is also proved by him that broilers may be affected by necrotic dermatitis, also known as Cellulitis, characterized by a chronic inflammation of the subcutis on abdomen and thighs. Wray *et al*. (2001) reported that Infectious bursal disease (IBD), Mycoplasmosis, Coccidiosis, Newcastle disease or Infectious bronchitis as well as nutritional deficiencies, all predispose the birds to this disease.

Swollen head syndrome (SHS), mainly a problem in broilers, causes oedema of the cranial and periorbital skin. SHS can cause a reduction in egg production of 2 to 3% and a mortality of 3 to 4% (Morley *et al*., 1984). Data on this disease are contradictory. Picault *et al*. (1987) and Hafez *et al*. (1990) considered SHS as a disease caused by avian pneumovirus (APV), usually followed by an opportunistic *E. coli* infection. Nakamura *et al*. (1998) however, reported that APEC were probably playing a significant part in the disease, but that the role of APV was not at all clear. This had been confirmed by Georgiades *et al.* (2001) who did not detect APV in any of the flocks affected by SHS during a field study, but instead detected infectious bronchitis virus (IBV), Avian adenovirus, Avian reovirus and Newcastle disease virus (NDV) as well as *Mycoplasma synoviae* and *M. gallisepticum* (MG).

Layers as well as broilers maysuffer from *E. coli*-induced acute or chronic salpingitis (Bisgard *et al*., 1980 and 1981). He also opined that salpingitis can be the result of an ascending infection from the cloaca. According to Barnes *et al*. (1997) the infection may also be seen in the left abdominal airsac. Although, Bisgaard and Dam (1980) considered the latter possibility is less likely than an ascending infection. They Bisgard and Dam (1981) also reported in chronic salpingitis the oviduct to have a yellowish-gray cheese-like content with a concentric structure. According to Barnes *et al*, (1997) in layers, salpingitis can cause egg peritonitis if yolk material is deposited in the peritoneal cavity.

Septicemia also affects chickens of all ages, and is mainly described in broilers. It is the most prevalent form of Colibacillosis, characterised by polyserositis (Dho-Moulin, 1999). It causes depression, fever and often high mortality. Although, its pathogenesis has not been elucidated, several routes of infection are possible: neonatal infections through skin lesions (Norton *et al*., 2000), infection of the reproductive organs (Barnes *et al*.,1997), infection of the respiratory tract (Pourbakhsh *et al*., 1997) and even infection *per os* (Leitner *et al*., 1992). When *E. coli* reaches the vascular system the internal organs and the heart are infected (Dho-Moulin *et al*., 1993). According to Barnes *et al*. (1997) and Dho-Moulin *et al*. (1999) the infection of the myocardium causes heart failure. Septicemia occasionally also leads to synovitis and osteomyelitis (Barnnes *et al*., 1997 and Dho-Moulin *et al*., 1993) and on rare occasions to panophthalmia (Barnes *et al*., 1997). Coligranuloma or Hjarre’s disease the rare form of Colibacillosis, is characterised by granulomas in liver, caeca, duodenum and mesenterium, but not in the spleen may also cause up to 75% mortality in affected flocks (Barnes *et al*., 1997).

**2.2.5.3 Post mortem lesions:**

The post mortem lesions according to Calnek *et al.,* 1997 that are found commonly in chickens are given below:

**Airsacculitis, Perihepatitis, and Pericarditis-** Secondary invaders as part of chronic respiratory disease results in white, friable material covering airsacs, liver and pericardial sac.

**Omphalitis- s**wollen, red crusted navels can be caused by contamination by egg shells through dirty setter fecal covered eggs and excessive moisture during storage of eggs.

**Septicemia-** hepatosplenomegaly (hepatitis), hemorrhages and necrosis on tissues.

**Salpingitis** **or peritonitis**- especially laying hens. Oviduct filled with yellow cheesy exudates.

**Cellulitis-** Scabby hip of broiler chickens, yellow exudates underneath the skin of hip, leg and breast**.**

**Synovitis or arthritis.**

**Hypopyon-** pus in the eye following *E. coli* septicemia.

**2.2.5.4 Virulence factors; bacteria-host interactions and pathogens:**

Virulence factors have not yet been described for *E. coli* and the suggestion of the existence of such mechanisms is therefore entirely a matter of speculation. The majority of economic impact results from mortality and decrease productivity of the affected birds (Otaki, 1995). Since avian pathogenic *E. coli* (APEC) and human uropathogenic *E. coli* (UPEC) may encounter similar challenges when establishing infection in extraintestinal locations, they may share a similar content of virulence genes and capacity to cause disease. In this regard, Rodriguez-Siek *et al*. (2005) compared 200 human uropathogenic *E. coli* (UPEC) and 524 avian pathogenic *E. coli* (APEC) isolates for their content of virulence genes (Table 2) including many implicated in extraintestinal pathogenic *E. coli* (ExPEC) virulence as well as those associated with APEC plasmids for assessing the potential of APEC to cause human extraintestinal diseases. They also opined that based on the well-documented ability of avian *E. coli* to spread to human beings, the potential for APEC to act as human UPEC or as a reservoir of virulence genes for UPEC should be considered.

Even though certain O-types are more frequently detected in APEC than in commensal *E. coli* (Blanco *et al.,* 1997) the isolates are very heterogenous. On the other hand, the prevalence of certain serotypes is linked with the geographical localisation of a flock (de Moura *et al.,* 2001).

**Table 2: A list of virulence factors contributing to the pathogenesis of Avian Colibacillosis.**

| Virulence facors | Reference |
| --- | --- |
| F (type 1) and P fimbrial adhesions | Dho- Moulin *et al.,* 1990; Dozois *et al.,* 1992; Vanden *et al.,* 1993 and Pourbaksh *et* *al*., 1997. |
| Curli | Maurer *et al.,* 1998 and Foley *et al.,* 2000. |
| Factors contributing to adhesion, resistance to immunologic defense, survival in physiologic fluids and cytotoxic effects | Dho-Moulin *et al*., 1999. |
| Factors conferring resistance to serum and phagocytosis | Dozois *et al.,* 1992; Pourbaksh *et al.,* 1997; Ellis *et al*., 1988 |
| Aerobactin siderophores | Dozois *et al.,* 1992 and Lafont *et al.,* 1987 |
| *hylE*, a hemolysin gene | Reingold *et al*., 1999 |
| The *tsh* gene encoding temperature sensitive hemagglutinin | Provence *et al.,* 1994 |
| K1 Capsular antigen | Bree *et al.,* 1989 |
| Cytotoxins | Emery *et al.,* 1992; Blanco *et al*., 1997 and Parreira *et al.,* 1998 |
| Outer membrane proteins | Chaffer *et al.,* 1974 |
| Coligenicity | Blanco *et al.,* 1997 |
| The heat-labile chick lethal toxin (CLT) | Truscott *et al.,* 1974 |
| Verotoxin-2 like toxin | Parreira *et al.,* 1998 |

Recently, Hughes *et al.* (2009) described a cross-sectional study of wild birds in northern England to determine the prevalence of *E. coli*-containing genes that encoded Shiga toxins (*stx1* and *stx2*) and intimin (*eae*), important virulence determinants of shiga toxin producing *E. coli* (STEC) associated with human disease and they stated that while wild birds were unlikely to be direct sources of STEC infections, they did represent a potential reservoir of virulence genes.

Further studies are needed to determine the role of newly identified putative virulence genes and genes with unknown functions as virulence markers of APEC to strengthen the current understanding of mechanisms underlying the pathogenesis of avian colibacillosis.

**2.2.5.5** [**Antibiotic resistance**](http://en.wikipedia.org/wiki/Antibiotic_resistance)

Bacterial infections are usually treated with [antibiotics](http://en.wikipedia.org/wiki/Antibiotic). However, the antibiotic sensitivities of different strains of *E. coli* vary widely. As [Gram-negative](http://en.wikipedia.org/wiki/Gram-negative) organisms, *E. coli* are resistant to many antibiotics that are effective against [Gram-positive](http://en.wikipedia.org/wiki/Gram-positive) organisms. Antibiotics which may be used to treat *E. coli* infection include [amoxicillin](http://en.wikipedia.org/wiki/Amoxicillin), as well as other semisynthetic penicillins, many [cephalosporins](http://en.wikipedia.org/wiki/Cephalosporin), [carbapenems](http://en.wikipedia.org/wiki/Carbapenems), [aztreonam](http://en.wikipedia.org/wiki/Aztreonam), [trimethoprim-sulfamethoxazole](http://en.wikipedia.org/wiki/Trimethoprim-sulfamethoxazole), [ciprofloxacin](http://en.wikipedia.org/wiki/Ciprofloxacin), [nitrofurantoin](http://en.wikipedia.org/wiki/Nitrofurantoin) and the [aminoglycosides](http://en.wikipedia.org/wiki/Aminoglycoside).

Avian pathogenic *E. coli* strains are often resistant to antimicrobials approved for poultry including cephradine (Rahman *et al.,* 2004), tetracyclines (Cloud *et al.,* 1985; Irwin *et al.,* 1989; Blanco *et al.,* 1997; Bass *et al.,* 1999 and Rahmn *et al.,* 2004), chloramphenicol (Rahman *et al*., 2004), sulfonamides (Cloud *et al*., 1985; Blanco *et al.,* 1997; Bass *et al.,* 1999; Li *et al.,* 2007), amino-glycosides (Dubel *et* *al.,* 1982; Irwin et al., 1989; Allan *et al.,* 1993; Blanco *et al*., 1997; Bass *et al.,* 1999) and β-lactam antibiotics (Cloud *et al.,* 1985; Blanco *et al*., 1997; Rahman *et al*., 2004; Li *et al.*, 2007).

Resistance to fluoroquinolones was reported within several years of the approval of this class of drugs for use in poultry (Blanco *et al.,* 1997; Li *et al*., 2007; White *et al.,* 2000; Van den *et al.,* 2001; Li *et al*., 2007). There is reason for concern that genes conferring resistance to extended-spectrum beta-lactams will emerge in avian pathogenic *E. coli* strains (Zhao *et al*., 2001) and reduce the efficacy of ceftiofur, which is currently used on a limited basis in poultry breeding flocks and hatcheries (Wooley *et al.,* 1992).

Wooley *et al.* (1992) conducted a study at the University of Georgia. They found 97 of 100 avian pathogenic *E. coli* isolates to be resistant to streptomycin and sulfonamide and 87% of these multiple antimicrobial resistant strains contained a class 1 integron.In 1999 Bass *et al,*reported that intI1, which carried multiple antibiotic resistance genes. Multiple antimicrobial resistance traits of avian pathogenic *E. coli* have also been associated with transmissible R-plasmids**.**

Antibiotic-resistant *E. coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as [*Staphylococcus aureus*](http://en.wikipedia.org/wiki/Staphylococcus_aureus), through a process called [horizontal gene transfer](http://en.wikipedia.org/wiki/Horizontal_gene_transfer). *E. coli* bacteria often carry multiple drug-resistant plasmids, and under stress, readily transfer those plasmids to other species. Thus, *E. coli* and the other [enterobacteria](http://en.wikipedia.org/wiki/Enterobacteriaceae) are important reservoirs of transferable antibiotic resistance (Salyers *et al.,* 2004).

[Antibiotic resistance](http://en.wikipedia.org/wiki/Antibiotic_resistance) is a growing problem. Some of this is due to [overuse of antibiotics](http://en.wikipedia.org/wiki/Overuse_of_antibiotic) in humans, but some of it is probably due to the use of antibiotics as growth promoters in animal feed (Johnson *et al.,* 2006). According to a study conducted by Perfeito *et al.* (2007) published in the journal [*Science*](http://en.wikipedia.org/wiki/Science_%28journal%29) in August 2007 found the rate of adaptative [mutations](http://en.wikipedia.org/wiki/Mutation) in *E. coli* is "on the order of 10−5 per [genome](http://en.wikipedia.org/wiki/Genome) per generation, which is 1,000 times as high as previous estimates," a finding which may have significance for the study and management of bacterial antibiotic resistance.

Hasan *et al.* (2011) studied the antibiotic resistance patterns in avian pathogenic *Escherichia coli* in Bangladesh. Of the 279 dead or sick poultry of different ages, 101 pathogenic *E. coli* strains were isolated from broilers and layer hens. The strains were screened to determine phenotypic expression of antimicrobial resistance against 13 antibiotics that are commonly used in both veterinary and human medicine in Bangladesh. Of the 101 pathogenic *E. coli* isolates, more than 55% were resistant to at least one or more of the tested compounds, and 36.6% of the isolates showed multiple–drug-resistant phenotypes. The most common resistances observed were against tetracycline (45.5%), trimethoprim-sulphamethoxazole (26.7%), nalidixic acid (25.7%), ampicillin (25.7%) and streptomycin (20.8%). Resistance to ciprofloxacin (12.9%), chlormaphenicol (8.9%), nitrofurantoin (2%) and gentamicin (2%) was also observed. One isolate was resistant to cefuroxime (1%), and cefadroxil (1%).

Hashem *et al*. (2012) reported *E. coli* to be highly sensitive to colistin sulphate (100%), intermediately sensitive to ciprofloxacin (50%), resistance to amoxicillin (100%), ampicillin (75%), oxytetracycilline (50%), penicillin (25%). Resistance also found against cotrimoxazole (100%), gentamycin (75%).

**2.2.5.6 Isolation and identification of *E. coli***

**Culture**

The [MacConkey agar](http://en.wikipedia.org/wiki/MacConkey_agar) or Eosin methylene blue ([EMB) agar](http://en.wikipedia.org/wiki/Eosin_methylene_blue) (or both) are specific media for detection of *E. coli*. On MacConkey agar, deep red colonies are produced, as the organism is [lactose](http://en.wikipedia.org/wiki/Lactose)-positive, and fermentation of this sugar will cause the medium's [pH](http://en.wikipedia.org/wiki/PH) to drop, leading to darkening of the medium. Growth on EMB agar produces black colonies with a greenish-black metallic sheen (Paton *et al*., 1998).

**Biochemical properties**

The biochemical properties of *E. coli* are

* They are [lysine](http://en.wikipedia.org/wiki/Lysine) positive, and grows on [TSI (Triple super Iron test) slant](http://en.wikipedia.org/wiki/TSI_slant) with a (A/A/g+/H2S-)
* *Escherichia coli* ferments glucose and lactose producing acid and carbon dioxide.  Acid causes the phenol red indicator in the agar to turn yellow.  Carbon dioxide is observed as bubbles or cracks in the agar. There is no hydrogen sulfide production, as indicated by the lack of black precipitate in the agar(Carter,1986)
* It is also, [IMViC](http://en.wikipedia.org/wiki/IMViC) {+ + –)(Indole positive,methyl red and Voges –prauskeur positive and citrate negative)
* *E. coli*; as it is [indole](http://en.wikipedia.org/wiki/Indole)-positive produced (red ring) in the peptone medium,(Cheesbough,1985)
* [Methyl red](http://en.wikipedia.org/wiki/Methyl_red)-positive (bright red)and VP-negative (no change-colourless in MR-VP([Methyl red](http://en.wikipedia.org/wiki/Methyl_red)- and Voges –prauskeur )broth (Cheesbough,1985).
* [Citrate](http://en.wikipedia.org/wiki/Citrate)-negative (no change-green colour) in citrate medium

Tests for toxin production can be done using mammalian cells in [tissue culture](http://en.wikipedia.org/wiki/Tissue_culture), which are rapidly killed by [shiga toxin](http://en.wikipedia.org/wiki/Shiga_toxin). Although sensitive and very specific, this method is slow and expensive (Paton *et al.,* 1998).

**Molecular Investigation**

The following molecular methods are usually used to identify *Escherichia coli*

* **Polymerase chain reaction (PCR)**

Candrian *et al.*, (1991) developed a PCR assay specific for *Escherichia coli* allowing rapid and unambiguous identification. It has a relatively s modified internal transcribed 16S rRNA gene sequence compared to other members of Enterobacteriaceae .

* **Pulsed field gel electrophoresis (PFGE)**

It is considered as the gold standard in typing bacterial isolates (Riley, 2004; Georging 2010). According to Georging (2010) the method involves the extraction of DNA in a plug followed by restriction digestion with the appropriate restriction enzymes (*Xba*1 *and NOT*I). The large fragments 30- 50 kbp produced as a result of restriction enzyme digestion is separated on agarose gel by altering field direction (current). This forces the small fragments to move faster than the large fragments on the agarose gel which results in a DNA pattern specific to each clone.

* **Multi Locus sequence Typing (MLST)**

Multi Locus sequence typing is a well established method for characterizing bacteria (Gordon, 2010). It is used for a variety of different bacteria to understand clonal groups and phylogenetic relatedness. In MLST seven housekeeping genes from the core genome are selected and 300-700 bp region of each of these genes are sequenced (Cooper and Feil, 2004). These sequence profiles can be used to determine the ancestry and relatedness of each strain (Gordon, 2010).

* **Random amplification of polymorphic DNA**

Random amplification of polymorphic DNA (RAPD) is a simple PCR based genotyping method that uses short oligonucleotides of random sequence (Williams *et al.,* 1990). The random sequences bind to random priming sites and amplify DNA segments of variable lengths (Hadrys *et al.,* 1992). This results in a pattern of small and large DNA fragments (Williams *et al.,* 1990). These patterns would be identical for clonal bacterial population (Gordon, 2010) and have been proposed as alternatives for using to characterize *Escherichia coli* isolates of avian origin (Chansiripornchai *et al.*, 2001 and de Moura *et al.,* 2001).

* **Clermont phylogenetic grouping**

Clermont phylogenetic grouping is a simple and rapid method that was used by Clermont *et al. (*2007) to identify the phylogenetic groups of *E. coli*. This method uses a triplex PCR to identify the phylogenetic group by examining the presence or absence of two genes (ChuA and YiaA) and a DNA fragment (TSPE4.C2).

**2.2.6 Staphylococcal infections**

*Staphylococcus aureus* was discovered in [Aberdeen](file:///\\wiki\Aberdeen), [Scotland](file:///\\wiki\Scotland) in 1880 by the [Surgeon](file:///\\wiki\Surgeon) Sir [Alexander Ogston](file:///\\wiki\Alexander_Ogston) in [pus](file:///\\wiki\Pus) from surgical abscesses (Ogoston, 1984), abbreviated to *S. aureus* or *Staph aureus* in medical literature. The bacterium *S. aureus* is widespread in the environment and causes a variety of opportunistic infections in poultry, especially in the tropics. These include foot abscesses, infections of joint membranes and dermatitis of combs and wattles. According to McCaughey (2007) it is found in United States, [Denmark](http://en.wikipedia.org/wiki/Denmark), [Finland](http://en.wikipedia.org/wiki/Finland), and the [Netherlands](http://en.wikipedia.org/wiki/Netherlands) as a public health hazard. Giasuudin *et al.* (2002) reported the disease to be found in Bangladesh as co-infection with other diseases.

*S. aureus* are capable of producing pigment according to their strain called [staphyloxanthin](file:///\\wiki\Staphyloxanthin), a [carotenoid](file:///\\wiki\Carotenoid) [pigment](file:///\\wiki\Pigment) that acts as a virulence factor. It has an [antioxidant](file:///\\wiki\Antioxidant) action that helps the microbe to evade death by [reactive oxygen species](file:///\\wiki\Reactive_oxygen_species) used by the host immune system. Staphyloxanthin is responsible for its characteristic golden colour in agar (Claudit *et al*., 2006). This pigment helps to survive incubation with an oxidizing chemical, such as [hydrogen peroxide](file:///\\wiki\Hydrogen_peroxide). For this reason, drugs designed to inhibit the bacterium's production of the staphyloxanthin may weaken it and renew its susceptibility to antibiotics (Liu *et al*, 2005).

**2.2.7 Host**

This is the commonest pathogen to all avian species including all fowl and especially the turkeys, chickens, game birds and water fowls are susceptible. The pathogen also affects mammals including human beings (Butcher *et al*., 1999).

**2.2.8 Habitat**

*Staphylococcus aureus* is considered to be normal inhabitant of skin, feathers and respiratory and intestinal tract of the chicken. It also can be isolated from litter, dust and environment (Butterworth, 1999). The organism can even cause disease by invading the circulatory system. The susceptibility of the disease increased by any injury e.g., beak trimming, toe trimming and needle injection. The major route of this organism is respiratory tract. Poor air quality or hot air vaccines increase the susceptibility of the disease.

**2.3 Epidemiology**

**2.3.1 Distribution**

*Staphylococcus aureus* have been reported from many countries around the world including the Asian continents. According to McCaughey (2007) it is found in the United States, [Denmark](http://en.wikipedia.org/wiki/Denmark), [Finland](http://en.wikipedia.org/wiki/Finland) and the [Netherlands](http://en.wikipedia.org/wiki/Netherlands) as a public health hazard. There were infrequent hospital outbreaks in Western Europe and Australia. Now a day it is a very common disease found in poultry in Bangladesh (Kamaruddin *et al,* 2007)

.

**2.3.2 Mode of transmission**

According to Butcher *et al*. (1999)Staphylococcosis is a soil borne disease and transmission of this disease occurs mainly after storms when birds are ranged within stagnant rain polls. According to Skeeles (1997) both vertical and horizontal transmission can occur in this disease. Horizontal transmission can occur due to stress. Stress can include overcrowding, insufficient access to feed & water, feed restriction, vaccination, other handling defects, on set of sexual maturity & egg production, inadequate clean out & bio- security procedures, extreme temperature and poor air quality.

**2.3.3 Prevalence**

Staphylococcosis is a very common disease of all ages of chicken. Skeeles (1997) differentiated the disease into four stages of a breeder`s life

* **0-2 weeks:** **Omphalitis** are very common with femoral head necrosis. The disease can be transmitted through eggs or hatchery contamination and minor surgeries.
* **4-6 weeks:** In this stage of life of a chicken, staphylococcosis can infect in hock and stifle joints. It may occur as a secondary infection with coccidiosis.
* **10-20 weeks:** Staphylococcsis also causes infection in hock and stifle joints at this stage of life. At this stage of life stress of vaccination, feed restriction, sexual maturation and overcrowding act as predisposing factors of the disease.
* **24-30 weeks:** At this stage of life Staphylococcsis usually causes infection in planter bones (bumble foot). Stress of movement, mating and onset of egg production predisposes the birds to the disease.

**2.3.4 Clinical sign:**

Butcher *et al*., (1999) described the disease, Staphylococcosis to appear in three forms i.e., the septicemic form (acute), arthritic form (chronic) and bumble foot. In the septicemic form of infection the birds become listless, there is loss of appetite and watery diarrhea may be seen. In this form of disease, black rot may pass through eggs. In case of arthritic form, birds show symptom of lameness and breast blisters as well as painful movement resulting in loss of production. Bumble foot is the chronic form of the disease, resulted with infection in the plantar bone. But, according to Skeeles (1997), there is another form of the disease called **omphalitis** which is seen at early stage of the life of chicken where wet navel is found.

**2.3.5 Post mortem lesions:**

According to Skeeles (1997), the post mortem lesions of staphylococcosis in chickens include

* **Omphalitis:** The disease is seen in the chicken of 0-2 weeks of age. After opening a caseous mass may be found in the navel.
* **Septicemia:** Generalized necrosis is found in the internal organs.
* **Gangreneous dermatitis:** The lesions arefound in young chicken.
* **Arthritis:** In this case, hock joint and stifle joint are involved. It is seen in all ages of chicken. In postmortem, the joint and surrounding tissues will contain white to yellow purulent exudates.
* **Bumble foot:** It is similar to arthritic form, but the infection occurs in plantar bones.

**2.4 Virulence Factors**

It is well known that S. aureus produces many virulence factors, such as hemolysins, leukocidins, proteases, enterotoxins, exfoliative toxins, and immune-modulatory factors (Manders,1998 ;Foster,2004 and 2005,  Rooijakkers *et al.,2*005). The expression of these factors is tightly regulated during growth. The agr system, known as the quorum-sensing system, is known to play a central role in the regulation of virulence factors (Cheung *et al.,2*004 **;** Novick,2003:  Novick and Geisinger 2008). *agr*AC is a two-component system (TCS) that consists of a histidine kinase and a response regulator. The *agr* system regulates the expression of the gene coding for small RNA, known as RNA III, which is localized divergently at the agr operon and regulates the expression of many virulence factors, such as hemolysins, leukocidins, and protein A( Novick,2003:  Novick and Geisinger 2008). In addition, RNA III, known as hld, encodes delta-hemolysin. The mechanisms of RNA III regulation for the expression of several virulence factors and regulatory factors, including alpha-hemolysin, coagulase, protein A, Map, and Rot, have been demonstrated to occur at the posttranscriptional level by RNA-RNA interaction (Chevalier C., *et al.* 2010;, Geisinger *et al*.,2006 ;  Liu Y, *et al,*2011; Morfeldt *et al*.,1995). However, in other virulence factors, the precise mechanism of regulation by RNA III remains unknown. Besides agr, many factors, including other TCSs (arl and sae) and transcriptional regulators (the sar family, rot, and mgr), have been demonstrated to be involved in the expression of virulence factors (Novick, 2003; Cheung *et al., 2004* ; Somervill *et al.,*2009).

Following table (Table 3) is a list of virulence factors that are contributing to the pathogenesis of avian stahylococcosis described in relation to avian species, which may be a subject for future studies.

**Table 3: A list of virulence factors contributing to the pathogenesis of avian stahylococcosis**

|  |  |  |  |
| --- | --- | --- | --- |
| Toxin | Species distribution | Gene | Reference |
| α | *Staphylococcus**aureus* biotype A, B, C | hla | Bhakdi & Tranum Jensen, 1991 |
| β | *Staphylococcus**aureus* biotype A, C | hlb | Smyth, Mollby & Wad Storm, 1975 |
| γ | *Staphylococcus aureus* | hlgA & hlB | Cole man et al., 1989; Fackrell & Wise Man, 1976; Cooney et al., 1988 |
| δ | *Staphylococcus aureus* | hld | Turner & Pickard, 1979; Fitton, Dell & Shaw, 1990 |
| Entero Toxin B | *Staphylococcus aureus* | Seb | Johns & Khan, 1988 |
| Entero Toxin C | *Staphylococcus* *aureus* | Sec | Altboum Hertman & Sarid, 1985 |
| Entero Toxin D | *Staphylococcus aureus* | Sed | Iandolo, 1989 |
| Entero Toxin G | *Staphylococcus aureus* | Seg | Bettley Borst & Ragga Sa, 1992 |
| Entero Toxin H | *Staphylococcus aureus* | Seh | Ren *et al*.,1994 |
| Staphylo coagulase | *Staphylococcus aureus* | Coa | Dru Mond & Tager, 1963 |
| Staphylo coccalnuclease | *Staphylococcus aureus* | nuc | Anfinsen Cautrecasas & Taniuchi, 1971; Davis *et al.,* 1977; Shortle, 1983 |
| Staphylo kinase | *Staphylococcus**aureus* biotype A, C |  | Dru Mond & Tager, 1963 |
| Hyaluronidase | *Staphylococcus aureus* | hysA | Rautela & Abram Son, 1973; Farrell, Taylor & Holland, 1995 |
| Serine protease | *Staphylococcus aureus* | sprV8 (Sasp) | Drapeu, 1978; Carmona & Gray, 1987; |

**2.5 Bacteria-host interactions and pathogens:**

The major infective route of entry for this organism is through the respiratory tract. Hills *et al.,* (1989) describe that economic impact of the disease results from morbidity rather than mortality and decrease in productivity of the affected birds. He also reported that with the impaired immune system due to Infectious bursal disease (IBD), Chicken Infectious Anemia (CIA) or Marek`s disease the incidence of the disease was increased. Jensen (1990), described that staphylococci have a high affinity for collagen rich surfaces e.g., articular surfaces of joints & tendons. As a result it infects young chickens rather than the older ones and causes femoral head necrosis & osteomyelitis.

**2.6** [**Antibiotic resistance**](http://en.wikipedia.org/wiki/Antibiotic_resistance)

The penicillin resistant Staphylococcus was first recorded in 1940 in UK but, by the year 1950, it became a worldwide problem (Blair *et al.,* 1960). In 1978, the scarlet fever, caused by staphylococcal infection**,** became a public health concern for women. According to Enright *et al. (*2002) and Chambers *et al*., (2009) the penicillin resistant *S. aureus* has become pandemic in hospitals. According to them *Staphylococcus aureus* is now [resistant](file:///\\wiki\Antibiotic_resistance) to many commonly used antibiotics and in the UK, only 2% of all *S. aureus* isolates are sensitive to penicillin, with a similar picture in the rest of the world. The β-lactamase-resistant penicillins (methicillin, oxacillin, cloxacillin, and flucloxacillin) were developed to treat penicillin-resistant *S. aureus*, and are still being used as first-line treatment.

Staphylococcal resistance to penicillin is mediated by [penicillinase](file:///\\wiki\Penicillinase) (a form of [β-lactamase](file:///\\wiki\Beta-lactamase)) production: an enzyme that cleaves the [β-lactam](file:///\\wiki\%CE%92-lactam) ring of the penicillin molecule, rendering the antibiotic ineffective. Penicillinase-resistant β-lactam antibiotics, such as [methicillin](file:///\\wiki\Methicillin), [nafcillin](file:///\\wiki\Nafcillin), oxacillin, [cloxacillin](file:///\\wiki\Cloxacillin), [dicloxacillin](file:///\\wiki\Dicloxacillin), and flucloxacillin, are able to resist degradation by staphylococcal penicillinase **.**

### According to Schneewind *et al.* (1995) the virulence factor for *S. aureus* mainly associated with protein A. The Protein A has an [IgG](file:///\\wiki\IgG)-binding protein that binds with [Fc region](file:///\\wiki\Fc_region) of an [antibody](file:///\\wiki\Antibody). As a result, *S. aureus* survive in the blood. There is another transpeptidase, such as the sortases responsible for penicillin resistance (Zhu *et al.,* 2008).

Resistance to methicillin is mediated via the *mec* [operon](file:///\\wiki\Operon), part of the staphylococcal cassette chromosome mec (SCC*mec*). Resistance is conferred by the *mecA* gene, which codes for an altered [penicillin-binding protein](file:///\\wiki\Penicillin-binding_protein) (PBP2a or PBP2') that has a lower affinity for binding β-lactams (penicillins, [cephalosporins](file:///\\wiki\Cephalosporin), and [carbapenems](file:///\\wiki\Carbapenem)). This allows for resistance to all β-lactam antibiotics, and obviates their clinical use during MRSA infections. As such, the [glycopeptide](file:///\\wiki\Glycopeptide) [vancomycin](file:///\\wiki\Vancomycin) is often deployed against MRSA.

[Aminoglycoside](file:///\\wiki\Aminoglycoside) antibiotics, such as [kanamycin](file:///\\wiki\Kanamycin), [gentamicin](file:///\\wiki\Gentamicin), [streptomycin](file:///\\wiki\Streptomycin), etc., were once effective against staphylococcal infections until strains evolved mechanisms to inhibit the aminoglycosides' action, which occurs via protonated amine and/or hydroxyl interactions with the [ribosomal RNA](file:///\\wiki\Ribosomal_RNA) of the bacterial [30S ribosomal subunit](file:///\\wiki\30S_ribosomal_subunit) (Carter *et al.,* 2000). Aminoglycoside-modifying enzymes inactivate the aminoglycoside by covalently attaching either a [phosphate](file:///\\wiki\Phosphate), [nucleotide](file:///\\wiki\Nucleotide), or [acetyl](file:///\\wiki\Acetyl) moiety to either the amine or the alcohol key functional group (or both groups) of the antibiotic. This changes the charge or sterically hinders the antibiotic, decreasing its ribosomal binding affinity. In *S. aureus*, the best-characterized aminoglycoside-modifying enzyme is aminoglycoside adenylyltransferase 4' IA (*ANT(4')IA*) (Sakon *et al.,* 1993).

The first case of *S. aureus* truly resistant to glycopeptide antibiotics was only reported in 2002 (Chang *et al.,* 2003). Three cases of VRSA(vancomysin resistant Staphylococcus aureus) infection have been reported in the United States as of 2005 (Menichetti, 2005). Glycopeptide resistance is mediated by acquisition of the *vanA* gene

Finally *et al.,* (2013) Pyzik, E and Marek, A., conducted a study on plasmid profile analysis and evaluation of antibiotic susceoptiblity of *Staphylococcus aureus* strains isolated from table chicken eggs and found out of 105 bacterial isolates the resistance against erythromycin to be (66.66%), tetracycline (66.66%), oxytetracycline (61.11%), Penicillin (50%) and amoxicillin (44.44%).

**2.7 Isolation and identification of Staphylococcus**

**2.7.1 Culture**

The cultural properties for *Staphylococcus* is given below- The cultural properties for *Staphylococcus* is given below-

**Calf blood agar:** Colonies are found surrounded by α, β, δ, and hemotoxic zone. This reaction occurs mainly due to hemotoxic reaction (Beer *et al.,* 1974).

**Mannitol Salt Agar:** Colonies with bright yellow zonedue to mannitol fermentation (Beer *et al.,* 1974).

**Baird-Parkar Agar:** Black, shiny, convex, 1-1.5mm diameter narrow white margin surrounded by a clear zone of 2 to 5mm width (Baird, 1969).

**Vagel -Johnson Agar:** White or gray black colonies are seen (Beer *et al.,* 1974).

**2.7.2 Biochemical properties.**

**The biochemical properties of this organism is given below-**

**Catalase test:** This test is done for evaluation of gas bubbles of Hydrogen peroxide (Cowan, 1974). The organism is catalase positive**.**

**Oxydase test-**

This test is done for oxydase –positive bacteria turned dark blue within 5-6 minutes (Faller and Schleifer, 1981)

**Coagulase test-**

This test is done for formation of clot (Kloss and lambe, 1991: Martin and Myer,1994).This test is only given by *staphylococcus aureus*

**Carbohydrate Dissimilation Test-**

The production of acid from maltose and mannitol under aerobic conditions indicator of Carbohydrate Dissimilation Test. It was described by Baird-Parkar, 1980.

**Biotyping:** The biotyping method is used for detection of strains, proposed by Devriese (1984). It is a simple method for detection of toxins. In this method, the organisms grow on crystal violet agar.

**2.8 Molecular Investigation**

The following molecular methods are usually followed to identify *Staphylococcus aureus.* It is divided into two parts: methods without DNA amplification and the methods with DNA amplification.

**2.8.1** **Methods without DNA amplification:**

* **Restriction endonuclease analysis:**

In this method, isolated total DNA is digested with the different restriction enzymes. The fragments generated are separated by agarose gel electrophoresis and visualized by ethidium bromide staininig. Restriction enzymes *Pst*I, *Eco*RI,and *Pvu*II,are used for staphylococci (Etienne *et al*., 1990; Haertl and Bandlow, 1990). Restriction fragments in the size range between 5-13Kb are separated by agarose gel electrophoresis (Matthews *et al*., 1992).

* **Pulsed field gel electrophoresis (PFGE)**

Pulse-field gel electrophoresis (PFGE) is known to be a highly discriminatory technique and is frequently used for characterizing genetic diversity and outbreak investigations of microbial pathogens. PFGE is a whole genome typing method based on DNA fragment patterns generated by restriction digestion,

so its stability may be insufficient for reliable studies of the evolution and phylogenetic relationships of bacterial strains (Hallin *et al.,* 2006). PFGE analysis was performed for all the *S. aureus* isolates.

It involves the extraction of DNA in a plug followed by restriction digestion with the appropriate restriction enzyme *Sma*I or *sp*I for *S. aureus* (Bannerman *et al*., 1995). The large fragments 30- 50 kbp produced as a result of restriction enzyme digestion is separated on agarose gel by altering field direction (current). This forces the small fragments to move faster than the large fragments on the agarose gel which results in a DNA pattern specific to each clone (Georging, 2010).

* **Multi Locus sequence Typing (MLST**)

Multilocus sequence typing (MLST), which involves sequencing of 7 housekeeping genes in each *S. aureus* genome and then comparing them with the established sequence information in MLST database, could be more accurate and reproducible. However, housekeeping genes are relatively stable by nature, and changes in these genes accumulate slowly over time (Peacock *et al*., 2002).

It has been suggested that combined assessment of virulence gene profiles and genetic background could increase the discriminatory ability of genetic investigations of *S. aureus* strains from geographically diverse locations (Varshney et al., 2009) . According to Campbell *et al*. (2008) *S. aureus* strains from different geographic regions had different profiles of virulence genes. Varshney *et al.*, (2009) also pointed out that MLST, spa typing, and PFGE were all less discriminatory than SE content in *seb*-positive strains in the U.S.

**2.8.2** **Methods with DNA amplification**

* **Gene Specific PCR:**

Amplification of conserved gene sequences by simplex PCR reaction is used for identification of Staphylococcal species and their genotypes. In *S. aureus* the genes encoding 16s rRNA (Saruta *et al*., 1995)factor A essential for methicillin resistance (femA) (Unal *et al*., 1992) and Staphylococcal thermonuclease (nuc) (Brakstad *et al.,* 1993 and Khan *et al.,* 1998) are frequently used for identification at the species level.

* **PCR-Restriction Fragment length Typing:**

A target DNA sequence corresponding to a specific gene or genomic region is amplified at high stringency using primers annealing to its terminal conserved regions. This amplicon is then cut with restriction endonucleases resulting in a specific RELP Pattern. This method is rapid, simple and reproducible but has shown only moderate discrimination (Goh *et al.,* 1992).

* **Real-time PCR:**

This method has recently been used for *S. aureus* species (Yang *et al*., 2002 and Palomares *et al.,* 2003) and 15 other species of coagulase negative staphylococci (Edward *et al.,* 2001). Rapid QPCR procedures were developed for identification of methicillin resistance (Reishel *et al*., 2000 and Elsayed *et al.,* 2003) and fluoroquinolones resistence genes (Lapierre *et al.,* 2003).

* **Multiplex PCR**

The multiplex PCR assay allows for simultaneous amplification of several genes in one reaction mixture. The use of multiplex PCR to characterize staphylococcal strains and their resistance to methicillin has been well documented (Barski *et al.,* 1996). Those reports focus on detection of the gene responsible for methicillin resistance (*mecA*) along with either the *femA*, 16S rRNA, *nuc* or IS*431* gene as a positive control(s) (Brakstad *et al*., 1993).

A recent study describes the use of two multiplex PCR assays for detection of *S. aureus* exotoxin genes: one is designed to detect the enterotoxin genes and the other is designed to detect the *tst*, *eta*, and *etb* genes (Becker *et al.,* 1998). They have used DNA enzyme immunoassays to validate the specificity of the PCR products, using oligonucleotide probes derived from the sequences of the *S. aureus* toxin genes.

* **Ribosomal Spacer PCR(RS-PCR):**

It is based on highly polymorphic nature of the 16s-23s rRNA internal transcribed spacer sequences which are amplified by primers containing conserved sequences from the adjacent 16s and 23s rRNA genes (Barry *et al*., 1991). Analysis of the intergenis spacer sequences proved useful in differentiating between species (Jensen *et al*., 1993; Mendoza *et al*., 1998, and Couto et *al.,* 2001).

**CHAPTER-III**

**METHODOLOGY**

**3.1 Duration of the study**

The study was conducted from August 2011 to May 2012 that arbitrarily covered three seasons (August, September, October and November was considered as Autumn; December, January, February as Winter and March, April, May as summer) for determining the prevalence of bacterial diseases of chickens in the Chittagong district of Bangladesh.

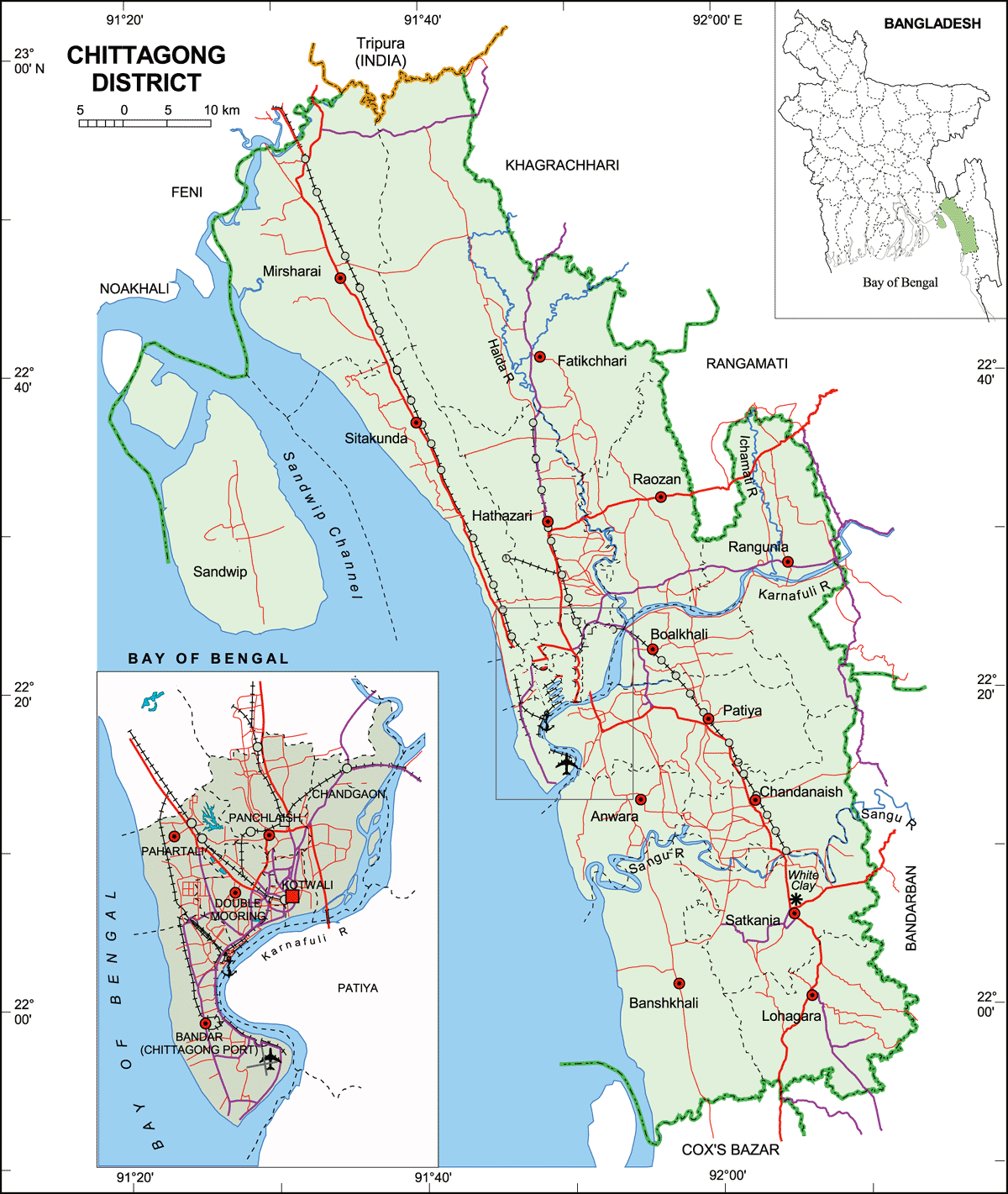
**3.2 Samples for investigation**

Dead chickens from different types of commercial poultry farms referred to the i) S. A. Quadery Teaching Veterinary Hospital (SAQTVH), Chittagong Veterinary and Animal Sciences University (CVASU); ii) Department of Pathology and Parasitology laboratory (PTHL), CVASU and iii) Chittagong lab, Khulshi, Chittagong for postmortem examinations were sampled.

All laboratory works related to this study were undertaken in the Animal Disease Diagnostic Laboratory of Poultry Research and Training Centre (PRTC), CVASU.

**3.3 Study area:**

The study areas included Chittagong metropolitan areas (AK Khan, CRB, Tigerpass, Vatiary, Cornelhat, Boddarhhat, Kotoali, Panchlaish, Doublemooring, Agrabad, Potenga, Santirhat); Different Upazilas of Chittagong district (Mirsharai, Boalkhali, Sitakunda, Patiya, Satkania, Raozan, Chandanaish, Anwara, Banshkhali, Lohagara, Hathazari, Fatikchhari, Kumira, Rangunia, Kattoli, Jorargang, Potiya), Chittagong Hill Tracts (Bandarban, Rangamati) and Cox`s Bazar as depicted in Figure 1.



Kumira

Cornelhat

Vatiary

Agrabad

Boddarhat

**Figure 1:** Map showing the location of the study areas (Red spots)

**3.4 Postmortem for clinical investigation**

Postmortem of the birds for preliminary diagnosis of diseases according to clinical history was done.

**3.5 Types of samples investigated**

Liver, lungs, trachea and pus from bumble foot were collected from the 320 dead birds for detection of *Escherichia coli* and *Staphylococcus* (Butcher *et al.,* 1999; *Norton et al,* 2000). The organ samples were collected into zipped bags containing ice to avoid putrefaction. Pus samples were collected from bumble foot using sterile cotton swab. For each sample supporting data were collected from the owner of the birds through a questionnaire designed for this study (Appendix).

**3.6 Isolation and identification of *Escherichia coli* and *Staphylococcus aureus***

The samples collected from birds preliminarily identified as to be the cases of colibacillosis and staphylococcosis, in postmortem investigation were considered for this study.

**3.6.1 Growth in nutrient broth**

Nutrient broth was prepared according to manufacturer’s direction (Oxoid, England). Using dry sterile cotton swabs or inoculation loops bacteriological samples were collected from each of the organs, taking all the aseptic measures and inoculated into nutrient broths. The inoculated broths were then incubated at 37oC overnight. The broths showing bacterial growth were sub-cultured twice on agar plates containing specific medium according to clinical history to obtain a pure culture of the specific organism.

**3.6.2 Isolation of *E. coli***

For the samples suspected of *E.coli* inoculation was done on Eosin Methylene Blue (EMB) (Oxoid, UK) agar plates. The plates were then incubated at 37oC for 24 hours to detect whether the bacterial population was positive for *E.coli* or not. Positive isolates showing metallic green sheen were then selected randomly and spot indole test was done according to standard method,by kovac`s reagent. To conduct the test, a small piece of Whatman filter paper was placed in a petri dish cover. Saturate paper with Kovács reagent (1 to 1.5 ml).  Smear the paper with cell paste from an 18- to 24-hour culture. If indole is present, a red pink color will develop within 1 to 3 minutes.  

**3.6.3 Isloation of Staphylococcus**

For the samples suspected of *Staphylococcus* infection the broth culture was inoculated on mannitol salt agar. The positive colonies with bright yellow zones (due to mannitol salt fermentation) were then re-inoculated into blood agar to detect the pathogenecity of *Staphylococcus aureus*. Blood agar was prepared with blood agar base (Oxoid, England) according to manufacturer’s direction, supplemented with 5% citrated caprine blood. All streaked blood agar plates were incubated at 37oC overnight to allow hemolysis to occur. Then, with the randomly selected positive isolates coagulase test was done, according to standard method. To conduct the coagulase test, whole blood from horse was collected into commercially available EDTA –treated lavender tops. Then blood was centrifuged at 2600 rpm for 10 minutes using a refrigerated centrifuge. The resulting supernatant which was plasma immediately transferred to clean 1.5 ml eppendorf tube using micropipette. The plasma was then stored at-20ºC.For slide coagulase test,dense suspensions of staphylococci from culture were made on two ends of clean glass slide. The two end was labeled as test and the other as control.The test suspension was treated with a drop of EDTA treated horse plasma and mixed well.Agglutination or clumping of cocci within 5-10 seconds was taken as positive.

**3.6.4 Preservation of the culture**

All positive isolates were inoculated into tryptic soy broth (TSB) (Oxoid, England), incubated overnight at 37oC and then preserved at -80oC with 15% glycerol in 1.5 ml eppendorf tubes for future investigation.

**3.7 Antimicrobial sensitivity test:**

All the positive isolates for *E. coli* and *Staphylococcus* were investigated for their diversity in antimicrobial susceptibility profiles. Bauer-Kirby disk-diffusion procedure (Bauer *et al.,* 1966) was used on Muller-Hinton (MH) agar, prepared according to the manufacturer`s instructions (Oxoid). A bacterial turbidity equivalent of 0.5 Mcfarland standard was used as inoculum for each isolate, for standardization. The standard was prepared by adding 0.5ml of 1% (11.75g/L) Bacl2.2H2O to 99.5ml 1% (0.36N) H2SO4 (Carter and Cole, 1990). The antibiotic resistance pattern for the panel of antibiotics was determined considering the zone of inhibition sizes for each of the antibiotics as "resistant (R)", "intermediately resistant (I)", and “sensitive (S)” against the test isolates as recommended by the Clinical and Laboratory Standard Institute (CLSI, 2007) shown in Table (4). A sterile swab was dipped into the inoculum prepared for antimicrobial sensitivity test and rotated against the side of the tube with firm pressure. Then after removing the excess fluid from the swab the dried surface of MH agar was inoculated by streaking the swab three times over the entire agar surface rotating the plates approximately at 60 degrees for each time to ensure an even distribution of the inoculums. The antimicrobial disks were then placed on the surface of the inoculated agar. A separate forceps was always used to dispense each of the antimicrobial disks. The disks were placed carefully on the surface of the agar with a gentle pressure to make a complete contact. After dispensing all the disks the agar plate was incubated at 35oC for 16 to 18 hours. At the end of incubation the size of zone of inhibition around a micro-disk was measured with a digital slide calipers and the result was deduced according to CLSI, 2007. Details of the antibiotic discs used for the experiment is presented in a tabular form (Table- 4).

**Table-4: Antimicrobial resistance pattern against *E. coli* and *Staphylococcus aureus***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Group of antimicrobials** | **Antimicrobial agents** | **Disk contents** | **Zone of inhibition (diameter in cm)** | | | **Manufacturers** |
| **R** | **I** | **S** |
| penicillin | Ampicillin &  Penicillin (PEN) | 10µg | ≤1.3 | 1.4-1.6 | ≥1.7 | Oxoid, Ltd. Basingstoke, Hampshire, England |
| β-lactamase inhibitor combination | Amoxicillin (AMC)-clavulanic acid | 20/10µg | ≤1.3 | 1.4-1.7 | ≥1.8 |
| Cephalosporins (1st generation) | Cephalexin (CFL) | 30 µg | ≤1.4 | 1.5-1.7 | ≥1.8-2.0 |
| Cefadrine (CH) | 30 µg | ≤1.5 | 1.5-1.7 | ≥1.7-2.0 |
| Amino glycoside | Neomycin (Neo) | 30 µg | ≤1.5 | 1.8-2.0 | 2.5-3 |
|  | Kanamycin (K) | 30 µg | ≤1.4 | 1.4-1.8 | 1.5-2 |
| Fluoroquinolones | Ciprofloxacin (CIP) | 5 µg | ≤1.5 | 1.5-1.8 | ≥2.0 |  |
| Enrofloxacin (ENR) | 5 µg | ≤1.1 | 1.1-1.5 | ≥2.1 |
| Polypeptide antibiotics | Colistin sulphate (CLT) | 10µg | ≤1.4 | 1.4-1.8 | ≥1.5-2 |
| Tetracycline | Doxycycline (DOXY) | 30 µg | ≤1 | 1-2 | ≥2 |

**3.8 DNA Extraction from bacterial culture for PCR test**

The preserved isolates identified as *E. coli* and Staphylococcus using conventional bacteriological culture and biochemical characterization method were removed from the freezer and thawed at room temperature. Then each of the isolates were again inoculated onto either EMB or Mannitol salt agar plates as described under 3.6.2 and 3.6.3, incubated at 37oC overnight. After incubation metallic sheen for *E. coli* or yellow zones for Staphylococcus was re-confirmed before initiating DNA extraction from the isolates. For DNA extraction boiling method was followed as per the steps given below:

1. Using a sterile inoculating loop, a loop full of fresh colonies (3-4) was picked up from each of the plates and transferred to 1.5 ml eppendorf tubes containing 100µl deionized water. The tubes were then vortexed to have a homogenous cell suspension.
2. Using a sterile needle a ventilation hole was made on the lids of each of the eppendorf tubes. The tubes with the cell suspension were then boiled in a water bath at 100oC for 10 minutes and immediately thereafter cooled by placing them into flaked ice for 10 minutes. This process of boiling and sharp cooling allowed the bacterial cell wall to break down thus releasing DNA.
3. Then the eppendorf tubes with the suspension were centrifuged at 10000 rpm (mention the maker of the centrifuge machine) for 10 minutes and the supernatants containing DNA were then collected in the fresh eppendorf tubes and preserved at -20oC until testing.
4. PCR tests were then performed using primers, described by Candarian *et al*. (1991) for *E.coli* and Forsman *et al*. (1997) for *Staphylococcus aureus* to detect 16S and 23S ribosomal RNA (rRNA) gene product for *E. coli* and *S. aureus*, respectively. The sequences along with the properties of the primer sets used in this study are shown in Table 5 and 8 for *E. coli* and *S. aureus*, respectively.

**3.8.1 Identification of *E.coli* by PCR**

**Table 5:** Oligonucleotide primers used in PCR to detect ***E. coli***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Primers | Sequence (5´-3´) | G/C content (%) | Melting temp oC (Tm) | Annealing Temp (oC) | Size of amplified product (bp) |
| ECO-1 (Forward) | GACCTCGGTTTAGTTCACAGA | 48 | 59.5 | 50ºC | 585 |
| ECO-2 (Reverse) | CACACGCTGACGCTGACCA | 63 | 61.6 |

At first the stock solution (100 picomole concentration) of each primer was diluted with molecular grade water to make a 20 picomole concentration to be used for a PCR test. The reaction mixture, with the constituents and their amounts, prepared for detection of *E. coli* using PCR test is shown in Table 6.

**Table 6:** Contents of each reaction mixture of PCR used to detect ***E.coli***

|  |  |
| --- | --- |
| **CONTENT NAME** | **AMOUNT** |
| Deionized water (Nuclease free) | 3µl |
| Thermo Scientific dream Taq PCR  Master Mix (2x) Ready to use | 5 µl |
| Forward Primer  Reverse Primer | 0.5µl  0.5µl |
| DNA template | 1µl |

Amplification (PCR) was performed in a thermo cycler (Applied Biosystem, 2720 thermal cycler, Singapore). All reactions were carried out in a final volume of 10 µl. The cycling conditions used for PCR (Wang *et al.,* 1997) are shown in Table 7. A total of 35 cycles were run.

**Table 7:** Cycling conditions used for PCR detection of *E. coli*.

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Steps** | **Temp. and Time** |
| 1 | Initial denaturation | 94 oC for 15 seconds |
| 2 | Final denaturation | 94 oC for 3 seconds |
| 3 | Annealing | 50 oC for 10 seconds |
| 4 | Extension | 74 oC for 5 seconds |
| 5 | Final extension | 74 oC for 2 minutes |
| 6 | Final holding | 4 oC |

**3.8.2 Identification of *Staphylococcus aureus* by PCR**

**Table 8** Oligonucleotide primers used in PCR to detect ***Staphylococcus aureus***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Primer | Sequence (5´-3´) | G/C content % | Melting temp ™ | Annealing Temp | Size of amplified product (bp) |
| STAA-AUI (Forward) | TCTTCAGAAGATGCGGAATA | 40 | 54 oC | 55 oC | 420 |
| STAA-AUII (Reverse) | TAAGTCAAACGTTAACATACG | 33.33 | 53 oC |

The reaction mixture, with the constituents and their amounts, prepared for detection of *S. aureus* using PCR test is shown in Table 9.

**Table 9.** Contents of each reaction mixture of PCR used to detect ***Staphylococcus aureus***

|  |  |
| --- | --- |
| **CONTENT NAME** | **AMOUNT** |
| Thermo Scientific Water (Nuclease free) | 1µl |
| Thermo Scientific dream Taq PCR  Master Mix (2x) Ready to use | 5 µl |
| Forward Primer  Reverse Primer | 1µl  1µl |
| DNA template | 1µl |

Amplification was performed in a thermo cycler (Applied Biosystem, 2720 thermal cycler, Singapore). All reactions were carried out in a final volume of 10 µl. The cycling conditions are shown in Table 10. A total of 40 cycles were run.

**Table 10:** Cycling conditions used for PCR detection of *S. aureus*

|  |  |  |
| --- | --- | --- |
| **Serial No.** | **Steps** | **Temp. and Time** |
| 1 | Initial denaturation | 94 oC for 2 minutes |
| 2 | Final denaturation | 94 oC for 30 seconds |
| 3 | Annealing | 55 oC for 30 seconds |
| 4 | Extension | 72 oC for 30 seconds |
| 5 | Final extension | 72 oC for 5 minutes |
| 6 | Final holding | 4 oC |

**3.8.3 Visualization of PCR Product for *E. coli* and *Staphylococcus aureus***

Agarose gel (1%) was prepared for electrophoresis of PCR- amplifications products. A gel tray was assembled with setting a proper teeth sized comb in the tray. Then 1 % agarose solution (Seakem ® LEagarose –Lonza) was prepared in 1x TAE buffer by boiling in a microwave oven for 2 minutes. The agarose gel thus prepared was then cooled to 40- 50oC in a water bath, having added with 1 drop of ethidium bromide at a concentration of 5µg per ml. Finally, agarose gel was poured into the gel tray and allowed about twenty minutes time for solidification of the gel.

The gel was shipped into an electrophoresis tank, already filled in with 50ml of 1x TAE buffer. Then 5μl of each of the PCR products for an isolate was loaded up into gel–holes. One hole was loaded with DNA marker (Thermo Scientific O’ Gene Rular 1 kb plus) to compare the amplicon size of the gene product. As a negative control one hole was loaded up with only 5μl of distilled water.

Electrophoresis was done at 110 volts, 80 Amp for 20 minutes. After completion of electrophoresis the gel was placed in a water bath for rinsing, just for a while.Finally the gel was examined under an UV transilluminator (BDA digital, biometra GmbH, Germany). Gel electrophoresis was repeated twice with the same PCR products.

As we had no previous PCR positive*Staphylococcus aureus and E. coli* isolates from any reference laboratory, so in first time electrophoresis no positive control was used and during second time a *E. coli* isolate that was found positive on first time reaction, used as a positive control. Finally, in this thesis paper I had submitted the PCR result that was found after second time electrophoresis and DNA product size of 1000 bp was deduced (in both time electrophoresis) for the presence of *E. coli .*

**3.9 Statistical analysis**

All data were entered into a spreadsheet programme (Excel 2007, Microsoft Corporation) and [STATA - GraphPad Software](http://www.google.com.bd/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&ved=0CCwQFjAA&url=http%3A%2F%2Fwww.graphpad.com%2Fquickcalcs%2F&ei=htP3Ub2xNsjDrAed-YHwCw&usg=AFQjCNF99CgZQm9cxNyH5DuA4f6-xTdWaA&sig2=k3xWpl4qqxP6cRfqylZJUg&bvm=bv.49967636,d.bmk) was used for data summary and descriptive statistics. Fisher’s exact χ2 test was used to compare the prevalence of bacterial diseases observed in chickens associated with two binary variables.

**Figure 2: Sequential steps for isolation and identification of bacterial disease in poultry**

Postmortem investigation for tentative diagnosis of poultry diseases

Collection of organ samples (Trachea, Lungs, Liver and Pus from bumble foot) with lesions suspected of causing from bacteriological infection

Inoculation into nutrient broth and incubation at 37°C in a CO2 incubator for overnight (24 hours)

|  |  |  |
| --- | --- | --- |
| Inoculation of positive samples onto specific media such as EMB agar for *E. coli*, and Mannitol salt agar followed by blood agar for *Staphylococcus aureus*  Positive colonies (metallic sheen for EMB, yellow zone for mannitol salt agar) were considered and sub cultured twice to obtain pure cultures. | | |
| Biochemical test such as Coagulase test for *Staphylococcus* *aureus* and spot indole tests for *E.coli* | | |
|  | |  |
| Antimicrobial sensitivity test was also done both for *E.coli and Staphylococcus aureus* | For molecular investigation, DNA extraction by boiling method from pure culture and then PCR test | |

**CHAPTER-IV**

**RESULTS**

In an attempt to know the prevalence of bacterial diseases of poultry with their risk factors in Chittagong and surrounding other hill districts and to develop fast and reliable techniques for diagnosis of the diseases a total of 320 dead chickens brought in the SAQTVH, PTHL and Chittagong lab for investigation were studied of the diseases was conducted on the basis of variation of occurrence of the diseases. Diagnosis of the diseases was made based on conventional method of isolation and identification of organisms using conventional bacteriological culture and biochemical tests. Attempts were then made to confirm the causal agents using PCR. Prevalence study diseases in poultry according to age, type of breed, season, management and region. The bacterial agents isolated and identified from the samples were also investigated for their antibiotic resistance pattern. An attempt was also made to establish a relationship of this resistance pattern with other factors like season, production type and age.

**4. Description of Data Set:**

**4.1 Sources of chickens:**

Among the 320 dead chicken samples most of the samples came from CP 117 (36.56%) and then from Kazi farms 84 (26.25%) followed by Provita 32 (10%), Nahar 24 (7.5%), Nourish and Aga 15 (4.69%), Paragon 13 (4.06%), Paharika 12 (3.75%), Goucea 7 (2.19%) and Jayson 1 (0.31%). The graphical presentation of the sources of chickens is depicted in Fig 3. The X -axis indicates the percentage of the sample and Y -axis is for the commercial farms from where the chickens were sourced.

**Fig 3: Sources of chickens (samples)**

**4.2 Hospitals where the postmortem was done for preliminary diagnosis before collecting samples:**

Among the 320 dead chickens subjected to postmortem for collection of samples for further investigation, 152 (47.5%) were obtained from SAQTVH (S. A. Quaderi teaching veterinary hospital), 126 (39.38%) from Pathology and Parasitology laboratory of CVASU and the rest 42 (13.13%) were from Chittagong lab. The graphical presentation of the postmortem sample collection from different hospitals is shown in Fig 4.



**Fig: 4 percent of postmortem samples collected from different hospitals**

**4.3 Tentative diagnosis of poultry diseases:**

In an attempt to determine the causes of death of the chickens (320) postmortem was done on them and a tentative diagnosis was made according to the clinical signs and postmortem lesions. Among them 48 % of the chickens were diagnosed tentatively to be the case of colibacillosis, 9% to be staphylococcosis and 2% co-infection with both *E.coli* and *Staphylococcus*. Infectious bursal disease was diagnosed in 28% of the samples and the other 4% of the samples were diagnosed to be the case of unknown viral diseases as those samples did not show any specific signs for any diseases and also latter on they were found nutrient broth negative. Rest of the samples diagnosed individually to be aspergillosis, mycoplasmosis, coccidiosis and New castle disease (ND) at the rate of 2% each and only 1% was diagnosed as aflatoxicosis. The graphical presentation of the result is shown in Fig 5.

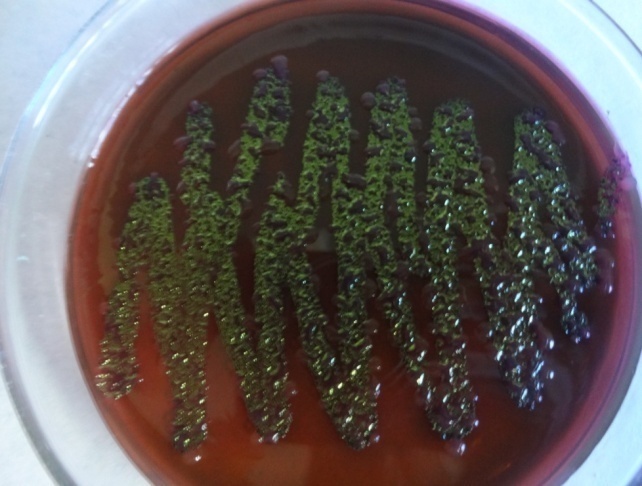
**Fig 5: Frequency of different poultry diseases diagnosed tentatively on the basis of clinical signs and postmortem lesions**

**4.4 Identification in bacteriological culture:**

All the samples collected during postmortem of the 320 chickens were inoculated into nutrient broth and from among them a total of 185 samples (58%) were found to be positive for bacterial growth. The rest 135 (42%) samples did not show any growth in the nutrient broth. The samples showing bacterial growth in the nutrient broth were then inoculated onto EMB agar and mannitol salt agar specific for *E. coli* and *Staphylococcus*, respectively. From among the isolates (48.44%) were positive in EMB agars and (Fig 7); 27 (8%) were positive in mannitol salt agars (Fig 8) which were preliminarily diagnosed as *E. coli* and *Staphylococcus*, respectively. Another 0.9% however, showed co-infection for both *E.coli* and *Staphylococcus.* The graphical presentation of the result is shown in Fig 6.



**Fig 6: Percent of samples positive and negative in different culture medium**

 **4.5 Identification in biochemical test:**

**Fig 8:** *Staphylococcus* in mannitol salt agar

**Fig 7:** *E. coli* in EMB agar

*E. coli* positive isolates showed metallic green sheen on EMB agar due to reaction of the dyes Eosin-Y and Methylene blue with the products released by *E. coli* from lactose or sucrose as carbon and energy source (Fig 7). Then, from positive samples 60 samples were randomly selected for spot indole test. The **indole test** is a biochemical test performed on [bacterial species](http://en.wikipedia.org/wiki/Bacteria) to determine the ability of the organism to convert [tryptophan](http://en.wikipedia.org/wiki/Tryptophan) into the [indole](http://en.wikipedia.org/wiki/Indole). Among them 40 isolates showed positive reaction to indole. The indole positive samples were then processed for PCR confirmation. For *Staphylococcus sp.* nutrient broth positive isolates were inoculated on to the mannitol salt agar. The colonies with bright yellow zones (due to mannitol salt fermentation) were taken as positive isolates (Fig 8). Only 27 samples (out of 185 isolates) showed positive reaction on mannitol salt agar which were then re-inoculated into blood agar to detect their pathogenesity but, none of them were positive on blood agar. All the samples were then subjected to coagulase test where all the samples showed positive reaction. The coagulase positive samples were used for PCR confirmation

**Table: 11 Association of different variables with samples positive in culture measured by chi square test**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **Categories** | **No. of observation** | | **No. positive (%)** | | **Chi square** | | **P value** |
| Season | Summer | | 139 | | 32 (23) | | 135.50 | <0.001 |
| Winter | | 77 | | 53 (69) | |
|  | Autumn | | 104 | | 100 (96) | |  |  |
| Production type | Broiler parent stock | | 7 | | 4 (57) | | 19.29 | <0.001 |
| Commercial Broiler | | 236 | | 153 (65) | |
| Commercial Layer | | 77 | | 28 (36) | |
| Litter | Saw dust | | 248 | | 163 (66) | | 42.92 | <0.001 |
| Rice husk | | 55 | | 10 (18) | |
| Others (cage/gunny bags) | | 17 | | 12 (71) | |
| Age (days) | ≤30 | | 275 | | 178 (65) | | 38.33 | <0.001 |
| >30 | | 45 | | 7 (16) | |

**4.6 Description of the association of different variables with samples positive in broth culture:**

Through this study association of different variables (Table 11) like season, production type, litter type and age of bird with the rate of bacterial infection in poultry was determined with the following findings:

* **Season:**

Three seasons (summer, winter and autumn) were investigated to determine the rate of occurrence of bacterial diseases in poultry. Autumn was found to be the worst season rendering poultry more susceptible to bacterial diseases, when out of 104 samples tested 100 (96%) were found to be positive in nutrient broth culture followed by the winter, when out of a total of 77 samples 53 (69%) showed positive result. During summer, the occurrence was found to be the lowest when out of 139 samples investigated only 32 (23%) showed positive growth in nutrient broth. The association between season and presence of bacterial diseases in the samples was found statistically significant (P<0.001).

* **Production type**:

The production types included in this study were broiler parent stock, commercial broilers and layers. Out of the 7 samples investigated from broiler parent stock 4 (57%) were found to be positive to bacterial diseases. But in case of commercial broilers out of 236 samples 153 (65%) were positivity to nutrient broth and incase of commercial layers out of 77 samples 28 (36%) were culturally positive. The results indicate that the commercial broilers are more sensitive to bacterial diseases than other production type of birds. The association between production types and the occurrence of bacterial diseases was found to be statistically significant (P<0.001).

* **Litter type**:

In an attempt to determine the effect of litters on the occurrence of bacterial diseases in poultry various types of litters (saw dust and rice husk) that were used in the source farms were considered for comparison in this study. The occurrence of bacterial diseases in no litter system (cage or gunny bag system) birds was also compared with the litter system birds. In this study, out of 248 birds from saw dust type litter system 163 (66%) were positive to bacterial diseases. In the other group of birds from rice husk system rearing, out of 55 samples 10 (18%) were positive to bacterial infection. The result indicates that out of the two types of litters the performance of rice husk was better. For cage and gunny bag system of rearing, out of 17 samples 12 (71%) were positive to bacterial diseases. The association between various types of rearing system (litter or no litter and saw dust or rice husk) with the presence of bacterial diseases in the samples was found statistically significant (P<0.001).

* **Age:**

To find out the relationship of birds with the occurrence of bacterial diseases in poultry the test birds were divided into two groups; one group was designated as the “equal to or less than 30 day group” and the other was “greater than 30 day group”. In less than 30 day group out of 275 samples tested 178 (65%) showed positive to bacterial diseases. On the other hand, in case of greater than 30 day group, out of 45 samples tested only 7 (16%) showed positive result to bacterial diseases. Result shows a clear indication of the association age and the presence of bacterial diseases in poultry with the difference to be statistically significant (P<0.001).

**Table: 12 Association of different variables with samples positive to colibacillosis in culture measured by chi square test**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **categories** | **No. of observation** | | **No. positive (%)** | | **Chi square** | | **P value** |
| Season | Summer | | 139 | | 29 (21) | 73.54 | <0.001 | |
| Winter | | 77 | | 46 (60) |
| Autumn | | 104 | | 77 (74) |
| Production type | Broiler parent stock | | 7 | | 4 (57) | 21.20 | <0.001 | |
| Commercial Broiler | | 236 | | 129 (55) |
| Commercial Layer | | 77 | | 19 (25) |
| Litter | Saw dust | | 248 | | 138 (56) | 29.47 | <0.001 | |
| Rice husk | | 55 | | 10 (18) |
| Others (cage/gunny bags) | | 17 | | 4 (24) |
| Age (days) | ≤30 | | 275 | | 150 (55) | 38.29 | <0.001 | |
| >30 | | 45 | | 2 (4) |
| Region | CMA | | 72 | | 27 (38) | 4.48 | 0.10 | |
| Ctg. district | | 228 | | 114 (50) |
| Hill | | 20 | | 10 (50) |

**4.7 Description of the association of different variables with culture positive for colibacillosis:**

In an attempt to find out the association of the occurrence of colibacillosis with the variation of season, production type, litter type, age of birds and region the data were analyzed (Table 12) with the following findings:

* **Season:**

Occurrence of colibacillosis was found to be highest during autumn with the rate of the disease being 74% (out of 104 samples 77 were positive) followed by winter and summer with the rates being 60% (out of 77 samples 46 were positive) and 21% (out of 139 samples 29 were positive), respectively. The association between season and presence of colibacillosis in the samples was found statistically significant (P<0.001).

* **Production type**:

In case of broiler parent stock, out of 7 samples 4 were (57%) positive to colibacillosis. But, in case of commercial broiler, out of 236 samples 129 (55%) showed positivity to the disease and incase of commercial layer, out of 77 samples 19 (25%) showed culturally positive to it. The association between production type and presence of colibacillosis in the samples was found statistically significant (P<0.001).

* **Litter type**:

In this study, where saw dust was used as litter, out of 248 observations 138 (56%) were found positive to colibacillosis. But, when rice husk was used as litter out of 55 samples 10 (18%) were positive to colibacillosis. This result indicates, so far as the occurrence of colibacillosis is concerned, saw dust is inferior to rice husk as litter. For gunny bag/cage rearing system, out of 17 samples 4 (24%) were positive to colibacillosis. The association between the litter types and presence of colibacillosis in the samples was found to be statistically significant (P<0.001).

* **Age:**

In the age group of less than or equal to 30 days, out of 275 samples 150 (55%) were positive for colibacillosis. But, in the age group of greater than 30 days the rate of occurrence of the disease was only 4% (out of 45 samples only 2 were positive to colibacillosis). The difference between the age group and the rate of colibacillosis in chickens were found to be statistically significant (P<0.001).

* **Region:**

In this experiment the study area was divided into three regions such as Chittagong metropolitan areas (Tigerpass, Panchlaich, Potenga, etc.); Chittagong district (various Upazilas of the district such as Chandanaish, Satkania, Sitakunda, etc.) and Chittagong Hill tracts (Rangamati, Khagrachori, Bandarban, etc). Occurrence of colibacillosis was found to be highest in the Hill tracts region where out of 20 samples 10 (50%) were positive to colibacillosis. In the Chittagong district region out of 228 samples 114 (50%) were positive to the disease and in the Chittagong metropolitan areas the rate was 38% (out of 72 samples 27 were positive to colibacillosis). However, the regional difference of the occurrence of colibacillosis was found not to be statistically significant (P>0.10).

**Table 13: Association of different variables with samples positive to *Staphylococcus* in culture measured by chi square test**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **categories** | **No. of observation** | | **No. positive (%)** | | **Chi square** | | **P value** |
| Season | Summer | | 139 | | 0 (0) | | 39.71 | 0.001 |
| Winter | | 77 | | 4 (5) | |
| Autumn | | 104 | | 23 (22) | |
| Production type | Broiler parent stock | | 7 | | 0 | | 1.89 | 0.39 |
| Commercial Broiler | | 236 | | 18 (8) | |
| Commercial Layer | | 77 | | 9 (12) | |
| Litter | Saw dust | | 248 | | 19 (8) | | 38.08 | <0.001 |
| Rice husk | | 55 | | 0 | |
| Others (cage/gunny bags) | | 17 | | 8 (47) | |
| Age (days) | ≤30 | | 275 | | 22 (8) | | 0.48 | 0.48 |
| >30 | | 45 | | 5 (11) | |
| Region | CMA | | 72 | | 4 (6) | | 2.70 | 0.25 |
| Ctg. district | | 228 | | 23 (10) | |
| Hill | | 20 | | 0 | |

**4.8 Description of the association of different variables with culture positive for staphylococcosis:**

Association of the occurrence of staphylococcosis with different risk factors/ epidemiological variables was analyzed (Table 13) with the following findings:

* **Season:**

The disease staphylococcosis was found to occur in chickens at a highest rate of 22% during autumn when, out of 104 samples tested 23 showed positive results to the disease. During winter the rate of occurrence of the disease was only 5% when, out of 77 samples only 4 showed positive result to the disease. Surprisingly, out of 139 samples tested during summer none was found positive for Staphylococcus. The seasonal variation of the occurrence of staphylococcosis was found to be statistically significant (P>0.001).

* **Production type**:

In case of broiler parent stock, no positive samples were found for staphylococcosis. But, in case of commercial broiler out of 236 samples tested 18 (8%) showed positive result for the disease. In commercial layer the rate of occurrence of the disease was found to be 12% (out of 77 samples tested 9 were positive for staphylococcosis). However, this difference in the rate of occurrence of staphylococcosis due to variation of production type was found not to be statistically significant (P>0.39).

* **Litter type:**

In case of chickens raised on saw dust as litter, out of 248 observations 19 (8%) were found positive to staphylococcosis. But, when rice husk was used as litter out of 55 samples none was found positive to staphylococcosis. For gunny bag/cage rearing system, out of 17 samples 8 (47%) were positive to staphylococcosis. The association between the litter types and presence of staphylococcosis in the samples was found to be statistically significant (P<0.001).

* **Age:**

In the age group of less than or equal to 30 days, out of 275 samples 22 (8%) were positive for staphylococcosis. But, in the age group of greater than 30 days the rate of occurrence of the disease was only 11% (out of 45 samples only 5 were positive to staphylococcosis). However, the difference between the age group and the rate of occurrence of staphylococcosis in chickens were found not to be statistically significant (P>0.48).

* **Region:**

Occurrence of staphylococcosis was found to be highest in the Chittagong district region where out of 228 samples tested 23 (10%) showed positive result to the disease and in the Chittagong metropolitan areas the rate was 6% (out of 72 samples 4 were positive to staphylococcosis). For Chittagong hill tracts region out of 20 samples tested none was found positive to the disease. But, the regional difference of the occurrence of staphylococcosis was found not to be statistically significant (P>0.25).

**4.9 Culture sensitivity test:**

In an attempt to determine the antimicrobial susceptibility pattern of the organisms isolated from poultry under this study culture sensitivity (CS) test was done using disc diffusion assay method described by CLSI, 2007. For analyzing data of the antimicrobial sensitivity test the antibiotics showing either 100% sensitivity or resistance were not considered, due to absence of variance. The antibiotic sensitivity tests are routine done to select best drug against *E. coli* and *Staphylococcus sp.* During this study 152 positive samples for *E. coli* and 27 for *Staphyococcus Sp* were subjected to CS Test. The activities of antibiotics against *E .coli and* *Staphyococcus Sp* showed varying levels of multiple antibiotic resistances. {Multiple drug resistance (MDR), multi-drug resistance or multiresistance is a condition enabling disease-causing microrganisms (bacteria, viruses, fungi or parasites) to resist distinct [antimicrobials](http://en.wikipedia.org/wiki/Antimicrobials), first and foremost [antibiotics](http://en.wikipedia.org/wiki/Antibiotics), but also [antifungal drugs](http://en.wikipedia.org/wiki/Antifungal_drug), [antiviral medications](http://en.wikipedia.org/wiki/Antiviral_medication), [antiparasitic drugs](http://en.wikipedia.org/w/index.php?title=Antiparasitic_drugs&action=edit&redlink=1), chemicals of a wide variety (US national library of medicine)

Ciprofloxacin, penicillin and ampicillin, showed 100% resistance; on the contrary, kanamycin and cephadrine showed 100% sensitivity to all the organisms isolated under this study (Fig 11).

For analysis of the response of different poultry diseases to antibiotics three types of diseases/conditions were considered that included colibacillosis, staphylococcosis and mixed infection with both. The activity of antibiotics was expressed as “intermediate”, “sensitive” and “resistant”. The graphical presentation of the result is shown in Fig 12.

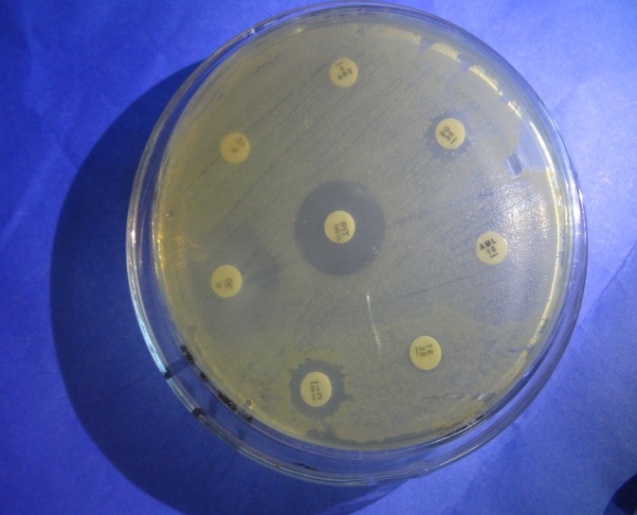
For cephalexin, intermediate sensitivity was shown by colibacillosis (due to *E. coli* infection), staphylococcosis (due Staphylococcus infection) and mixed infection (with both) at the rate of 86%, 81% and 100% respectively. For staphylococcosis it showed intermediate sensitivity, 19% resistance to it. Among the total positive infections for cephalexin intermediate reaction shows, 267, & resistant 33)

For doxycyline, staphylococcosis showed 100% sensitivity but, colibacillosis and mixed infection showed intermediate sensitivity at the rate of 69% and 100% respectively**.** (Among the total positive infections for doxycycline intermediate reaction shows, 169, & resistant 30, sensitive 100.67.)

For amoxycyline, colibacillosis and mixed infection showed 84.11% and 100% resistance respectively. On the contrary, staphylococcosis responded with 100% sensitivity to the antibiotic. (Among the total positive infections, for amoxycycline intermediate reaction shows, 15.89, & resistant 184.11, sensitive 100)

For colistin sulphate colibacillosis and mixed infection showed 100% sensitivity. However, staphylococcosis was found to be 63% resistant to the same antibiotic. (Among the total positive infections, for colistin sulphate intermediate reaction shows, 37& resistant 63, sensitive 200.)

For enrofloxacin colibacillosis, mixed infection and Staphylococcosis showed resistance at the rates of 45.14%, 50% and 96.3%, respectively. (Among the total positive infections, for enrofloxacin intermediate reaction show, 108.56, & resistant 191.44, and no sensitive reactions were found here.)



**Fig:10 Antimicrobial sensitivity test for Staphylococcus**

**Fig:9 Antimicrobial sensitivity test for *E.coli***

For neomycin, colibacillosis and mixed infection showed 100 % sensitivity. But, staphylococcosis showed 100 % resistance. **(**Among the total positive infections, for Neomycin no intermediate reaction shows, resistant 100, and sensitive reactions 200 were found here.)

**Fig: 11 Frequency (%) of samples sensitive to different antibiotics**

**Fig: 12 Frequency of antimicrobial sensitivity test of different poultry diseases**

**4.10 Association of epidemiological factors of colibacillosis and Staphylococcosis with the antimicrobial sensitivity**

To find out whether various epidemiological factors have any association between poultry diseases and antibiotic resistance the association of enrofloxacin, doxicycline, Colistin sulphate and Cephalexine was compared with the epidemiological factors of colibacillosis and staphylococcosis.

**Table: 14 Association of enrofloxacin resistance in colibacillosis with different variables**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Variables* | *Categories* | *No. of observation* | ***No. resistant*** *(%)* | *Chi square* | *P value* |
| *Season* | *Summer* | 21 | 21 (100) | 34.04 | <0.001 |
| *Winter* | 46 | 11 (24) |
| *Autumn* | 77 | 33 (43) |
| *Production type* | *Broiler parent stock* | 4 | 0 | 3.55 | 0.16 |
| *Broiler* | 121 | 57 (47) |
| *Layer* | 19 | 8 (42) |
| *Age (days)* | *≤30* | 142 | 63 (44) | 2.46 | 0.11 |
| *>30* | 2 | 2 (100) |

**4.11 Description of the association of enrofloxacin resistance (antimicrobial sensitivity test) for colibacillosis (Table 14):**

* **Season:**

During summer, out of 21 isolates of colibacillosis 21(100%) showed resistance to enrofloxacin. For winter, out of 46 isolates 11(24%) showed resistance to the antibiotic. For autumn, out of 77isolates 33(43%) showed resistance to the antibiotic.

The association between season and enrofloxacin resistance for colibacillosis was found statistically significant (P<0.001).

* **Production type**:

In case of broiler parent stock, no resistance was found. But in case of commercial broiler, out of 121 samples 57 (47%) showed resistance to enrofloxacin. And in case of commercial layer, out of 19 samples 8 (42%) showed resistance to the antibiotic. The association between production type and enrocin resistance was not statistically significant (P>0.16).

* **Age:**

In case of less than or equal to 30 days groups, out of 142 samples 63 (44%) showed resistance to the antibiotic enrofloxacin. In case of greater than 30 days group, 100% resistence to enrofloxacin was found. However, this relationship between age and enrofloxacin resistance was found not to be statistically significant (P>0.11).

**Table 15: Association of doxycycline resistance in colibacillosis with different variables**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Variables* | *Categories* | *No. of observation* | ***No. resistant*** *(%)* | *Chi square* | *P value* |
| *Season* | *Summer* | 26 | 13 (50) | 10.69 | 0.03 |
| *Winter* | 46 | 17 (37) |
| *Autumn* | 77 | 15 (20) |
| *Production type* | *Broiler parent stock* | 4 | 0 | 5.44 | 0.22 |
| *Broiler* | 128 | 43 (34) |
| *Layer* | 17 | 2 (12) |
| *Age (days)* | *≤30* | 147 | 43 (29) | 4.68 | 0.09 |
| *>30* | 2 | 2 (100) |

**4.12 Description of the association of doxycycline resistance (antimicrobial sensitivity test) for colibacillosis (Table 15):**

* **Season:**

In case of summer season, for colibacillosis, out of 26 samples 13 (50%) showed resistance to doxycycline. For winter season, out of 46 samples 17 (37%) showed resistance to the antibiotic and for autumn out of 77 samples 15 (20%) showed resistance to doxycycline. The association between season and doxycycline resistance for colibacillosis was found statistically significant (P<0.03).

* **Production type**:

In case of broiler parent stock, no resistance was found. But, in case of commercial broiler, out of 128 samples 43 (34%) showed resistance to doxycycline. In case of commercial layer farm, out of 17 samples only 2, (12%) showed resistance to the antibiotic. The association between production type and doxycycline resistance was not statistically significant (P>0.22).

* **Age:**

In case of less than or equal to 30 days groups, out of 147 samples 43 (29%) showed resistance to doxycycline and in case of greater than 30 days groups 100% resistance was found. The association between age and doxycycline resistance was not statistically significant, (P>0.09).

**Table: 16 Association of enrofloxacin resistance in *Staphylococcus* with different variables.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Variables* | *Categories* | *No. of observation* | ***No. resistant*** *(%)* | *Chi square* | *P value* |
| *Season* | *Autumn* | 23 | 22 (96) | 0.18 | 0.67 |
| *Winter* | 4 | 4 (100) |
| *Production type* | *Broiler parent stock* | 0 | 0 | 0.51 | 0.47 |
| *Broiler* | 18 | 17 (94) |
| *Layer* | 9 | 9 (100) |
| *Age (days)* | *≤30* | 22 | 21 (95) | 0.23 | 0.62 |
| *>30* | 5 | 5 (100) |

**4.13 Description of the association of enrofloxacin resistance (antimicrobial sensitivity test) for *Staphylococcus* (Table-16)**

**Season:**

No *Staphylococcus* was isolated during summer however, during autumn out of 23 isolates of *Staphylococcus* 22 (96%) showed resistance to enrofloxacin and during winter out of 4 isolates 4 (100%) showed resistance to the antibiotic. The association between season and enrofloxacin resistance for *Staphylococcus* was not statistically significant, (P>0.67).

* **Production type**:

No *Staphylococcus* was isolated from broiler parent stock. In case of commercial broiler, out of 18 samples 17 (94%) showed resistance to enrofloxacin and in case of commercial layer the resistance was100%. However, the association between production type and enrofloxacin resistance for *Staphylococcus* was not statistically significant (P>0.47).

* **Age:**

In case of less than or equal to 30 days age group, out of 22 samples 21 (95%) showed resistance to enrofloxacin and for greater than 30 days group the resistance was 100%. The association between ageand enrofloxacin resistance was not statistically significant (P>0.62).

**Table: 17 Association of Colistin sulphate resistance in *Staphylococcus* with different variables**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Variables* | *Categories* | *No. of observation* | ***No. resistant*** *(%)* | *Chi square* | *P value* |
| *Season* | *Autumn* | 23 | 13 (57) | 2.76 | 0.09 |
| *Winter* | 4 | 4 (100) |
| *Production type* | *Broiler parent stock* | 0 | 0 | 1.98 | 0.15 |
| *Broiler* | 18 | 13 (72) |
| *Layer* | 9 | 4 (44) |
| *Age (days)* | *≤30* | 22 | 17 (77) | 10.43 | 0.001 |
| *>30* | 5 | 0 (0) |

**4.14 Description of the association of Colistin sulphate resistance (antimicrobial sensitivity test) for Staphylococcus (Table-17)**

* **Season:**

Out of a total of 23 isolates of *Staphylococcus* isolated during autumn 13 (57%) showed resistance to colistin sulphate and during winter the resistance was 100%. The association between season and cloistin sulphate resistance was not statistically significant (P>0.09).

* **Production type**:

No Staphylococcus was isolated from broiler parent stock. But, in case of commercial broiler, out of 18 isolates 13 (72%) showed resistance to Colistin sulphate and in case of commercial layer, out of 9 samples 4 (44%) showed resistance. The association between production type and Colistin sulphate resistance was not statistically significant (P>0.15).

* **Age:**

In case of less than or equal to 30 days group of chicken, out of 22 samples 17 (77%) showed resistance to Colistin sulphate and in greater than 30 days group no resistance was found. The association between age and Colistin sulphate for *Staphylococcus* resistance was statistically significant (P<0.001).

**Table: 18 Association of** **Cephalexine resistance in S*taphylococcus* with different variables**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Variables* | *Categories* | *No. of observation* | ***No. resistant*** *(%)* | *Chi square* | *P value* |
| *Season* | *Autumn* | 23 | 5 (22) | 1.06 | 0.30 |
| *Winter* | 4 | 0 (0) |
| *Production type* | *Broiler parent stock* | 0 | 0 | 6.01 | 0.01 |
| *Broiler* | 18 | 1 (6) |
| *Layer* | 9 | 4 (44) |
| *Age (days)* | *≤30* | 22 | 5 (23) | 1.39 | 0.23 |
| *>30* | 5 | 0 (0) |

**4.15 Description of the association of Cephalexine resistance (antimicrobial sensitivity test) for *Staphylococcus* (Table-18)**

* **Season:**

From the autumn samples out of 23 isolates of Staphylococcus 5 (22%) showed resistance to Cephalexine however, from the winter samples out of 4 none showed resistance to the antibiotic. The association between season and cephalexine resistance was not statistically significant, which was (P>0.30)

* **Production type**:

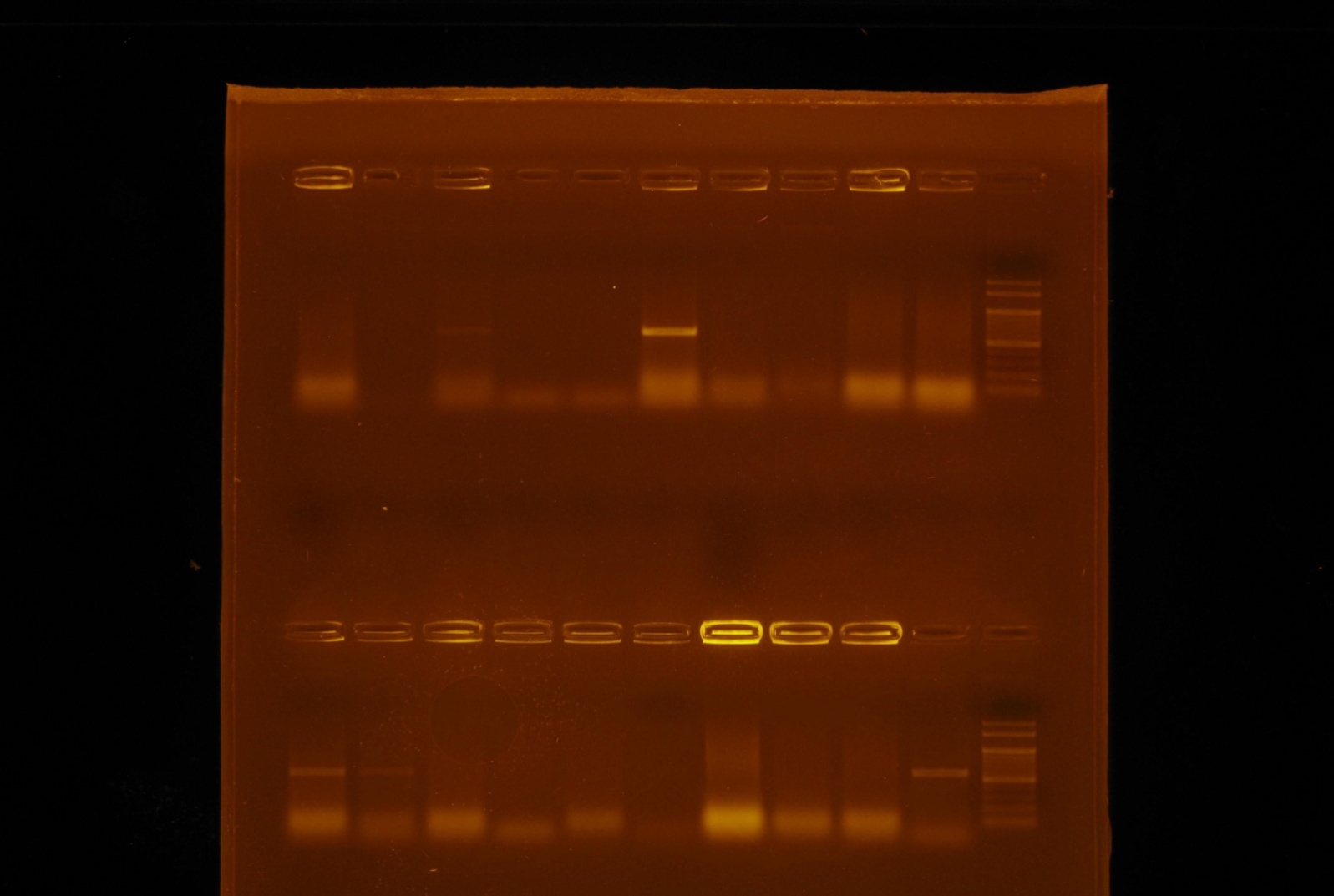
No Staphylococcus was isolated from broiler parent stocks. However, in case of commercial broiler, out of 18 isolates only one (6%) showed resistance to Cephalexine. But, for the samples collected from commercial layer chickens out of 9 isolates 4 (44%) showed resistance to the antibiotic. The association between production type and Cephalexine resistance was statistically significant, (P>0.01).

* **Age:**

In case of less than or equal to 30 days age group of chickens, out of 22 samples 5 (23%) showed resistance to Cephalexine and in case of greater than 30 days groups there was no resistance. However, the association between age and Cephalexine resistance was not statistically significant (P>0.23).

**4.16 Confirmation of colibacillosis and staphylococcosis using PCR:**

In order to develop a quick and reliable confirmatory diagnostic test using molecular method PCR was tried for diagnosis of collibacillosis and staphylococcosis. For molecular detection from among the culturally and biochemically positive samples 40 isolates from each of either *E. coli* the causal agent of colibacillosis and 20 isolates from *Staphylococcus* the causal agent of staphylococcosis were randomly selected for DNA extraction. PCR amplification of specific DNA fragments was then conducted using specific primer sets for each of the organisms (targeting 16S and 23S rDNA genes for *E. coli* and *S. aureus* respectively). The PCR products were then run in 1% agarose gel with ethedium bromide, incorporating 100bp size markers and visualized under UV light.

For *E. coli* out of **40** isolates only 16 isolates produced expected bands at 585bp region confirming the isolates to be *E. coli.* The results obtained from PCR assay for *E. coli* is displayed in Figure 13. The attempt for PCR amplification of 23S rDNA gene for detection of *Staphylococcus aureus* was however failed due to unknown reasons. The experiment could not be repeated as there was limitation of time.

9 8 7 6 5 4 3 2 1 N M

500 bp

400 bp

300 bp

200 bp

100 bp

**Figure 13.** Results of PCR for 16s rRNA gene of ***E. coli*** ; Lane M: 100 bp ladder; Lane N: Negative control; Lane 1-5: 16S rRNA gene-sized (585bp) amplicon.

**CHAPTER – V**

**DISCUSSION**

The present study was conducted to determine the prevalence of bacterial diseases among the commercial poultry farms of Chittagong district. A total of 320 organ samples were collected during August 2011 to May 2012 encompassing the seasons **autumn (August to November), winter** **(December to February)** and **summer (March to May)**. To determine the cause of death of the birds first of all a tentative diagnosis was made on the basis of observation of clinical signs & postmortem lesions. Then the organ samples were subjected to bacteriological culture methods to determine the true existence of bacterial infection among the culture-positive samples followed by confirmation of the causative organisms using biochemical tests. For further confirmation Polymerase Chain Reaction (PCR) was attempted. The bacterial agents isolated and identified from the samples were also investigated for their antibiotic resistance pattern. An attempt was also made to establish a relationship of this resistance pattern with other factors like season, production type and age.

According to clinical signs and postmortem lesions 48% of the chickens were diagnosed tentatively as the case of Colibacillosis**.** This finding is close but slightly lower than the findings of Ahmed *et al*. (2009) and Hashem *et al*. (2012) which were 52.26% and 54.55% respectively. Hossain *et al*. (2013) recorded slightly lower infection rate (30.43%) to our finding**.** On the contrary, Giasuddin *et al*. (2002), Islam *et al*. (2003) and Uddin *et al*. (2010) recorded the disease at a very lower rate which was 4.42%, 5.17% and 5.70% respectively. Rahman *et al*. (2004) however, recorded the disease at a level of 21% (147out of 697 birds). We found Staphylococcosis at the rate of 9% which was about 10 times higher (0.81%) than the findings ofRahman *et al*. (2004) and around 5 times lower (47.83%) than Hossain *et al*. (2013). This type of variation of the occurrence of Collibacillosis and Staphylococcosis is attributable to geographic variation of the region from where the samples were collected, types of birds examined, variation of the techniques adopted by different laboratories for conducting the experiments and skill as well as experience of the researchers in observing the clinical signs and symptoms to draw a conclusion in support of a disease. Hasan *et al*. (2010) found Colibacillosis at the rate of 34% and 31.82% in broilers and layers respectively in clinical diagnosis. The same samples when tested using bacterial culture method they found the infection rate of 25.53% and 25% in broiler and layer chickens respectively.They concluded thatclinical diagnosis is not always accurate like laboratory diagnosis. However in our case, tentative diagnosis on the basis of clinical signs and symptoms did not vary with the confirmatory diagnosis using bacteriological culture and biochemical test methods (48% in tentative diagnosis as oppose to 48.44% in confirmatory tests for *E. coli* infection and 9% in tentative diagnosis as oppose to 8% in confirmatory diagnosis for *Staphylococcus* infection.

Association of bacterial infection with season, production type, litter type and age was determined. Autumn was found to be the worst season rendering poultry more susceptible to bacterial diseases (96%) followed by winter (69%). During summer, the infection rate was found to be the lowest (23%). The association between season and presence of bacterial diseases in the samples was found statistically significant (P<0.001). Islam *et al*. (2012) also found the occurrence of bacterial diseases to be significantly (p<0.01) lower in summer (14.3%) than winter (23.1%) and rainy (46.7%) seasons in layer birds. However in broiler, they found bacterial diseases to occur at a higher rate in winter (40%) followed by summer (25.8%) and rainy (20.5%) seasons. In case of production type prevalence was significantly (P<0.001) higher (65%) in commercial broiler than commercial layers (36%). However, Islam *et al.* (2012) did not find any significant difference in the occurrence of bacterial diseases in broiler (28.6%) and layer (27.6%) birds. Prevalence varied among different types of litter used for rearing chickens. In this study, saw dust type litter system showed significantly (P<0.001) higher rate of bacterial infection (66%) than rice husk system rearing (18%) indicating that out of the two types of litters the performance of rice husk is better. We failed to find any paper comparing the prevalence of bacterial diseases in relation to the use of various types of litter for rearing chickens; therefore we could not compare our findings with other woks of similar nature. Prevalence of bacterial diseases varied among different age group of birds and the highest (65%) was found in ≤30 days age groups with the lowest (16%) being in >30 days age group of birds. Islam *et al*. (2003) also reported in general, the highest number poultry diseases to occur in the age group of 8-21 days (42.60%), followed by 22-35 days age group (26.62%), 0-7 days age group (26.10%), 36-60 days age group (1.03%) and over 60 days age group (3.62%) of Poultry. Similarly, according to Uddin *et* *al.* (2010) the highest number of cases were recorded in the age group of 8-20 days which was 42.64%, followed by 35.76% in 21-35 days age group, 16.12% in 0-7 days age group, 1.52% in 36-60 days age group and 3.96% in >60 days age group of poultry.

The (Table 11) shows that, there was significant (p< 0.01) difference in the prevalence of bacterial diseases in the commercial farms of Chittagong district with different variables ,season, production type, litter management system and age groups .Statistically significant (p < 0.001) difference in prevalence of bacterial was found among the commercial poultry farms.

Prevalence of Colibacillosis in relation to season, production type, litter type, age and region was determined. Avian colibacillosis was found to occur covering all the three seasons studied and highest prevalence of the disease was found in autumn (74%) followed by winter (60%) and then summer (21%). Bhattacharjee *et al.* (1996) also reported avian colibacillosis to occur in all the seasons of the year in Bangladesh. Pandey *et al.* (1998) reported outbreak of *E. coli* infection during November to March, and Lambie *et al.* (2000) reported higher *E. coli* infection during rainy season. Rahman *et al.* (2004) reported Colibacillosis to occur at a significantly (P <0.01) higher rate during summer (40.82%) season. This type of variation in the infection rate of colibacillosis might be resulted from the differences in the geographic variation of the study region and the study populations.

In case of production type prevalence was higher in commercial broilers (55%) than in commercial layers (25%). This finding corresponds to the findings of a prevalence rate of 52.26% & 52% and 25% respectively. Hasan *et al.* (2010) however, reported a 25.53% prevalence rate of colibacillosis in broiler birds. A recent Ahmed *et al.* (2009) & Roy *et al.* (2012) for broiler and Hasan *et al*. (2010) for layer, who reported study conducted by Hashem *et al.* (2012) in broiler chickens also support our work in the way that they also found a 54.55% prevalence rate of colibacillosis in Chittagong.

Prevalence of colibacillosis in chickens under the study varied among different litter management systems and the highest (56%) was found in saw dust litter management system with the lowest (18%) in rice husk management system. Due to lack of information related to litter management system we failed to compare our findings with others. Prevalence also varied among different age groups with the highest (56%) being found in ≤30 days age groups and lowest (4%) in >30 days age groups. This finding correlate with the findings of Talha *et al.* (2001) who reported higher proportionate prevalence rate of colobacillosis in growing chickens in comparison to adults. On the contrary, Rahman *et al*., (2004) reported avian colibacillosis to be widely prevalent in all age group of chickens (9.52 to 36.73%) with specially high prevalence rate in adult layer birds (36.73%).Rahman *et al.* (2007) also found colibacillosis in 0-8 weeks age group of chicks at the rate 13.36% and in grower group (9-20 weeks) of birds at the rate of 11.93% but in adult chicken (21-78 weeks) they did not find any colibacillosis infection. Bhattacharjee *et al.* (1996) reported wide prevalence of colibacillosis in both the brooding (12.82%) and pre-peak-post production layer chickens (5.49 to 8.78%). Khaton *et al*., 2008 however, reported the disease to occur at the rate of 60% in commercial broiler and layer birds.

Attempts were made to determine whether the occurrence of colibacillosis varies with the regional variations. For this the study area was divided into three regions such as Chittagong metropolitan areas (Tigerpass, Panchlaich, Potenga, etc.); Chittagong district (various Upazilas of the district such as Chandanaish, Satkania, Sitakunda, etc.) and Chittagong Hill tracts (Rangamati, Khagrachori, Bandarban, etc). Occurrence of colibacillosis was found to be in the Hill tracts region (50%). In the Chittagong district region the occurrence was 50% followed by 38% in the Chittagong metropolitan areas. However, the regional difference of the occurrence of colibacillosis was found not to be statistically significant (P>0.10). There being no report on the occurrence of colibacillosis in the Hill tracts region we failed to compare and validate our finding. However, it is thought that due to small number of samples from Hill tracts region the statistical calculation showed insignificant result for variation. Further study is needed to be conducted for obtaining a true picture of the occurrence of colibacillosis in the Chittagong district involving Hill tracts regions.

**Predisposing factors**

According to Whittam & Wilson (1988) the risk for colibacillosis increases with the increase of infection pressure in the environment. A good hygiene in the poultry houses and avoiding overcrowding are very important. Other principal risk factors that may include are the duration of exposure, virulence of the strain, breed, and immune status of the bird (Gross *et al.,* 1980; Rosenberger *et al.*, 1985; Gross, 1992; Pourbakhsh *et al.*, 1997; McGruder & Moore, 1998). Specifically for layers, Kohlert (1968) reported a lowered resistance in chickens after treatment with progesterone. She proposed that in the case of increased egg production an imbalance exists between the oestrogen and progesterone level, which also induces a lowered resistance against infections. Animals exposed to stress run an increased risk of colibacillosis (Barnes & Gross, 1997). Leitner & Heller (1992) showed that stress promotes infection *per os*: they induced bacteraemia after inoculation of APEC *per os*, after fasting the animals for 36–48 hours or exposing them to high temperatures. Every damage to the respiratory system favours infection with APEC. Several pathogens, like NDV, IBV, MG, both wild type and vaccine strains, may play a part in this process. An unfavourable housing climate, like an excess of ammonia or dust, renders the respiratory system more susceptible to APEC infections through deciliation of the upper respiratory tract (Barnes & Gross, 1997). In broilers, the disease is age related. If the breeder flock is vaccinated or infected with APEC, there is a clear maternal resistance before the age of four weeks, which however may be breached after debilitation of the respiratory epithelium by other pathogens or an unfavourable housing climate. About three to four weeks after introduction of the flock in the house, the maternal antibody titre becomes minimal and the bacterial infection pressure in the environment maximal. Viral diseases mostly occur around the age of three weeks. The biggest losses in broilers therefore are suffered in the age category of five to twelve week (Foley *et al.*, 2000). All those factors discussed above might have played vital roles for the variation of prevalence rates of colibacillosis reported by the different authors.

Prevalence of staphylococcosis in relation to season, production type, litter type, age and region was determined. Prevalence of staphylococcosis was found to occur in chickens at the rate of 22% during autumn. During winter the rate of occurrence of the disease was only 5%. Surprisingly, during summer no positive case for Staphylococcus infection was found. The seasonal variation of the occurrence of staphylococcosis was found to be statistically significant (P>0.001). Due to paucity of papers describing seasonal variation of the occurrence of staphylococcosis we could not verify our work with others. In case of production type prevalence of staphylococcosis was found to be 12% in commercial layer and 8% in commercial broiler. However, the seasonal variation of the occurrence of staphylococcosis was found not to be statistically significant (P>0.39). Our work was supported by Skeeles (1997) and Butcher *et al.* (1999) and according to them staphylococcal infection mainly found in layers rather than broiler as the infection starts from mainly 4th week of the chicken life. Hossain *et al*., (2013) recorded the prevalence of *Staphylococcus* sp., to be 47.83% and Rahman *et al.* (2004) found only 0.8% prevalence rate in chicken. However, they did not mention about the production type they have included for their study. Prevalence varied among different litter type, very high prevalence was found in gunny bag/cage rearing system (47%) followed by saw dust type litter system (8%). But, in case of rice husk litter system out of 55 samples none was found positive to staphylococcosis. The reason for very high prevalence rate of Staphylococcus infection in gunny bag/cage rearing system could be explained to be due to very small size that might have affected the result and need further investigation. Prevalence varied among different age groups, highest (11%) prevalence was found in >30 days age groups and lowest (8%) in age group ≤30 days. This finding relating to age of birds correlate with the finding of Skeeles (1997), who reported the disease to occur 4th week onward in chicken. Prevalence of staphylococcal diseases was found to be highest in the Chittagong district region with the rate of 10% and in the Chittagong metropolitan areas the rate was 6%. For Chittagong hill tracts region out of 13 samples tested none was found positive to the disease. However, the regional difference of the occurrence of staphylococcosis was found not to be statistically significant (P>0.25).

In Culture sensitivity (CS) test the organisms under study were found 100% sensitive to cephradin (CH) and kanamycine (Kacin) but completely resistant to ampicillin, penicillin and ciprofloxacin. This finding correlates with the findings of Blanco *et al.* (1997), Witte *et al.* (2000), Van den *et al.* (2001), Li *et al.* (2007) who reported fluoroquinolones to become resistance within several years of the approval of this class of drugs for use in poultry

For *E. coli* cephalexin (86%) and doxycycline (69%) showed intermediate sensitivity but, amoxycilline and enrocin or enrofloxacin showed 84.11% and 45.14% resistance respectively. This finding was supported by Cloud *et al.* (1985), Irwin *et al.* (1989), Blanco *et al*. (1997), Bass *et al.* (1999), and Li *et al.* (2007). For colistin sulphate and neomycine the organism showed 100% sensitivity. Nazir *et al.* (2005) and Hashem *et al*. (2012) also got similar result who reported *E. coli* to be highly sensitive to colistin sulphate (100%) and ciprofloxacin to be intermediately (50%) sensitive. However, Rahman *et al.* (2004) reported *E. coli* to be highly sensitive to ciprofloxacin but, they opined that it may be due to that ciprofloxacin was then newly introduced in the treatment of poultry in Bangladesh when the organism did not have enough time to grow resistance against the drug. Nasrin *et al.* (2007) also reported *E. coli* to be sensitive to ciprofloxacin and opined for the variation to be possibly due to the presence of different clones of organisms. (Ciprofloxacin and enrofloxacin both are belongs to fluoroquinolones group. Resistance to fluoroquinolones was reported within several years of the approval of this class of drugs for use in poultry (Blanco *et al.,* 1997; Witte *et al*., 2000; Van den *et al.,* 2001; Li *et al*., 2007)

For staphylococci cephalexin showed intermediate sensitivity (81%) but, doxycycline and amoxycillin showed 100% sensitivity. On the other hand colistin sulphate, enrofloxacin and neomycin showed 63%, 96.3% and 100 % resistance respectively. Andrew *et al.* (2011) reported resistance (intermediate and complete) of *S aureus* to tetracycline, ampicillin, penicillin, and erythromycin including other important antimicrobials like quinupristin/dalfopristin, fluoroquinolones, oxacillin, daptomycin, and vancomycin. Nasrin *et al.* (2012) reported staphylococci to be sensitive to ampicillin, amoxicillin, ciprofloxacin, gentamycin, erythromycin, chloramphenicol, kanamycin, and resistant to tetracycline and nalidixic acid. Roy *et al.* (2012) found *Staphylococcus* spp to be sensitive to ciprofloxin, cloxacillin, amoxicillin, chloramphenicol, neomycin erythromycin, ampicillin and colistin sulphate. Aziz *et al*. (2013) found their isolates of *S aureus* to be resistant to cephradine, ampicillin, bacitracin, amoxacillin and recommended ciprofloxacin (70%), gentamycin (70%), levofloxacin (70%) and penicillin (70%) to be of better value in the treatment of *S aureus* infection for their susceptibility to the antibiotics. Jackson *et al.* (2013) reported MRSA isolates to be 100% resistant to ampicillin, ciprofloxacin, erythromycin, levofloxacin, oxacillin and penicillin. Pyzik & Marek (2013) reported *Staphylococcus aureus* strains to be 66.66% resistance against tetracycline and 61.11% against oxytetracycline, 44.44% against amoxicillin, and 100% sensitivity was shown by cephalexin. All these reports cited above shows very different antibiotic resistance pattern to staphylococci and the reason for this is due to the fact that difference in the resistance of *S aureus* against various antibiotics is usually determined by the presence of plasmids containing various genes for resistance to those antibiotics (Lyon and Skurray 1987).

This study demonstrates alarming high individual and multiple resistances to antibiotics in *E. coli* and *Staphylococcus. Sp.* The problem of antibiotic-resistances of avian *E. coli* isolates is of particular importance in Bangladesh where exists a high risk of human contamination because of manual slaughtering of animals. Antibiotic resistances are frequently encoded by conjugative plasmids or transposons, thus *E. coli* of avian origin could act as a possible source for the transfer of antibiotic resistances to other bacterial species including human pathogens. Thus, an increase in the reservoir of antibiotic resistant bacteria could heavily impair the treatment of human diseases.

It is a well known fact that the resistance pattern of antibiotics should not vary significantly with the variation of epidemiological factors (season, production type and age of birds). But, to avail the opportunity of proving this fact we have analyzed the resistance pattern of enrofloxacin and doxycycline for *E. coli* (colibacillosis) as well as enrofloxacin, colistin sulphate and cephalexine for Staphylococcal (staphylococcosis) infection to determine the association, if any, of the epidemiological factors with the resistance pattern of the antimicrobials. As expected, the resistance pattern of the said antibiotics against *E. coli* and Staphylococcus in relation to the season, production type and age of the birds was found in most of the cases not to vary significantly. However, there were some exceptions in the way that the seasonal variation of enrofloxacin resistance was found to be statistically significant (P<0.001) for *E. coli* isolates. Resistance against enrofloxacin was found to be 100% for the samples collected during summer followed by autumn (43%) and winter (24%).Likewise, for Staphylococcal isolates variation of resistance due to the variation of age against colistin sulphate and variation of production type against cephalexin was found to be significant with p values of P<0.001 and P>0.01respectively. These variations with statistical significance are thought to be due to small sample size that might have played a significant role. Due to lack of information in relation to this type of work we could not compare our work for validation. Further investigation is needed with bigger sample size to rule in or rule out the present findings.

In order to develop a quick and reliable confirmatory diagnostic test using molecular method PCR was tried for diagnosis of colibacillosis and staphylococcosis. The PCR is used as a highly sensitive and specific test for detecting the presence of pathogenic bacteria in clinical specimens (Cohen *et al.,* 1993). PCR is also a more rapid and reliable test method than that of the traditional culture methods (Carli *et al*., 2001). For molecular detection from among the culturally and biochemically positive samples 40 isolates from each of either *E. coli*, and 20 isolates from each of either *Staphylococcus sp*, the causal agent of colibacillosis and staphylococcosis , were randomly selected for DNA extraction. The extracted DNA after amplification by PCR using ECO-f and ECO-r primer targeting *E. coli* 16S ribosomal DNA a 585 bp amplicon was found after 1% agarose gel electrophoresis confirming the isolates to be *E. coli*. The similar result was also found by other authors (Amith-Romach *et al.,* 2004 and Tonu *et al.,* 2011). The attempt for PCR amplification of rRNA-spacer gene (16S-23S rRNA intergenic sequencs) for detection of *Staphylococcus aureus* was however failed due to unknown reasons. The experiment could not be repeated as there was limitation of time.

However, Staphylococci producing coagulase enzyme are usually pathogenic (Quinn *et al.,* 2002). Though they showed Coagulase positive result, but they may be include *S. aureus, S. intermedius* and*, S. hyicus,* and coagulase negative may include *S. chromogens, S haemoliticus and S warneri.* These results were similar to that of the Addis *et al.,* 2011 in similar studies earlier. But they showed no positive result on blood agar.

Ultimately the results were complemented with that of PCR based analysis. Further species specific PCR analysis can elucidate the extent of infection by each different type of *E .coli* and *Staphylococci.*

PCR is a powerful tool where DNA template and Specific primers are used for molecular identification of any specific bacteria. Here PCR optimization is required in case of no.of cycles and reaction temperature time .Strong band was found at 1µl. For all subsequent reactions similar proportions were used. During performing PCR some difficulties were there such as primer dimer formation, band at negative control, band below the 100 bp, overlapping band were found due to contamination in various ways.

According to PCR results, 40% (16/40) of indole positive samples of *E .coli* were confirmed. This result is in agreement with the earlier report by Tonu *et al*., 2011 and Wang *et* *al*., 1997

However, biochemical test may confirm in case of any isolates of Staphylococci that they were coagulase positive but in PCR they did not show positive result, indicating limitation of biochemical test.

Again coagulase negative (only 7 isolates) may be Methicillin resistant *S. aureus* or rare *Staphylococci* ,(:Koneman *et al.,*1997 National standard method of SOP,UK)

Prevalence of *E .coli* and *Staphylococcus* *sp i*n biochemical tests in Chittagong zone commercial farm varies that indicates that not all chicken samples contain similar diseases and this is a clear indication of variation from zone to zone due to cleanliness and management system.

**CHAPTER-VI**

**CONCLUSION**

The prevalence of bacterial diseases was about 58% in the commercial chickens in Chittagong region. Among the various diseases 48**.**44%of the chickens were positive to colibacillosis and 8% to staphylococcosis. Prevalence of colibacillosis was high in autumn (74%) and commercial broilers were found to be more susceptible (55%) than layers (25%). Chicks and growers were found to be more susceptible (55%) to the disease than adults (4%). The disease was more prevalent in the hill tracts region (50%) and Chittagong district, than other 38% were found from various upazilas of Chittagong Metropolitan areas respectively. The rate of occurrence of staphylococcosis was also high during autumn (22%). On the contrary, for staphylococcosis layers were more susceptible (12%) than broilers (8%) and chicks & growers were less susceptible (8%) than adults (11%).

The *E.coli* isolates were susceptible to colistin sulphate and neomycin with an intermediate sensitivity to Cephalexin and Doxycyline. The staphylococcal isolates were susceptible to amoxycyline and doxycyline with an intermediate susceptibility to Cephalexin. The organisms showed 100% and 96% resistance to neomycin and enrocin respictevly. This knowledge of antibiotic sensitivity could be used in prescribing antibiotics for the treatment of colibacillosis and staphylococcosis in the Chittagong region.

PCR detection method for *E. coli* was successfully established through this project that could be used in the laboratory for routine diagnosis of the organism. However, for staphylococcus further work is needed for establishing a molecular diagnostic method.

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**CHAPTER-VII**

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**APPENDIX**

**Questionnaire relating to Bacterial diseasesand probable lesions associated with the isolations of the organisms**

**SECTION A: GENERAL**

Name of the Farmer:

Address: Village: ……………………………… Upazilla: ………………....…………

District:………………………………… Contact number:………………….…………

**SECTION B: FARM OPERATION**

1. Information of the farm: Commercial. Small or large
2. Management system: i) Litter ii) Cage iii) Gunny bag or others
3. Which litter is used : i) Rice husk ii) Saw dust

**Information about flock:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Shade | Type of bird | Breed | flock size | Hatchery of origin | Age |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

**SECTION C: HISTORY OF OUTBREAK AND RELATED INFORMATION**

i. Is there any disease problem in your farm? Yes No

If yes, how …………………………………………………………………………

ii. Did you report to any one? Yes No

iii. How long did it take you between disease infection and reporting? ………...……

iv. If previvously antibiotic is used? Yes No

v. If yes, how long it is used?

iv. Number of birds affected, died and culled (date wise)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Date** | **Shed** | **Number affected** | **Number died** | **Number culled** |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

* **Clinical sign:**

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…………………………………………………………………………………………

* **Necropsy findings:**

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…………………………………………………………………………………………

…………………………………………………………………………………………

* **Lab test result:**

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…………………………………………………………………………………………

* **Tentative Diagnosis**

…………………………………………………………………………………………

…………………………………………………………………………………………

…………………………………………………………………………………………

**Vaccination history of affected flocks:**

|  |  |  |  |
| --- | --- | --- | --- |
| Shed | Vaccinated against | Age at vaccination/date of vaccination | Name of vaccine |
|  |  |  |  |
|  |  |  |  |

**Source of feed:**

a) Use commercial feed. Yes ⁭ No ⁭

b) Used self made feed. Yes ⁭ No ⁭

Is there any thing you want to say about?

…………………………………………………………………………………………

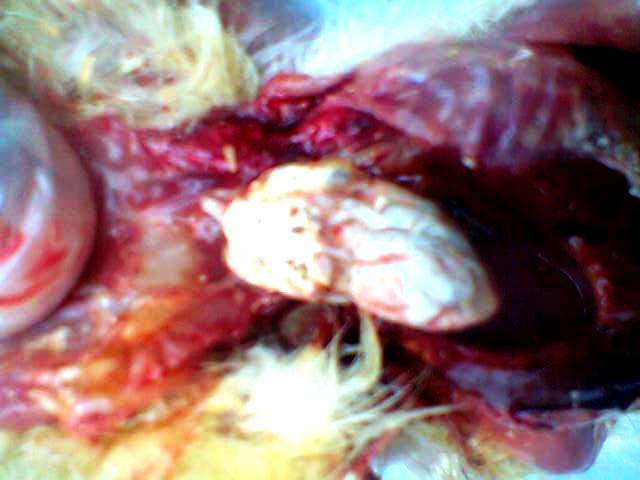
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Thank you for your time.

**Signature of data collector**

**POSTMORTEM LESIONS**

**Fig: Fragile Liver in staphylococcosis**

**Fig: Colisepticaemia**

**Fig: Typical Pericarditis in colibacillsis**



**Fig: Horse plasma**



**Fig: Coagulase test**