ISOLATION, IDENTIFICATION AND ANTIBIOTIC SENSITIVITY PROFILING OF *ESCHERICHIA COLI* AND *SALMONELLA* SPP. FROM DEAD BROILER IN LOCAL MARKET OF DHAKA CITY

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CERTIFICATE

This is to certify that the thesis entitled **"ISOLATION, IDENTIFICATION AND ANTIBIOTIC SENSITIVITY PROFILING OF *ESCHERICHIA COLI* AND *SALMONELLA* SPP. FROM DEAD BROILER IN LOCAL MARKET OF DHAKA CITY"** submitted to the Department of Medicine & Public Health, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (M.S.)** in **MEDICINE**, embodies the result of a piece of bonafide research work carried out by **MD. KHAIRUL ISLAM**, Registration No. **13-05379** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

December, 2020
Dhaka, Bangladesh

(Prof. Dr. K. B. M. Saiful Islam)
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DEDICATED TO
MY
BELOVED PARENTS
AND TEACHERS

DECLARATION

I declare that the thesis hereby submitted by me for the MS degree at the Sher-e-Bangla Agricultural University is my own independent work and has not previously been submitted by me at another university/faculty for any degree.

Date: 05.01.2022 **MD. KHAIRUL ISLAM**

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LIST OF ABBREVIATIONS AND ACRONYMS

ABBREVIATION		FULL MEANING
AMP	:	Ampicillin
AMX	:	Amoxicillin
BG	:	Brilliant Green
CL	:	Colistin
CXM	:	Cefuroxime
CFT	:	Ceftriaxone
CFM	:	Cefixime
COT	:	Co-trimoxazole
CLSI	:	Clinical and Laboratory Standards Institute
CFU	:	Colony Forming Unit
DLS	:	Department of Livestock Services
EMB	:	Eosin Methylene Blue
et al.	:	And others
e.g.	:	That is
E.coli	:	*Escherichia coli*
FAO	:	Food and Agricultural Organization
etc.	:	Etcetra
Fig.	:	Figure
GM	:	Gentamicin
H_2O_2	:	Hydrogen peroxide
H_2S	:	Hydrogen Sulphide
hrs.	:	Hours
HEQEP	:	Higher educational quality enhancement project
IN	:	Intermediate
Lbs.	:	Pound
Ltd.	:	Limited
M. S.	:	Master of Science

LIST OF ABBREVIATIONS AND ACRONYMS

ABBREVIATION		FULL MEANING
MC	:	MacConkey
Mg	:	Milligram
MH	:	Muller Hinton
Ml	:	Millilitre
Mm	:	Milimeter
Min.	:	Minute
MR	:	Methyl Red
NB	:	Nuttrient Broth
No.	:	Number
PBS	:	Phosphate buffered solution
R	:	Resistant
S	:	Sensitive
S	:	Streptomycine
SAU	:	Sher-e-Bangla Agricultural University
Spp	:	Species
SS	:	Salmonella Shigella
TE	:	Tetracycline
TSI	:	Tripple Sugar Iron
USA	:	United states of America
µg	:	Microgram
µl	:	Microlitre
VP	:	Voges-Proskauer
WHO	:	World Healtth Organization
yrs.	:	Years

LIST OF SYMBOLS

SYMBOLS		FULL MEANING
^{o}C	:	Degree Celsius
&	:	And
+	:	Positive
-	:	Negative
%	:	Percentage
®	:	Registard trade mark
<	:	Less than
>	:	Greate r than
≥	:	Greater than equal
≤	:	Less than equal

ISOLATION, IDENTIFICATION AND ANTIBIOTIC SENSITIVITY PROFILING OF *ESCHERICHIA COLI* AND *SALMONELLA* SPP. FROM DEAD BROILER IN LOCAL MARKET OF DHAKA CITY

ABSTRACT

This study was carried out in Sher-e-Bangla Agricultural University, Dhaka for isolation, identification and antibiotic sensitivity profiling of *Escherichia coli* and *Salmonella* spp. from dead broiler in local market of Dhaka city. A total of 150 liver and heart samples were collected from dead broiler. The microorganisms of *Escherichia coli* and *Salmonella* spp. were isolated and identified. Samples were collected aseptically and inoculated onto various culture media for isolation of *Escherichia coli* and *Salmonella* spp. Identification of bacteria from positive samples was performed by cultural characteristics and biochemical tests. Hundred percent prevalence with microorganism was found from all the samples. The prevalence of *E. coli* and *Salmonella* spp. in liver samples was (45.33%) and (40%) and in heart samples it was (44%) and (38.67%) respectively. The overall prevalence of *E. coli* and *Salmonella* spp. in broiler was (44.67%) and (39.33%). Antibiotic sensitivity profiling of the isolated *E. coli* and *Salmonella* spp. was performed by the disc diffusion method against 10 commonly used antibiotics. The highest rate of sensitivity against *E. coli* was found with Ceftriaxone (52.24%) followed by Gentamicin (49.25%) and Streptomycin (44.78%). The highest rate of resistance was recorded in Tetracycline (52.24%) followed by Ampicillin (50.75%), Cefuroxime (47.76%), Amoxycillin (46.27%) and Co-Trimoxazole (46.27%). The highest rate of antibiotic sensitivity against *Salmonella* spp. was found with Ceftriaxone (52.54%) followed by Gentamicin (50.85%) and Streptomycin (47.46%). The highest rate of resistance of *Salmonella* was recorded in Cefuroxime (54.24%) followed by Ampicillin (52.54%), Cefixime (50.85%), Tetracycline (49.15%), Amoxycillin (47.46%) and Co-Trimoxazole (45.76%). Based on the present study, it may be concluded that Ceftriaxone should be best result against both *E. coli* and *Salmonella* spp. followed by Gentamicin and Streptomycin under the present study.

CHAPTER I

INTRODUCTION

Poultry industry in Bangladesh plays a vital role in the rural socio-economic system by contributing significantly on economic growth and simultaneously creating numerous employment opportunities. Bangladesh is one of the key players in the south Asian livestock industry, especially in poultry industry (Daily Star, 2011). Chicken meat is also relatively cheap and affordable source of animal protein (Yami and Dessie, 1997). The livestock sector is the integral part of the agro-based economy. Poultry industries play an important role in poverty alleviation and economic development of Bangladesh. Poultry meat contributes approximately 37% of total animal protein supplied in the country (Rahman and Rahman, 1998).

According to WHO-FAO joint survey, meat consumption per head in Bangladesh is 15.23 kg per year and poultry contributes 35.25% of total meat supply (Akbar *et al*., 2013). The poultry sector employs about 5 million people and has experienced a long-term growth rate of about 4.50%, which is highest in the economy (BLRI Report, 2009). It is an important component of farming system and plays a significant role to 80% rural people of Bangladesh. In common terms, a disease is an abnormal condition that is caused by infection, basic weaknesses, or environmental stress. A disease is defined by a specific group of signs or symptoms. Diseases prevent affected animals from functioning normally (Islam *et al*., 2009; Ravichandran and Mohamed, 2015). Health is the overall condition of an animal at a given time. Disease causes this condition to weaken. This can result in poor productivity and reduced quality of the affected animals. It could even lead to the death/loss of one or all of the birds in a flock (Ravichandran and Mohamed, 2015; Islam *et al*., 2003).

Traditionally in Bangladesh, poultry rearing is one of the most important sources of income for rural women especially for landless and marginal farmers (Paul *et al*., 1990). But it is true to say that this profitable sub-sector is seriously interrupted by a number of infectious and contagious diseases such as Newcastle disease(ND), Infectious bursal disease (IBD), Salmonellosis, Fowl cholera, Infectious Coryza, Chronic respiratory disease, Aspergillosis, Coccidiosis, Helminthiasis etc. The poultry sector in Bangladesh is now in a great challenge of these harmful diseases (Hossain *et al*., 2004). Diseases can be categorized by common causes, such as genetic, mechanical, toxic, and nutritional. Infectious diseases are caused by viruses, bacteria, and fungi. Parasitic diseases are caused by protozoa, worms, and external parasites such as mites and lice (Rashid *et al*., 2013).

From last few years commercial poultry farming has been developing very rapidly but several factors reduce the growth rate of this sector and raise mortality of chickens. Bangladesh's long-term outlook as a leading producer of poultry products remains bright despite the diseases outbreak that has held off its potential. The number of poultry farmers has reduced to about 90,000 from 1.5 Lakh due to 2007 - 08 outbreak of the bird flu scare (Daily Star, 2011). It is well known that poultry diseases are the major constraints for developing the poultry industry (Karim, 2003). Among different causal agents of disease, bacteria are one of the most destructive which can damage poultry seriously and sometimes 100% losses can be occurred (Rashid *et al*., 2013).

Development of poultry sector in Bangladesh is being hampered by a number of factors, of which the diseases are considered as the major factor causing 30% mortality of chicken per year (Das *et al*., 2005). Important bacterial diseases of poultry in Bangladesh are pullorum disease, colibacillosis and fowl cholera (Samad, 2000) which are responsible for high percentage of morbidity and mortality.

Escherichia coli are 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% and morbidity over 50% but in layer it affects the reproductive tract resulting failure of egg productivity and fertility (Barens and Gross, 1997). It may cause about 28% death in Sonali variety birds of Bangladesh (Biswas *et al*., 2006). *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).

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 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, 2006), 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 (Hasan *et al*., 2010).The gross lesions in chicks are unabsorbed yolk sac and turbid yellow color fluids in the peritoneal cavity and in adult peritonitis, discrete, small, white necrotic foci in the liver and enteritis (Hasan *et al*., 2010; Cheesbrough, 2006).

Escherichia coli are one of the common microbial floras of gastrointestinal tract of poultry and human being **(**Jawetz *et al.*, 1984). Although most isolates of *Escherichia coli* are nonpathogenic but they are considered as indicator of fecal

contamination in food and about 10 to 15% of intestinal coliforms are opportunistic and pathogenic serotypes (Barnes *et al.,* 1997) and cause a variety of lesions in immune-compromised hosts as well as in poultry. Infection with bacteria genus *Salmonella* is responsible for a variety of acute and chronic disease in poultry reported in Bangladesh (Bhattacharjee *et al.,* 1996). Many epidemiological studies and research have implicated foods of animal origin as major vehicles associated with illnesses caused by *Escherichia coli*, *Campylobacter*, *Salmonella* and *Yersinia spp*. (Cretikos *et al.,* 2008).

Antibiotics have been used successfully in poultry for different purposes such as growth promotion, prophylaxis, or therapeutics. However, their use in animal production and human therapy has resulted in increased bacterial resistance to many (Castanon, 2007). Some previous studies described the high gene load of resistance determinants in the bacterial community in chicken litter (Lu *et al.,* 2003; Nandi *et al.,* 2004). Acquired multi drug resistance to antimicrobial agents creates an extensive trouble in case of the management of intra and extra intestinal infections caused by *E. coli* and *Salmonella* spp. which are major source of illness, death, and increased healthcare costs (Gupta *et al.,*2001). Since bacteria acquire most resistance genes through horizontal transfer, conjugative genetic elements such as plasmids and transposons are common vectors for the dissemination of antimicrobial resistance genes to the diverse microorganisms.

Therefore, the present study was designed to isolate *E. coli* and *Salmonella* spp. strains from broiler for assessing their susceptibility and resistance patterns to some selected antimicrobials with the following objectives:

1. To isolate and identify *Escherichia coli* and *Salmonella* strains from dead broiler samples
2. To perceive the performances of antibiotic against *Escherichia coli* and *Salmonella* isolated from dead broiler

CHAPTER II

REVIEW OF LITERATURE

Poultry, in animal husbandry, birds rose commercially or domestically for meat, eggs, and feathers. Chickens, ducks, turkeys, and geese are of primary commercial importance, while guinea fowl and squabs are chiefly of local interest. The poultry sub-sector is an important avenue in fostering agricultural growth and reduce malnutrition for the people in Bangladesh (Da-Silva and Rankin, 2014). It is an integral part of farming system in Bangladesh and has created direct, indirect employment opportunity including support services for about 6 million people (Ansarey, 2012). Eggs come from hens raised specifically to lay eggs, but chickens that are raised for meat are called "broilers." These chickens are typically white, and are bred specifically for optimal health and size to produce a quality product for the consumer. Under the present study, related findings pertaining to the investigation is reviewed below under the following headings:

2.1 Importance of poultry

Bangladesh is an agricultural based densely populated country. About 71% of the population lives in rural areas (BBS, 2010a). The average per capita income is only US$751 (BBS, 2010b). The majority of people are engaged in agricultural operations, particularly crops, fish and livestock, of which both native and exotic poultry are now main stream. Approximately 20% of the protein consumed in developing countries comes from poultry meat and eggs (Alders and Pym, 2009).

The poultry sub-sector has proved as an attractive economic activity, thereby, indicating its` importance for the entire economy. The sector accounts for 14% of the total value of livestock output and is growing rapidly (Raihan and Mahmud, 2008). It is finding out that poultry meat alone contributes 37% of the total meat production in Bangladesh. Poultry contributes about 22-27% of the total animal protein supply in the country (Prabakaran, 2003). It is stated that in Asia, poultry

manure is used as feed for fish where poultry are raised on top of the ponds as part of an integrated system for example, fish-cum-duck farming (FAO, 2014). Development of poultry has generated considerable employment through the production and marketing of poultry and poultry products in Bangladesh (Da-Silva and Rankin, 2014).

As a developing country, poverty, unemployment and malnutrition are the major problems of Bangladesh. Forty four percent of this country's population lives below the absolute poverty line and the number of landless poor people has been increasing by 3.7 percent per annum (GOB, 2009). Poultry is one of the most important sub-sectors of agriculture in Bangladesh. The rural people have been keeping indigenous chicken for centuries under semi-natural conditions mainly for their domestic consumption with very little commercial motives. At present, a large number of poultry farms have been established on commercial basis in and around the cities and towns and are operating under intensive management. Poultry meat can efficiently and rapidly fill in the shortage of body requirement. At present a total of 0.15 million commercial farms have been established throughout the country. About 6 million people are engaged directly and indirectly in poultry industry. About 3500 million of eggs, 250 million of broiler day-old chick, 25 million of layer day-old chick and more than 200 million tons of poultry feed are being produced per year in the country (Rahman, 2004).

Bangladesh is a densely populated country. Malnutrition and hunger are serious problems in this country. Fifty percent of the new born are low birth weight and more than 90 percent of the children (aged < 5years) suffers from mild to severe forms of malnutrition. Egg, meat and milk, the three important protein foods originate from the poultry and livestock sector. On an average every person should consume at least 100 eggs, 43.5 kg of meat and 90 liter of milk per annum to prevent malnutrition (Hossain *et al*., 2010). Therefore, it is essentially needed to

increase the production of eggs, meat and milk and there are good prospects to increase the production of poultry and livestock products.

Broiler farming has a great potential for providing additional income to both male and female of rural and urban areas through creation of employment opportunities. Broiler, however, has a shorter life cycle and its production requires less capital compared to other meat producing animals (Rahman, 2004). Since the majority of the people irrespective of caste or religion prefer chicken, its demand is very high. As a result, the prices of those products have gone up. Having received the signal of higher price and demand in home market, recently a tendency to establish small-scale commercial farm is observed among some people both in rural and urban areas. Poultry is no more a backyard farming now. It is shaping up as an industry. So, an efficient production system is required for supporting commercial broiler farming in the country (Hossain *et al*., 2010).

2.2 Common bacterial disease of broiler

2.2.1 Colibacillosis

Escherichia coli are Gram negative bacteria, normal inhabitants of the intestinal tract of birds. Pathogenic strains can cause diseases such as air sac disease, salpingitis, omphalitis, etc. alone or in combination with other pathogenic agents (viruses, Mycoplasma) (Kaper *et al*., 2004 and Lutful, 2010).

Colibacillosis, and especially its respiratory form is of major importance in poultry production, as it can cause severe economic losses with mortality, loss of egg production or impaired growth (Rahman *et al*., 2004 and Barbour *et al*., 1985).

This disease can be treated by antimicrobials effective against Gram negative bacteria (Rahman *et al*., 2004 and Lutful, 2010).

2.2.2 Salmonellosis

Avian Salmonellosis is a large group of acute or chronic diseases caused by Salmonella (Gram negative bacteria, more than 2000 serotypes are known) (Lutful, 2010 and Rahman *et al*., 2004).

Besides some specific types of Salmonella (*S. gallinarum, s.pullorum, S. arizona*) which cause particular diseases in birds, numbers of paratyphoid serotypes are common to birds and to other animal species (Rahman *et al*., 2004). These serotypes can cause diseases, especially in young birds (e.g., *S. typhimurium*), but are more often carried by birds not manifesting any symptoms. This fact is of major public health significance, as poultry meat and eggs are possible sources of food-borne Salmonellosis in humans (Berhe *et al*., 2012).

Salmonella are very resistant in the environment and complex globalized control programs must be implemented to control Salmonella spread. Authorities and poultry producers make a priority of this control, which involves complementary measures such as compulsory slaughter, monitoring, antibiotics, feed pelleting, competitive exclusion products and, of course, hygiene (Berhe *et al*., 2012 and Rahman *et al*., 2004).

2.2.3 Pasteurellosis

This disease is caused by *Pasteurella*, which are Gram negative bacteria. Different types of *Pasteurella* can cause diseases. The most important ones are *P. multocida* (fowl cholera), *P. anatipestifer* and *P. haemolytica* (Rahman *et al*., 2004 and Addis and Sisay, 2015).

These types can infect chickens, turkeys, ducks and other birds' species. Mortality can be high, especially in older birds; the difficulty is that symptoms appear usually only briefly before death. Symptoms are anorexia, ruffled feathers, diarrhea, and respiratory difficulties. A chronic form can also occur, with secondary localizations (joints, foot pads, sternal bursa). Hyperemia occurs in the

acute form, more localized lesions appear in the chronic one (Pneumonia in turkeys, etc.) (Rahman *et al*., 2004).

Antimicrobials and vaccines are available to treat and prevent the disease. Strict hygienic procedures must be undertaken to eliminate *Pasteurella* organisms from a contaminated poultry house (Rahman *et al*., 2004 and Addis and Sisay, 2015).

2.2.4 Bordetellosis

This is a highly contagious upper respiratory tract disease in poultry (especially in turkeys), caused by *Bordetella avium* (a Gram-negative bacteria) (Yami and Dessie, 1997).

It can be complicated by *Escherichia coli* and then cause severe economic losses. *Bordetella* alone causes sneezing, oculonasal discharge, mouth breathing, stunted growth and predisposes to other infections (Yami and Dessie, 1997).

Antibiotic treatments plus strict biosecurity measures help the treatment and control of the disease (Addis and Sisay, 2015 and Yami and Dessie, 1997).

2.2.5 Infectious Coryza

This is an acute respiratory disease, caused by *Haemophilus paragallinarum*. It can occur in growing chickens and layers, causing increased numbers of culls and marked reduction (10-40%) in egg production (Yami and Dessie, 1997).

All ages are susceptible, chronic and healthy carrier birds can serve as a reservoir for the infection. Symptoms are mostly nasal discharge, facial oedema and conjunctivitis, growth impairment and loss in egg production (Yami and Dessie, 1997).Vaccination and antibiotics can be used to prevent or treat this infection (Yami and Dessie, 1997 and Addis and Sisay, 2015).

2.2.6 Tuberculosis

This is caused by *Mycobacterium avium,* Gram-positive bacteria. It is not a common disease in modern poultry farms. All bird species can be infected (Addis and Sisay, 2015).

It is a chronic disease, and causes economic losses by decreased egg production and death (Addis and Sisay, 2015 and Yami and Dessie, 1997). The birds appear depressed, lose weight, pectoral muscles are often atrophied, feathers have a ruffled appearance; affected birds die within a few months or survive (Dashe *et al*., 2003 and Yami and Dessie, 1997).

Antimicrobials are not often used (long treatments are required). Bio-security hygiene and vaccination can help towards disease control (Dessie, 1997).

2.2.7 Campylobacteriosis

This disease is caused by members of the genus Campylobacter, especially *C. jejuni*, *C. coli* and *C. laridis*, which are Gram negative bacteria (Dashe *et al*., 2003).

This disease is of public health importance, as Campylobacter can cause serious disease in humans. Campylobacteriosis in humans is a food-borne disease and poultry meats are possible carriers. Campylobacter usually induces depression and diarrhea, or even mortality. It is difficult to eradicate Campylobacter presence in poultry houses and birds (Dashe *et al*., 2003 and Havelaar *et al*., 2015).

Much work is done to improve the control of this infection, through hygiene, biosecurity, antibiotics or competitive exclusion products (Havelaar *et al*., 2015).

2.2.8 Mycoplasmosis

This disease is caused by *Mycoplasma* spp., which is neither a virus nor bacteria. The most significant *Mycoplasma* species in poultry production are *M. gallisepticum*, *M. synoviae*, *M. meleagridis*, *M. iowae* (Dashe *et al*., 2003).

These diseases are widely spread all over the world and have a major economic significance, even if no public health importance (Dashe *et al*., 2003).

Hygiene and antibiotherapy, together with slaughter of some infected breeder flocks help towards the control of this disease (Dashe *et al*., 2003).

2.2.9 Erysipelas

This is caused by *Erysipelothrix rhusiopathiae,* Gram-positive bacteria. Turkeys are affected mostly, especially in backyard or free-range stocks (Addis and Sisay, 2015 and Havelaar *et al*., 2015).

Erysipeloid in humans in contact with infected birds can occur. In chickens, clinical signs are mainly weakness, depression, diarrhea and sudden death. In turkeys, sudden death, swollen snood and dewlap, weakness and anemia (Havelaar *et al*., 2015).

Antibiotics active against Gram positive bacteria (e.g., penicillin) can treat the disease successfully (Havelaar *et al*., 2015).

2.2.10 Clostridial disease

These are caused by some Clostridium, which are Gram positive bacteria. *C. colinum* causes ulcerative enteritis, *C. perfringens* causes necrotic enteritis or gangrenous dermatitis, *C. botulinum* causes botulism, *C. septicum* causes gangrenous dermatitis (Dashe *et al*., 2003).

Avian botulism is usually manifested by paralysis of various muscles and its public health significance is minimal (Addis and Sisay, 2015).

These diseases are not always easy to manage, even with appropriate antibiotics (Addis and Sisay, 2015).

2.2.11 Staphylococcosis

This is caused by *Staphylococcus aureus*, Gram-positive bacteria, which is mostly present in joints or skin (Dashe *et al*., 2003).

It is a possible source of infection for humans. When ill, birds have ruffled feathers, difficulties in walking and fever. Gangrenous dermatitis can also be induced by *S. aurous* infection (Dashe *et al*., 2003).

Antibiotics can treat this infection successfully (Havelaar *et al*., 2015 and Dashe *et al*., 2003).

2.2.12 Steptococcosis

Streptococcus, Gram-positive bacteria, is a normal inhabitant of the avian intestinal flora, but can sometimes cause acute or chronic infections, with ensuing mortality (Dashe *et al*., 2003 and Addis and Sisay, 2015).

In the acute form, depression, ruffled feathers, diarrhea and death can occur. In the chronic form, depression, loss of weight and head tremors can occur (Dashe *et al*., 2003 and Havelaar *et al*., 2015).

Together with good management, antibiotics can treat successfully the disease, especially in its early stages (Dashe *et al*., 2003).

2.3 Common features of Colibacillosis

Among the first reports of infections in poultry caused by coliform organisms were those of Gross (1994) and Huq (2002). Later Wray (2001**)** reported the isolation of *E. coli* from ‘air sac disease’. Pathogenic sero-groups of *E. coli* are common in the environments in which poultry are raised and may cause airsacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis, cellulitis or yolk sac infection. Collectively, these diseases constitute a major economic loss. Colibacillosis refers to any localized or systemic infection caused entirely or partly by *E. coli*, including septicemia, granuloma, air sac disease, chronic respiratory disease, avian cellulitis, swollen head syndrome, peritonitis, salpingitis, synovitis, panophthalmitis, and omphalitis. Colibacillosis in mammals is most often a primary enteric disease, whereas Colibacillosis in poultry is typically a

secondary localized or systemic disease occurring when host defense has been impaired or overwhelmed (Barnes and Gross, 1997).

Collectively, infections caused by *E. coli* are responsible for significant economic losses to the poultry industry. For example, 43% of broiler carcasses condemned for disease at processing had lesions consistent with *E. coli* septicemia (Yogaratnam, 1995).

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

2.3.1 Environmental distribution

The most important reservoir of *E. coli* is the intestinal tract of animals, including poultry. In chickens, there are about 109 colony forming units of bacteria per gram of feces. Of these, 106 CFU are *E. coli*, 10-15% of which are pathogenic sero-groups (Gross, 1994) and probably infect most mammals and birds thus having a cosmopolitan distribution. At times, coliforms may be transmitted between poultry and humans (Ojeniyi, 1989).

Egg transmission of pathogenic *E. coli* is common and can be responsible for high chicken mortality. Pathogenic coliforms are more frequent in the gut of the newly hatched chicks than in eggs from which they hatched (Barnes and Gross, 1997), suggesting rapid spread after hatching. The most important source of egg infection seems to be fecal contamination of the surface with subsequent penetration of the shell and membranes. Coliform bacteria can be found in litter and fecal matter. Dust in poultry houses may contain 105-106 *E. coli*/g. These bacteria persist for long periods, particularly when dry (Barnes and Gross, 1997).

Feed is often contaminated with pathogenic coliforms, but these can be destroyed by hot pelleting process. Rodent droppings often contain pathogenic coliforms.

Pathogenic serotypes can also be introduced into poultry flocks through contaminated well water (Nagi and Raggi, 1972).

2.3.2 Incidence of Colibacillosis in Bangladesh

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

2.3.3. Disease syndromes

2.3.3.1 Yolk sac infection

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

2.3.3.2 Respiratory tract infection

Respiratory disease complex, involving a secondary infection with *E. coli* (Huq, 2002), usually occurs between 2 and 12 weeks of age, with most losses occurring between 4 and 9 weeks. This is one of the most common poultry diseases with losses at times exceeding 20%.

Economic loss results from reduced growth and feed efficiency, increased mortality and increased condemnation at processing. Poultry frequently inhale pathogenic *E. coli* in dust derived from feces, but the normal host defense prevents respiratory tract infection. However, following infection with respiratory tract agents such as New Castle Disease virus (NDV), Infectious Bronchitis Virus (IBV) and *Mycoplasma gallisepticum* alone or in combination, certain *E. coli* are able to establish in the respiratory tract (Gross, 1994).

Vaccine viruses (NDV and IBV) are as important as the more virulent field strains **(**Gross, 1994).High level of environmental stress also increases the severity of the

respiratory infection (Gross, 1994).Several host and environmental factors influence susceptibility of chicks to *E. coli*. Resistance to *E. coli* was the greatest in a strain termed LA chickens (Gross, 1984a).

Resistance to *E. coli* increased as the level of environmental stress increased until protection was close to complete. Further increases in the severity of environmental stress resulted in increased susceptibility (Gross, 1984a).Under very low level of stress, birds became extremely susceptible. Socialization also resulted in increased resistance (Gross and Siegel, 1982).

Exposure to ammonia and dust resulted in declination of the epithelium of the respiratory tract, which allowed coliforms to invade (Nagaraja *et al.,* 1984). Control of the disease by preventing the predisposing respiratory infections has been much more successful than treatment of the secondary *E. coli* infection. *Mycoplasma gallisepticum* has been eradicated from all commercial breeding stocks and is seldom seen under good management conditions. Most respiratory viruses now resemble the vaccine strains (Alexander *et al.,* 1987) and the severity of these viral infections can be reduced by raising birds under a relatively low level of environmental stress and by socializing the birds to their handlers. In some birds, respiratory tract infection is not controlled and the *E. coli* infection becomes bacteraemic.

In most bacteraemic birds, infection spreads to the myocardium and later to the pericardial sac. Myocardial infection results in changes in the electrical conductivity of the myocardium resulting in major changes in the electrocardiogram (Gross, 1994).

2.3.3.3 Acute septicemia of chickens

Acute *E. coli* septicemia is an infection of mature chickens characterized by a firm dark or greenish liver and congested pectoral muscles. Sometimes small necrotic

foci can be seen on the liver. The crops are usually full and the birds are in good flesh. In some cases, pericarditis and peritonitis are also present.

2.3.3.4 Salpingitis

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

2.3.3.5 Peritonitis

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

2.3.3.6 Swollen head syndrome

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

2.3.3.7 Cellulitis

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

2.3.3.8 Enteritis

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

2.3.4 Isolation and Identification of *E. coli*

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

2.1.4.1 Gross lesions

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

2.1.4.2 Staining properties of *E. coli*

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

2.1.4.3 Colony morphology of *E. coli*

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

2.1.4.4 Biochemical character of *E. coli*

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

2.4 Common features of Salmonellosis

The genus *Salmonella* (of the family Enterobacteriaceae) named for the eminent United States Department of Agriculture veterinarian and bacteriologist Daniel E. Salmon, consist of more than 2300 serologically distinguishable variants (Gast, 1997). Towards the end of the 19th century, infectious enteritis causing heavy mortality in chicken was described in Europe and North America (Jordan and Pattison, 1996).

Initially the causal agent was called *Bacillus gallinarum* and the name fowl typhoid was applied in 1902 (Shivaprashad, 1997). *Salmonella pullorum* was first isolated from chicks suffering from severe diarrhea and was described by Rettger and Stone burn in 1909 (Marchant and Packer, 1983).

The disease had been previously known as bacillary white diarrhea (BWD), but as white diarrhea is not always a clinical feature, it becomes known pullorum disease (Jordan and Pattison, 1996).

The disease is caused by gram negative bacteria known as *Salmonella pullorum.* This organism belongs to a family known as Enterobacteriaceae. Organism is motile and looks like slender rod measuring 0.3-0.5×1-2.5μm. It is no liquefying, non-chromogenic, non-sporogenic facultative anaerobe (Snoeyenbos and Willims, 1994). It grows on beef agar or broth very readily. MacConkey agar can be very used for growth. The organism is non-lactose fermenter. The organism is resistant to heat and many chemicals. In suitable environment the organism contains a thermostable toxin. *S. gallinarum* is a short bacillus 1-2μm broad, which does not possess flagella (Jordan and Pattison, 1996).

Pullorum disease is caused by bacterium *S.pullorum* (Shivaprasad, 1997).In addition to *S. gallinarum, S. pullorum*, other Salmonallae such as *S. enteritidis, S. panama and S. Dublin* also belongs to the sero-group D1 (Le Minor, 1984). The various motile and non-hosts adapted highly invasive serotypes such as *Salmonella enteritis* and *Salmonella typhimurium* are commonly referred to as paratyphoid Salmonellae (Gast, 1997).

2.4.1 Environmental Distribution

Salmonellosis is a serious systemic disease of domestic poultry which cause large scale economic losses through mortality, morbidity and reduction in egg production (Junior *et al.*, 2000). The disease occurs sporadically and enzotically in most countries of the world including Bangladesh. It causes severe economic losses of the poultry with morbidity and mortality varying in chicken from 10-50% or more (Pomeroy, 1984).

Salmonellosis is distributed in many countries of the world, and has economic significance (Barrow *et al.*, 1992).They are mainly distributed in Latin America,

the Middle East, the Indian Subcontinent, Africa and perhaps other part of the world Shivaprasad, (1997).

Salmonellosis has also been reported in many countries of South-East Asia including Bangladesh (Bhattacharjee *et al*., 1996 and Begum *et al*., 1993), India (Ghosh, 1988; Kumar and Kaushik, 1988), Pakistan (Javed and Hameed, 1989; Muneer*et al*, 1988) and Nepal (Jha *et al.,* 1994). Salmonellosis is common in both backyard chickens and in commercial poultry (Fricker, 1987).

2.4.2 Incidence in Bangladesh

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

2.4.3 Mode of Transmission:

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

2.4.4 Disease Syndromes

2.4.4.1 Pullorum Disease

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

2.4.4.2 Fowl Typhoid

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

2.4.5 Isolation and Identification of *Salmonella*

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

2.4.5.1 Gross lesions

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

2.4.5.2 Staining properties of *Salmonella*

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

2.4.5.3 Colony morphology of *Salmonella*

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

2.4.5.4 Biochemical Character of *Salmonella*

In TSI agar it produces acidic (Yellow) butt and alkaline slant (Red) with blackening due to production of H_2S gas (Waltman *et al.*, 1998).

2.6 Role of bacterial diseases

Rahman and Adhikary (2016) conducted a pathological study on poultry diseases occurring at Sylhet district of Bangladesh to know the disease pattern of poultry. The diagnosis of different diseases was performed on the basis of history of the affected flock, visual examinations, post mortem examinations, pathological findings, isolation and identification of the causal agents, serology and age of affected birds. Cases found in FDIL were Newcastle disease (9.85 %), Infectious bursal disease (16.43 %), colibacillosis (14.51 %), omphalitis (10.14 %), salmonellosis (7.32 %), fowl cholera (3.11 %), aspergillosis (7.33 %), duck plague (9.16 %) and duck cholera (7.03 %). Disease prevalence was recorded as 38.04 % among the age group of >2 - 8 weeks followed by 28.24 % among 0 - 2 weeks of age, 6.66% among >8 - 20 weeks of age and 3.39 % among >20 weeks of age. It was found that prevalence of poultry diseases was significantly highest in summer season (47.36 %) followed by rainy season (25.4 %) and winter (24.02 %). Prevalence of poultry diseases were significantly higher in summer (40.5 %) followed by rainy season (32.11 %) and winter (27.2 %). The study may help to know the prevalence of poultry diseases for the improvement of poultry production in Sylhet region.

Rahman *et al*. (2017) conducted a study to determine the prevalence of broiler and layer diseases at Gazipur sadar upazilla veterinary hospital in Bangladesh. A total of 296 either dead or sick birds were considered for the present study. Diagnosis of different diseases was made on the basis of history, owner's complains, age of birds, presenting signs, clinical and necropsy examination findings. The prevalence of diseases was recorded in broiler (n=189) 63.9% and layer (n=107) 36.1%. In broiler the prevalence of Colibacillosis (33.4%) was higher followed by

Omphalitis (23.8%), Infectious Bursal Disease-IBD (15.3%), Newcastle disease-ND (9.0%), mixed infection (7.4%), Gout (5.3%) and Coccidiosis (5.8%). In case of layer highest prevalence was recorded in Salmonellosis (19.6%). while other diseases were Colibacillosis (15.9%), Mycoplasmosis (12.1%), ND (10.3%), IBD (8.4), Mareks (9.3%), mixed infection (8.4%), Fowl Cholera (4.7%) and Mycotoxicosis (4.7%). The present findings will help poultry researchers and practitioner to know the present status of infectious diseases of commercial chickens in the study area and also help to establish flock-based control strategy.

Hasan *et al*. (2010) carried out a study with a view to compare clinical and laboratory diagnoses of various bacterial diseases of poultry. 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 for 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.

Hassan *et al*. (2016) conducted a study with a view to investigate the prevalence of poultry diseases in Gazipur district of Bangladesh. A total of 679 poultry birds (313 layers, 338 broilers and 28 cockerels) either dead or live were examined. The diseases were diagnosed on the basis of history, clinical signs and post-mortem findings. The diseases encountered in layers were bacterial diseases 52.29% (salmonellosis 38.56%, colibacillosis 6.7%, fowl cholera 4.79% and necrotic enteritis 1.60%), viral diseases 23.95% (avian influenza 2.56%, Newcastle disease 16.61%, infectious bronchitis 3.19% and avian leucosis 0.64%), mycoplasmal disease (mycoplasmosis 14.70%) and protozoal disease (coccidiosis 5.75%). Salmonellosis was most prevalent disease in age group of >20 weeks, while Newcastle disease most common in 8 to 20 weeks of age group. In case of broiler, bacterial diseases 28.99% (salmonellosis 21.30% and colibacillosis 7.69%), viral diseases 53.24% (infectious bursal disease 28.99%, Newcastle disease 8.87% and infectious bronchitis 15.38%), mycoplasmal disease (mycoplasmosis 7.1%) and protozoal disease (coccidiosis 6.5%). In cockrels, the most prevalent disease was colibacillosis 35.71% followed by salmonellosis 28.57%, Newcastle disease 14.28% and mycoplasmosis14.28%. So, among the diseases, salmonellosis is most prevalent disease followed by infectious bursal disease and mycoplasmosis in different kinds of poultry.

Al-Mamun *et al*. (2019) conducted a study to determine the occurrence of different poultry diseases in broilers, layers and sonali chickens. A total of 1981 chickens either infected or dead were collected and examined to diagnose the diseases. Diagnosis of diseases was made on the basis of history, clinical signs, and postmortem findings and in some cases laboratory examination was performed to confirm the diseases. Overall incidence of infectious bursal disease was the highest (29.32%) in broiler chickens followed by salmonellosis (14.29%), new

castle disease (11.78%), infectious bronchitis (9.27%), coccidiosis (6.93%), colibacillosis (6.43%), chronic respiratory disease (4.85%), visceral gout (4.68%), necrotic enteritis (1.59%), mycotoxicosis (0.67%) and infectious coryza (0.08%). In layer chickens prevalence of salmonellosis was higher (30.60%), followed by new castle disease (17.54%), infectious bursal disease (9.16%), coccidiosis (9.16%), chronic respiratory disease (9.16%), colibacillosis (7.01%), fowl cholera (5.26%), infectious bronchitis (4.09%), necrotic enteritis (2.92%), egg peritonitis (1.94%), aspergillosis (1.75%), deficiency disease (1.75%), mycotoxicosis (1.75%), helminth parasites (1.36%), fowl pox (0.97%), infectious coryza (0.97%) and lymphoid leukosis (0.78%). In case of Sonali Chickens infectious bursal disease was the highest (33.95%) followed by salmonellosis (27.31%), new castle disease (19.56%), chronic respiratory disease (11.07%), coccidiosis (10.70%), colibacillosis (8.11%), deficiency disease (4.80%), fowl cholera (3.32%), necrotic enteritis (2.56%), aspergillosis (2.21%), fowl pox (0.74%), helminth parasites (0.74%) and mycotoxicosis (0.37%).

2.5. Antibiotic Resistance in *E. coli* and *Salmonella*

Escherichia coli, a member of the normal gut flora of humans and animals, possess many beneficial functions. Nonetheless, their pathogenic role is also well recognized as they cause many bacterial infections including urinary tract infection (UTI), diarrhea, meningitis and pneumonia (Lim *et al*., 2009). There are several reports on resistance of *E. coli* to several antibiotics such as tetracycline, nalidixic acid, cefotaxime, chloramphenicol, gentamicin, ampicillin, kanamycin, trimethoprim/sulfamethoxazole, etc. (Adzitey, 2011; Sukhumungoon *et al*., 2011 and Lim *et al*., 2009). The traditional method of treatment for E. coli infections was a combination of an aminoglycoside and ampicillin but has developed extreme resistance to many drugs (beta lactams, tetracycline, and aminoglycosides) (Enne *et al*., 2001 and Sunde *et al*., 1998). Salmonella is a Gram-negative bacterium capable of causing disease in humans as well as in

domestic animals. Salmonellosis is one of the common foodborne disease caused by Salmonella spp. It is a significant pathogen of food producing animals and these animals are the primary source of salmonellosis (For shell and Wieup, 2006). As Salmonella is an intracellular pathogen, the effective way to eradicate this organism is to use antibiotics that have intracellular activity. The earliest groups of drugs used in the treatment of Salmonella infections were neomycin and colistin. This was followed by the use of absorbable drugs such as ampicillin, amoxicillin, chloramphenicol, tetracycline, and co-trimoxazole, which unfortunately do not have substantial intracellular activity. But strains of Salmonella resistant to several antimicrobial agents have been reported worldwide (Angulo and Griffin, 2000 and Breuil *et al*., 2000). Several studies have reported the prevalence of multi resistance genes in different serotypes of Salmonella (Aarts *et al*., 2001). Among the members of the genus Vibrio many are pathogenic to humans and are implicated in foodborne diseases (Tavakoli *et al*., 2012).

Ibrahim *et al*. (2021) reported that *Salmonella* species (spp.) and *Escherichia coli* (*E. coli*) are the most common infectious pathogens in poultry. Antimicrobials are given either as growth promoters or as treatment, thereby increasing the possibility of the emergence of antimicrobial resistance (AMR), the prevalence of AMR for both pathogens isolated were determined from broiler farms. A total of 384 cloacal swabs were collected, followed by bacterial isolation, confirmation, and antimicrobial susceptibility tests. The overall prevalence of *Salmonella* spp. and *E. coli* were 6.5% and 51.8%, respectively. *Salmonella* spp. and *E. coli* displayed resistance towards the following antimicrobials: erythromycin (100% for both pathogens), chloramphenicol (76.2% and 84.5%, respectively), tetracycline (62% and 94.6%, respectively), ampicillin (47.7% and 87%, respectively), sulfamethoxazole/trimethoprim (42.9% and 83.3%, respectively), ciprofloxacin (4.8% and 23.8%, respectively), nalidixic acid (9.6% and 60.7%, respectively), streptomycin (19% and 66%, respectively), kanamycin (28.6% and 57%,

respectively), cephalothin (0% and 11%, respectively), and gentamicin (0% and 20.2%, respectively). Multidrug resistance (MDR) was recorded in 82% of *Salmonella* spp. and 100% of *E. coli*. These findings could be attributed to the excessive use of antimicrobial agents by poultry farm owners. Enhanced control measures and a strong monitoring system should be urgently implemented in order to reduce the emergence of antimicrobial resistance.

Ngai *et al*. (2021) reported that contaminated poultry feeds can be a major source of *E. coli* and *Salmonella* infections in poultry. This study aimed at determining microbial load, prevalence and antimicrobial resistance profiles of *Salmonella* spp. and *E. coli* and associated resistance genes among isolates from poultry feeds. A total of 150 samples of different poultry feed types were randomly collected from selected sites within Rurik Sub-County. The microbial load was determined, *Salmonella* spp. and Escherichia coli were isolated and antimicrobial susceptibility test carried out. Antimicrobial resistance genes were also screened among the resistant isolates. Out of analyzed samples, 58% and 28% contained *Escherichia coli* and Salmonella spp. respectively. Bacterial load ranged between 3.1×105 and 3.0×106 cfu/g. Highest resistance was against ampicillin (41%) for *Salmonella* spp. and (62%) for *E. coli* isolates. Ampicillin resistant isolates carried TEM and SHV genes. In addition, strB and Dfr resistance genes associated with streptomycin and co-trimoxazole were detected. All the isolates were susceptible to chloramphenicol and ciprofloxacin. The study reveals high bacterial contamination, presence of beta-lactamase, aminoglycoside and sulphonamide resistance genes across isolates from poultry feeds. Therefore, contaminated poultry feeds with bacteria are likely to lead to increase in antimicrobial resistant strains across the community.

Phiri *et al*. (2020) conducted a study aimed to determine the occurrence of antibiotic-resistant *Salmonella* spp. and *E. coli* in broiler chickens at farm level, abattoirs, and open markets. A cross-sectional study was undertaken to determine

the resistance profiles of *Salmonella* spp. and *E. coli* obtained from broiler chickens at farms, abattoirs, and open markets. A total of 470 samples were collected which include; litter, cloacal swabs, and carcass swabs. Samples were inoculated into buffered peptone water and incubated for 24 hours then sub-cultured onto MacConkey and Xylose Lysine Deoxycholate agar plates. Identification of *Salmonella* spp. and *E. coli* was done using the API-20E kit and confirmation by 16S rDNA sequencing. Confirmed isolates were tested against a panel of 09 antibiotics using the Kirby-Bauer disc diffusion method and interpreted according to the Clinical Laboratory Standards Institute guidelines. Data analysis of the antibiotic sensitivity test results was done using WHONET 2018 software. Overall, 4 *Salmonella* spp. and 280 *E. coli* were isolated. One of the *Salmonella* spp. was resistant to ampicillin (25%), amoxicillin/clavulanic acid (25%), and cefotaxime (25%). *E. coli* antibiotic resistance was highest to tetracycline (81.4%) and 100% susceptibility to impanel. The antibiotic susceptibility profile revealed 75.7% (237/280) multidrug-resistant (MDR). The highest MDR profile was observed in 8.2% (23/280) isolates in which 6 out of the 9 classes of antibiotics tested were resistant. Out of the 280 isolates, 11.4% (32/280) exhibited Extensive Drug resistance (XDR). Conclusion: The study found antimicrobial resistance to E. coli and Salmonella spp. in market-ready broiler chickens which were resistant to important antibiotics and is of public health concern.

Rahman *et al*. (2017) conducted this study to investigate the prevalence of *E. coli* in milk, chicken meat and beef and to determine the multi-drug resistance profile of *E. coli* in Mymensingh district, Bangladesh. A total of 169 samples including milk (n=108), chicken meat (n=51) and beef (n=10) were collected from Bangladesh Agricultural University (BAU) dairy farm, American dairy farm, Gazipur and retail markets of municipal area during July 2016 to June 2017. *E. coli* were isolated and identified by colony characteristics on selective agar like

Eosine-methylene blue (EMB) agar, Salmonella-Shigella (SS) agar, Gram staining, biochemical test and Polymerase Chain Reaction (PCR). The overall prevalence of E. coli in all food samples was 37.86%. A total of 32 (29.63%) milk, 25 (49.02%) chicken meat and 07 (70%) beef samples were E. coli positive through conventional method. Among 64 samples only 23 samples (35.94%) were confirmed by PCR. Multi-drug resistant *E. coli* were detected by disc diffusion test using 10 commonly used antibiotics. Antibiogram study showed that *E. coli* isolated from chicken meat were resistant to oxytetracycline (92%), sulphonamide-trimethoprim (84%), amoxycillin (76%) and erythromycin (60%). *E. coli* isolated from beef sample were resistant to erythromycin (85.71%) and oxytetracycline (71.43%) and sensitive to ciprofloxacin (100%), gentamicin (100%) and neomycin (100%). However, all isolates of E. coli were found sensitive to amikacin (100%). E. coli isolated from milk sample were 100% sensitive to gentamicin followed by neomycin, ciprofloxacin, azithromycin, oxytetracycline and erythromycin. Overall 50% of *E. coli* isolates of food were found multi-drug resistant. About 28.13%, 57.14% and 76% of the E. coli isolates originated from milk, beef and chicken meat respectively were multi-drug resistant. The higher prevalence of E. coli in chicken meat, beef and milk indicates unhygienic production and processing of these foods. Presence of multi-drug resistant *E. coli* in these foods might pose serious public health threats. The antibiogram profile of the isolates will help therapeutic decision making in the treatment of colibacillosis in cattle and poultry in Bangladesh.

Khanal *et al*. (2017) conducted a study with the objective to determine antibiotic resistance in commercial poultry of Nepal; this study was designed taking *Escherichia coli* as a flagship bacterium. The commercial layers and broilers birds brought to veterinary teaching hospital of Agriculture and Forestry University by commercial poultry producers for disease diagnosis and treatment were considered as clinical examination of birds were carried out followed by post mortem

examination (PME). Those layer/broiler birds which were not taking antibiotic orally or parenterally for last 2 weeks and diagnosed with collibacillosis on PME were included in sampling frame. *Air sacculitis*, *fibrinous pericarditis*, *fibrinous perihepatitis*, and *coligranuloma* were major criteria for presumptive diagnosis of colibacillosis on PME. The first 40 for both broiler and layer birds totaling 80 that fulfilled the criteria were selected as samples, each representing a commercial farm. All necessary information on daily management practices and previous treatments were obtained from farmer's record book or sheets or face to face interview. Avian pathogenic *E. coli* was isolated from aseptically collected liver samples and confirmed by biochemical tests. Antibiogram of the isolates were investigated by means of Kirby-Bauer disc diffusion method. *E. coli* was isolated from all liver samples taken for the culture. It was found that *E. coli* were resistant most substantially towards Cephalexin (81.2%) and Amoxycillin (81.2%) followed by Tetracycline (78.8%), Colistin sulphate (n=50, 62.5%). Chloramphenicol (61.2%), Ciprofloxacin (55.0%), Enrofloxacin (53.8%), Levofloxacin (28.8%), however, no resistance was found against amikacin. The proportion of E. coli isolates that were resistance against Colistin sulphate, Tetracycline, Ciprofloxacin, Enrofloxacin and Gentamicin were significantly higher in layers compared to that of broilers. In conclusion, avian pathogenic E. coli were resistant towards several antibiotic molecules commonly used in commercial poultry of Nepal, and the resistance was higher in layers compared to broilers.

Akinbowale *et al.,* (2006) reported that bacteria from the aquatic sources and environment were found resistant to different types of antibiotics to a great extent, even significant level of multi-drug resistance also observed. This indicating the highest possibility of transfer of resistance gene from aquaculture isolates to human pathogens, some assessment of risk of transfer of resistant organisms to

humans via the food chain and the threats imposed by environmental contamination with antibiotic resistant bacteria.

Barton, (2004) reported that *E coli* strains showed widespread resistance to tetracycline and moderately common resistance (30-60%) to ampicillin and sulphadiazine. Resistances to more than one antibiotic were common. Barton also reported in 2000 that the development of antibiotic resistance in bacteria has been linked to the use of antibiotics in agriculture in overseas studies, particularly for intensively housed species such as pigs, poultry and feedlot cattle.

Biswas *et al.,* (2001) reported that 100% of his poultry *E. coli* isolates were resistant to tetracycline but 72% isolates were found to susceptible to Gentamycin but 20% were found resistant to Gentamycin.

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

Jesus *et al.*, (1997) indicated increasing incidences of antibiotic-resistant *E. coli* strains isolated from chickens with Colibacillosis. However, the high percentage of *E. coli* strains that were resistant to Trimethoprim-sulfamethoxazole (67%) and to the new fluoroquinolones (13 to 24%) in our study was surprising. Ellen K. Silber geld in2007 reported that occupational exposure to antimicrobial-resistant *E. coli* from live animal contact in the broiler chicken industry might be an important route of entry for antimicrobial-resistant *E. coli* into the community.

Germon *et al.,* (2005) reported that the ideal gene, which encodes a virulence factor of *E. coli* strains that can cause neonatal meningitis in humans were recently detected in avian pathogenic *E. coli* (APEC). Caya *et al.,* (1999) reported that the Virulence determinants common to both APEC and human isolates Expect (extra intestinal pathogenic *E. coli*) were previously identified, leading to the conclusion that APEC are potential human pathogens.

Rahman *et al.,* (2009) reported that 150 *Salmonella* isolates were 100% sensitive to Gentamycin followed by Amoxicillin (90%), Colistin (70%), Co-trimoxazole (60%) and Furazolidone (40%) but the isolates were highly resistant to Norfloxacin, Flumequine, Ciprofloxacin and Enrofloxacin. The study demonstrated that the *Salmonella gallinarum* were more sensitive to Gentamycin than Amoxicillin or Colistin.

Molla *et al.,* (2003) reported that fifty-one (63.7%) of the 80 *Salmonella* strains were resistant to one or more antimicrobials of which 42 (52.5%) displayed multiple-drug resistance. Among the strains, 51.2% were resistant to sulfisoxazole, 46.2% to spectinomycin, 45% to amoxycillin-clavulanic acid and ampicillin, 41.2% to tetracycline and 30% to chloramphenicol. Less than 27.5% of the strains showed resistance to florfenicol, streptomycin, co-trimoxazole and to trimethoprim. *S. typhimurium* var. Copenhagen (100%), *S. anatum* (62.5%), *S. typhimurium* (33.3%) and *S. braenderup* (34.3%) showed multiple antimicrobial resistance to up to eight antimicrobials. None of the strains were resistant to amikacin, apramycin, gentamicin, kanamycin, neomycin, tobramycin, quinolones, cephalosporins and nitrofurantoin. They also indicated the potential importance of chickens as source of multiple antimicrobial-resistant *Salmonella* for human infections.

Humphrey, (2000) mentioned that a wide range of food borne illness attributable to *Salmonella enterica*. Poppe, (2000)mentioned that Poultry is widely acknowledged to be a reservoir for *Salmonella* infections in humans due to the

ability of *Salmonella* to proliferate in gastrointestinal tract of Chicken and subsequently survive on commercially processed Broiler carcasses and edible giblets.

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

Gregova *et al.,* (2012) investigate the antibiotic resistant *E. coli* strains isolated from bioaerosols and surface swabs in a slaughterhouse as a possible source of poultry meat contamination. The highest air coliforms contamination was during shackling, killing and evisceration of poultry. The strains showed resistance to ampicillin (89%), ceftiofur (62%) and cefquinome (22%), while resistance to ampicillin with sulbactam was only 6%. Resistance to streptomycin and gentamicin was detected in 43% *vs.* 14% isolates; to tetracycline 33%; to chloramphenicol and florfenicol in 10% vs. 18% isolates; to co-trimoxazole in 35% isolates; to enrofloxacin in 43 % isolates.

Hemen *et al.,* (2012) conducted a study aimed at isolating and identifying *Shigella*, *Salmonella* and *Escherichia coli* bacteria associated with poultry litter. The antibiotic sensitivity patterns of the isolated bacteria tested against Septrin,

Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxicillin, Augmentin, Gentamycin, Pefloxacin, Triviid and Streptomycin. *Shigella* and *Salmonella* were completely resistant to chloramphenicol, Augmentin, pefloxacin, amoxicillin. *Shigella* was also resistant to all the antibiotics except Septrin and ciprofloxacin showed they are intermediate to the drugs. Percentage antibiotics susceptibility pattern of gram-negative bacteria isolated from poultry litter showed all bacterial isolates (100%) were resistant to Chloramphenicol while most of the isolates were susceptible to Amoxicillin. All the bacterial isolates showed high level (10.2 MAR index) antibiotic resistance.

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

Begum *et al.,* (2010) mentioned 100% isolated strains *of Salmonella* were found to be sensitive Ceftriaxone, Ciprofloxacin, Cephalexin, Gentamycin and Chloramphenicol. On the other hand, strains have shown resistance to Co-trimoxazole, Nalidixic acid, Ampicillin, Tetracycline and Kanamycin. However, it was found that strains isolated from intestinal and environmental were more antibiotic resistant than egg isolated.

Hayes *et al.,* (2004) mentioned that his prevalence of resistance among isolates of *E. faecalis was* comparatively higher among glycosamide, macrolide, and tetracycline anti-microbial, while isolates of *E. faecium* were observed to be more frequently resistant to fluoroquinolones and penicillin. Notably, 63% of the *E. faecium* isolates were resistant to the streptogramin quintuplicating-dalfopris-tin, while high-level gentamicin resistance was observed only among the *E. faecalis* population, of which 7% of the isolates were resistant.

Islam *et al.,* (2008) stated that50% isolates of *S. typhi* and 83.33% isolates of *S. Para typhi A* were multidrug resistant. All of the isolates of *S. typhi* were sensitive

(100%) to Aztreonam Amikacin and Gentamycin and all of the isolates of *S. Para typhi A* were sensitive (100%) to Aztreonam, Amikacin, Cefaclor, Cefixime, Ceftazidime, Ceftriaxone, Gentamycin, Mecillinam. All of the isolates of *Salmonella typhi* and *Salmonella Para typhi A* were resistant to Nalidixic acid (100%). In addition, isolates of *S. Para typhi A* were also resistant to Azithromycin, Netilmicin. Decreased susceptibility of *S. typhi*and *S. Para typhi A* was observed in case of ciprofloxacin 73.33% and 70% respectively.

CHAPTER III

MATERIALS AND METHODS

3.1 Methods

3.1.1 Experimental design

The entire study was divided into two major steps: The first step included selection of sources, collection of samples, isolation, identification and characterization of microorganisms on the basis of their colony morphology, staining properties, motility and biochemical characteristics & molecular identification. In the second step, the current status of drug sensitivity and resistance pattern of a total of 150 isolates of microorganism isolated from broilers was determined.

3.2 Study area and duration

The study was conducted on dead broiler at Dhaka city in Bangladesh which is one of the most concentrated poultry areas of Bangladesh, during the period of July to December, 2020.

3.3 Diagnosis of disease

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

3.4 Study population

A total of 150 dead samples from different local market of Dhaka city were subjected to postmortem during the study period at lab of Medicine & Public Health, SAU, Dhaka.

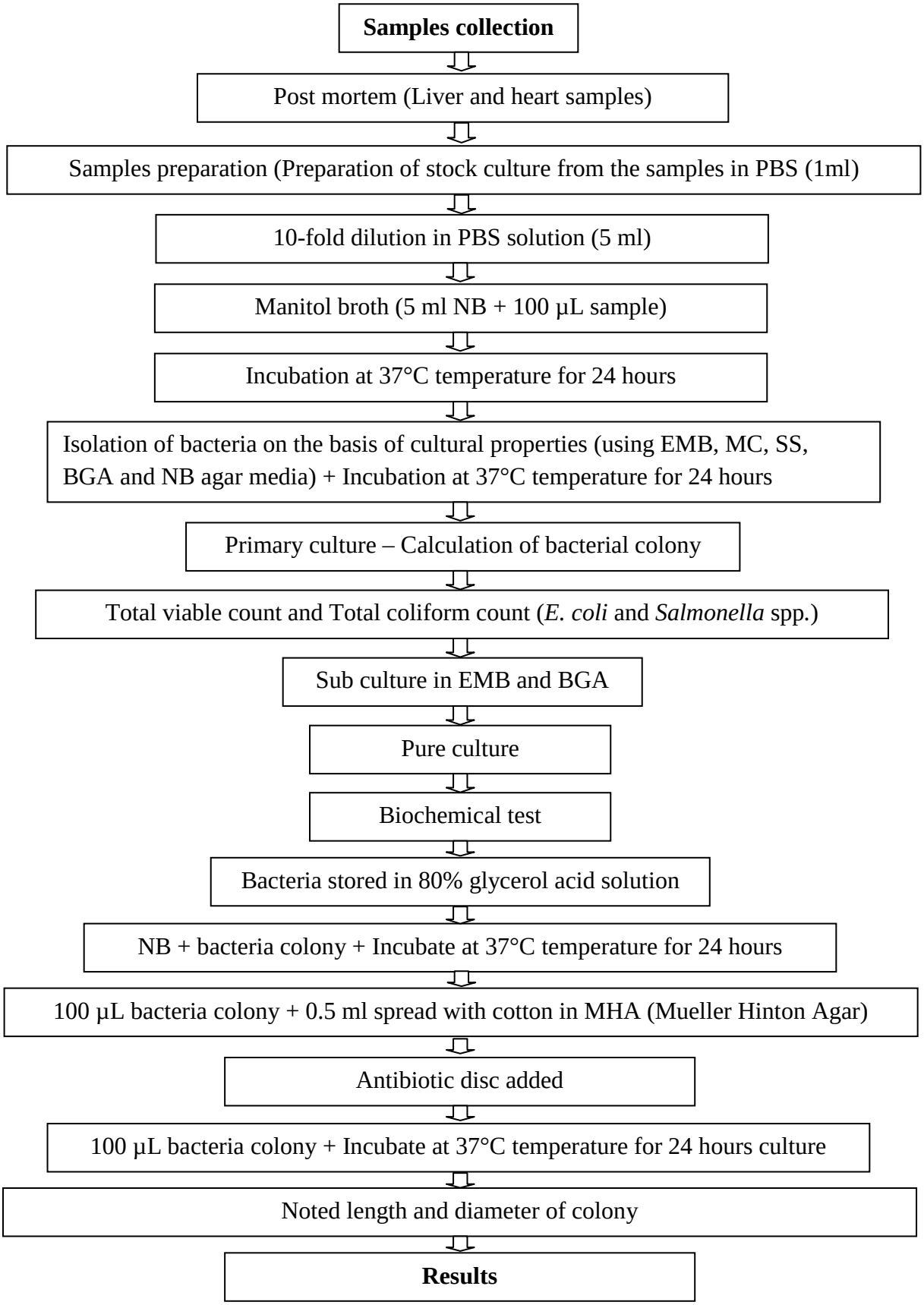

Fig.1. Layout of the experimental design

3.5 Samples Collection

A total number of 150 liver & heart samples were collected (Table 1) from totally different local market of Dhaka city, Bangladesh.

Table 1. Sampling sites and number

SL. No	Name of the places of sample collection in Dhaka district, Bangladesh	Total dead broiler	No. of samples		No. of total samples
			Liver	Heart	
1	Kawran Bazar	5	5	5	10
2	Shyamoli	5	5	5	10
3	Adabor	5	5	5	10
4	Mohammadpur Town Hall	5	5	5	10
5	Dhanmondi	5	5	5	10
6	Agargaon	5	5	5	10
7	Mirpur-1	5	5	5	10
8	Mirpur-10	5	5	5	10
9	Gabtali	5	5	5	10
10	Jatrabari	5	5	5	10
11	Sadarghat	5	5	5	10
12	Hemaetpur	5	5	5	10
13	Amin Bazar	5	5	5	10
14	Savar	5	5	5	10
15	Ashulia	5	5	5	10
Total		75	75	75	150

3.6 Samples preservation

The collected samples were transferred to the Medicine and Public Health Laboratory, SAU via cool-chain maintaining in cool box and stored at -20°C temperature until working (Lab work).

3.7 Post-mortem examination

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

3.8 Samples collection for microbiological test

The liver and heart samples were collected aseptically and used for microbiological test.

3.9 Isolation and identification of collected samples

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

3.10 Bacteriological media

3.10.1 Agar media

Agar media used for bacteriological analysis were Eosin Methylene Blue (EMB) agar, MacConkey (MC) agar, Brilliant Green (BG) agar, Salmonella shigella (SS) agar, Nutrient agar and Mueller Hinton (MH) agar.

3.10.2 Liquid media (broth)

The liquid media used for this study were Nutrient broth, Peptone broth, Methyl-Red and Voges-Proskauer broth (MR-VP broth) and Sugar media (dextrose, maltose, lactose, sucrose and mannitol).

3.10.3 Phosphate Buffered Saline (PBS)

For preparation of phosphate buffered saline, 8 gm. of sodium chloride (NaCl), 2.89 gm. of disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$), 0.2 gm. of potassium chloride (KCl) and 0.2 gm. of potassium hydrogen phosphate (KH_2PO_4) were suspended in 1000 ml of distilled water. The solution was heated to dissolve completely and pH was adjusted with the help of pH meter. The solution was then sterilized by autoclaving and stored at 4^{o}C for future use.

3.11 Chemicals and reagents

The chemicals and reagents used for this study were 0.1% Peptone water, Phosphate buffered saline (PBS), reagents for Gram's staining (Crystal Violate, Gram's iodine, Safranin, Acetone alcohol), 3% Hydrogen peroxide, Phenol red, Methyl red, 10% Potassium hydroxide, Kovac'sindole reagent (4-dimethylamino-benzaldehyde, concentrated HCl), Mineral oil, Normal saline and other common laboratory chemicals and reagents.

3.12 Glass wares and other appliances

The following glass wares and appliances were used during the course of the experiment. Test tubes (with or without Durham's fermentation tube and stopper), petridishes, conical flask, pipette (1 ml, 2 ml, 5 ml, 10 ml) & micro-pipettes (1ml, 200µl, 100µl, 10 µl) slides and cover slips, hanging drop slides, immersion oil, compound microscope, bacteriological loop, sterilized cotton, cotton plug, test tube stand, water bath, bacteriological incubator, refrigerator, sterilizing instruments, thermometer, ice carrier, hand gloves, spirit lamp, match lighter, laminar air flow, hot air oven, syringe, needle, tray, forceps, scalpel, scissors etc.

3.13 Antimicrobial discs

Commercially available antimicrobial discs (OXOID Limited, Canada) were used for the test to determine the drug sensitivity and resistance pattern and to interpret their disease potential. This method allowed for the rapid detection of the efficacy of drugs against the test organisms by measuring the diameter of the zone of inhibition that resulted from diffusion of the agent into the medium surrounding the discs inhibiting the growth of the organisms. The following antimicrobial agents with their disc concentration were used to test the sensitivity and resistance pattern of the selected *E. coli* and *Salmonella spp*. isolates from liver and heart of broiler.

Table 2. Drugs with their disc concentration for the Enterobacteriaceae family (Source: CLSI= The Clinical and Laboratory Standards Institute, 2020)

Antibiotics	Disc concentration (µg /disc)	Zone Diameter Interpretive Standard (mm)		
		Resistance (%)	Intermediate (%)	Sensitive (%)
GEN-10	10	≤12	13-14	≥15
S-10	10	≤11	12-14	≥15
TE-30	30	≤11	12-14	≥15
CXM-30	30	≤14	15-17	≥18
CFM-5	5	≤14	15-17	≥18
CTR-30	30	≤19	20-22	≥23
CL-10	10	≤11	12-14	≥15
AMX-30	30	≤13	14-17	≥18
AMP-25	25	≤13	14-16	≥17
COT-25	25	≤10	11-15	≥16

GEN-10 = Gentamicin, S-10 = Streptomycin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

3.14 Preparation of various bacteriological culture media

3.14.1 Nutrient Broth

Nutrient Broth was prepared by Suspended 25 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Sterilized by autoclaving at 15 lbs. pressure (121°C) for 30 minutes. The broth was filled in test tubes & incubated at 37ºC for overnight to check their sterility and stored at 4ºC in the refrigerator until used.

3.14.2 Nutrient Agar

Nutrient agar was prepared by dissolving 28 grams of dehydrated nutrient agar (Hi Media, India) in to 1000 ml of distilled water and was sterilized by autoclaving at 121ºC under 15 lbs. pressure per square inch for 15 minutes. Then the agar was

dispensed into Petridis (90 mm and 100 mm) and was incubated at 37ºC for overnight to check their sterility and stored at 4ºC in the refrigerator until used.

3.14.3 MacConkey's agar

A quantity of 49.53 grams of Bacto MacConkey agar (Hi Media, India) was suspended in to 1000 ml of cold distilled water and was heated for boiling to dissolve the medium completely. It was then poured in to sterile petridishes and allowed to solidify. After solidification of the medium in the plates, the plates were then incubated at 37ºC for overnight to check their sterility.

3.14.4 Eosin Methylene Blue (EMB) agar

A quantity of 36 grams powder of EMB agar base (HI Media, India) was suspended in 1000 ml of distilled water. The suspension was heated to boil for few minutes to dissolve the powder completely with water. The medium was autoclaved for 30 minutes to make it sterile. After autoclaving the medium was put in to water bath at 45ºC to cool down its temperature at 40ºC. From water bath 10-20 ml of medium was poured in to small and medium sized sterile petridishes to make EMB agar plates. After solidification of the medium in the plates, the plates were incubated at 37ºC for overnight to check their sterility.

3.14.5 Brilliant Green (BG) agar

According to the direction of manufacturer (HI Media, India) 58 grams of dehydrated medium was suspended in 1000 ml distilled water and heated for boiling to dissolve the medium completely. The medium was sterilized by autoclaving. After autoclaving the medium was put in to water bath of 45ºC to decrease its temperature. After solidification of the medium in the petridishes, the petridishes were allowed for incubation at 37ºC for overnight to check their sterility and then stored at 4ºC in a refrigerator for future use.

3.14.6 Salmonella-Shigella (SS) agar

According to the direction of manufacturer (HI Media, India) 60 grams of dehydrated medium was suspended in 1000 ml distilled water and heated for boiling to dissolve the medium completely. After heating the medium was put in to water bath of 50ºC to decrease its temperature. After solidification of the medium in the petridishes, the petridishes were allowed for incubation at 37ºC for overnight to check their sterility and then stored at 4ºC in a refrigerator for future use.

3.14.7 Mueller Hinton Agar

A quantity of 38.0 grams in 1000 ml distilled water &heated to boiling to dissolve the medium completely. After the sterilization by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. Cooling was done to 45-50°C.Then it was mixed well and poured into sterile Petridishes. After solidification of the medium in the petridishes, the petridishes were allowed for incubation at 37ºC for overnight to check their sterility and then stored at 4ºC in a refrigerator for future use.

3.14.8 Triple Sugar Iron (TSI) agar

A quantity of 65 grams of dehydrated medium (Difco, USA) was mixed with 1000 ml cold distilled water in a flask and heated for boiling to dissolve the medium completely. The solution was distributed in tubes which were plagued with cotton. The tubes were then sterilized by autoclaving and slanted in such a manner as to allow a generous butt. After solidification tubes were incubated at 37ºC for overnight to check sterility. The sugar solutions were sterilized in Arnold's steam sterilizer at 100ºC for 30 minutes for three consecutive days. An amount of 0.5 ml of sterile sugar solution was added aseptically in each culture tubes containing sterile peptone water. The sugar solutions were incubated at 37ºC for 24 hours to check sterility. These solutions were used for biochemical test.

3.14.9 Methyl Red and Voges–Proskauer (MR-VP) broth

A quantity of 3.4 gm. of MR-VP medium (HI Media, India) was dissolved in 250 ml of distilled water, distributed in 2 ml quantities in test tube and then autoclaved. After autoclaving, the tubes containing medium were incubated at 37ºC for overnight to check their sterility and then stored at 4ºC for future use.

3.15 Isolation of bacteria

3.15.1 Collection, transportation and preparation of sample

3.15.1.1 Liver

A number of 75 samples of liver were collected through using sterile stick in 2 ml Eppendorf tube filled with PBS from the broiler from Sher-e-Bangla Agricultural University campus. The collected samples were immediately carried to the laboratory in an ice box containing ice and processed for isolation and characterization of bacteria.

3.15.1.2 Heart

A number of 75 samples of heart were collected through using sterile stick in 2 ml Eppendorf tube filled with PBS from the broiler from Sher-e-Bangla Agricultural University campus. The collected samples were immediately carried to the laboratory in an ice box containing ice and processed for isolation and characterization of bacteria.

3.15.2 Serial dilution for bacterial culture (10-fold dilution method)

Serial dilution of the stock sample was done to lowering the bacterial count for the total viable count (TVC) and total coliform count (TCC). It was done by taking 8 (1-8) Eppendorf tube filled with 900µl of PBS. 100µl of stock sample was transferred from the stock tube (2ml) to the Eppendorf tube next to the stock tube. Then 100µl of diluted sample is transferred from the first Eppendorf tube to the

next. Successive dilution should be made in the same way to the last tube and from the last tube 100µl of diluted sample should be discarded. From the last tube 25µl of liquid sample should be transferred to the nutrient Agar media and MacConkey agar to elucidate the total viable count & total coliform count. Enumeration of Salmonella was done by transferring same amount of liquid sample in the Salmonella –Shigella agar.

3.15.2.1 Primary culture of microorganism

Primary growth of all kinds of bacteria present in the collected samples was performed in nutrient broth. The samples were inoculated in nutrient broth and incubated for overnight at 37ºCfor the growth of the organisms.

3.15.2.2 Isolation in culture media

After primary culture of the organism, a small amount of inoculums from Nutrient broth was streaked on the MacConkey agar and Brilliant green agar & Salmonella-Shigella agar to observe the colony morphology of the isolates. Characteristic colony morphology of the organisms indicating E. coli was selected for subculture on selective media such as EMB agar and Salmonella on Salmonella-Shigella agar. Morphological characteristics (shape, size, surface texture, edge and elevation, color, opacity etc.) of the suspected colonies on different agar media developed within 18 to 24 hours of incubation were carefully recorded.

3.15.3 Identification of isolated *E. coli* & *Salmonella* spp. by using specific biochemical tests

Several biochemical tests were performed for confirmation of E. coli & Salmonella isolates.

3.15.3.1 Carbohydrate fermentation test

The carbohydrate fermentation test was performed by inoculating 0.2 ml of nutrient broth culture of the isolated organisms into the tubes containing different sugar media (five basic sugars such as dextrose, maltose, lactose, sucrose and mannitol) and incubated for 24 hours at 37ºC. Acid production was indicated by the color change from red to yellow and gas production was noted by the accumulation of gas bubbles in the inverted Durham's tube (Cheesbrough, 2006).

3.15.3.2 Catalase test

For this study 3 ml of catalase reagent (3% H_2O_2) was taken in a test tube. Single colony from the pure culture of E. coli was taken with a glass rod and merged in the reagent. The tube was observed for bubble formation. All of the isolates were catalase positive; formation of bubble within few seconds was the indication of the positive test, while the absence of bubble formation indicated negative result (Cheesbrough, 2006).

3.15.3.3 Methyl Red test

The test was conducted by inoculating single colony from the pure culture of the test organism in 5 ml sterile MR-VP broth. After 5 days incubation at 37ºC, 5 drops of methyl red solution was added and observed for color formation. Development of red color was positive and indicated an acid pH of 4.5-6 resulting from the fermentation of glucose. Development of yellow color indicated negative result (Cheesbrough, 2006).

3.15.3.4 Voges-Proskauer (V-P) test

The test *E. coli* organisms were grown in 3 ml of sterile MR-VP broth at 37ºC for 48 hours. Then 0.6 ml of 5% alpha-naphthol and 0.2 ml of 40% potassium hydroxide containing 0.3% creatine was added per ml of broth culture of the test organism. Then shaking well and allowed to stand for 5-10 minutes to observe the

color formation. Positive case was indicated by the development of a bright orange red color. In negative cases there was no development of pink color (Cheesbrough, 2006).

3.15.3.5 Indole test

The test organisms were cultured in test tubes having 3 ml of peptone water containing tryptophan at 37ºC for 48 hours. Then 1 ml of diethyl ether was added, Shaked well and allowed to stand until the ether rises to the top. Then 0.5 ml of Kovac's reagent was gently run down the side of the test tube so that it forms a ring in between the medium and the ether layer and observed for the development of color of the ring. Development of a brilliant red colored ring indicated indole production. In negative case there is no development of red color (Cheesbrough, 2006).

3.16 Maintenance of stock culture

Stock culture was prepared by adding 1ml of 80% sterilized glycerol in 1 ml of pure culture in nutrient broth and it was stored in -200C.

3.17 Antimicrobial sensitivity pattern of *E. coli* and *Salmonella* spp. isolated from liver and heart samples of broiler

A total of 66 *E. coli* isolates and 51 *Salmonella* spp. Collected from 75 liver and 75 heart samples of dead broiler were used for disc sensitivity testing. The antimicrobial sensitivity testing of each isolate was carried out by the Kirby-Bauer disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) procedures. Antibiotic sensitivity discs used were GEN-10 = Gentamicin, S-10 = Streptomycin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole. This method allowed for the rapid determination of the efficacy of a drug by measuring the

diameter of the zone of inhibition that results from diffusion of the agent in the medium surrounding the disc. The suspension of the test organism was prepared in a test tube containing 5 ml nutrient broth by overnight incubation in shaking incubator. By micropipette 100µl of broth culture of the test organism was poured on Muller-Hinton agar plate. Sterile glass spreader was used to spread the culture homogenously on the medium. Inoculated plates were closed and allowed to dry for approximately 3-5 minutes. Then the antibiotic discs were applied aseptically to the surface of the inoculated agar plates at a special arrangement with the help of a sterile forceps. The plates were then inverted and incubated at 37ºC for 24hours. After incubation the plates were examined and the diameter of the zone of complete inhibition was measured by mm scale. The zone diameters for individual antimicrobial agents were translated in to sensitive, intermediate and resistant categories by referring to an interpretation table.

3.18 Antibiotic Sensitivity analysis

The antibiotic sensitivity of the isolated strain at different concentration was performed by using standard paper disc diffusion method described by NCCLS (2009). Antibiotics selected for susceptibility testing included a panel of antimicrobial agents of interest to the poultry industry and human public health authorities. From the range of antimicrobial drugs, 10 were selected on the basis of their range of activity against enterobacteria and on their use in local poultry farming. Veterinary antibiotics were chosen due to their use as therapeutic, prophylactic or growth promoting agents in poultry industry. Human antibiotics were selected on the basis of their use and /or importance in human medicine.

The following antibiotics and disc potencies were used:

1. GEN-10: Gentamicin
2. S-10: Streptomycin
3. TE-30: Tetracycline
4. CXM-30: Cefuroxime
5. CFM-5: Cefixime
6. CTR-30: Ceftriaxone
7. CL-10:Colistin
8. AMX-30: Amoxycillin
9. AMP-25: Ampicillin
10. COT-25: Co-Trimoxazole

The antibiotic susceptibility tests were performed in Mueller-Hinton agar by micro-disc diffusion techniques. By the standard method of inoculation, the top of a single and well-isolated colony was touched with a sterile loop and the growth was inoculated into 2ml of Mueller-Hinton broth. The broth culture was then allowed to incubate at 37°C for 4 hours to obtain the young culture. The turbidity of actively growing broth cultures was then adjusted to a 0.5 McFarland standard and then a sterile cotton swab was dipped into the adjusted suspension within 15 minutes and excess broth was purged by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then spread evenly over the entire surface of the plate of LB agar to obtain uniform inoculums.

The plates were then allowed to dry for 3-5 minutes. Antibiotics impregnated discs were then applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. Even distribution of discs and minimum distance of 24 mm from center to center were ensured. Five discs were placed in each Petridis. Within 15

minutes of the application of the discs, the plates were inverted and incubated at 37°C. After 16-18 hours of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate and resistant categories according to the interpretation table of the Becton Dickinson Microbiology Company, USA. Measurement of the growth inhibition zone permitted the classification of each isolate as susceptible, intermediate and resistant according to data provided by CLCI. The result of antibiotic sensitivity test was then recorded, analyzed and discussed.

CHAPTER IV

RESULTS AND DISCUSSION

The study was conducted for clinical investigation of common bacterial causes of death of broiler in small holder farming system in Bangladesh. The results presented below demonstrated the isolation and identification of bacteria isolates from liver and heart samples of broiler from different farm and households around Dhaka district. Sensitivity and resistance pattern of the isolates to different drugs are also shown in results of the study with the help of table and graphs and possible interpretations given under the following headings:

4.1 Total viable count and coliform count from the isolated samples

There were 150 broiler samples investigated for identification of *E. coli* and *Salmonella* in different local market of Dhaka district (Table 1). Total viable count and total coliform count from the isolated sample of broiler are presented in Table 3. Among the 150broiler samples, 24 samples (16%) showed no viable and coliform count. Sample viable and coliform count done in the Laboratory is presented in Fig. 2.

Table 3. Total viable count and total coliform count from the isolated sample of broiler

Sl. No.	Name of sample	Total viable count (TVC) (CFU/ml)	Total coliform count (TCC) (CFU/ml)
1	B1	8.4×10^{7}	5.2×10^{6}
2	B2	2.9×10^{8}	1.9×10^{9}
3	B3	1.3×10^{7}	1.9×10^{6}
4	B4	2.2×10^{6}	1.7×10^{8}
5	B5	1.4×10^{8}	6.8×10^{7}
6	B6	3.4×109	Nil
7	B7	1.4×10^{8}	5.2×10^{8}
8	B8	4.1×10^{7}	1.4×10^{6}
9	B9	2.1×10^{8}	4.1×10^{9}
10	B10	2.7×10^{8}	1.4×10^{5}

Sl. No.	Name of sample	Total viable count (TVC) (CFU/ml)	Total coliform count (TCC) (CFU/ml)
11	B11	7.4×10^{7}	2.2×10^{5}
12	B12	2.9×10^{8}	1.9×10^{6}
13	B13	3.4×10^{9}	Nil
14	B14	2.2×10^{6}	1.7×10^{8}
15	B15	1.4×10^{7}	6.1×10^{6}
16	B16	3.4×10^{9}	1.4×10^{6}
17	B17	1.4×10^{8}	5.1×10^{8}
18	B18	4.1×10^{7}	1.4×10^{6}
19	B19	3.4×10^{9}	Nil
20	B20	2.7×10^{6}	1.4×10^{8}
21	B21	4.4×10^{7}	4.2×10^{6}
22	B22	2.9×10^{8}	1.9×10^{5}
23	B23	1.3×10^{7}	5.6×10^{4}
24	B24	2.2×10^{6}	1.9×10^{6}
25	B25	1.4×10^{5}	3.9×10^{7}
26	B26	3.4×10^{7}	1.7×10^{7}
27	B27	3.4×10^{9}	Nil
28	B28	4.1×10^{7}	5.2×10^{4}
29	B29	2.1×10^{8}	1.9×10^{6}
30	B30	2.7×10^{5}	1.9×10^{4}
31	B31	3.4×10^{9}	Nil
32	B32	2.9×10^{7}	6.2×10^{6}
33	B33	6.3×10^{6}	1.4×10^{8}
34	B34	2.2×10^{6}	6.2×10^{5}
35	B35	1.4×10^{7}	1.4×10^{7}
36	B36	3.4×10^{8}	2.1×10^{5}
37	B37	3.4×10^{9}	Nil
38	B38	4.1×10^{5}	1.9×10^{6}
39	B39	3.1×10^{7}	8.1×10^{6}
40	B40	2.7×10^{7}	1.7×10^{5}
41	B41	5.4×10^{8}	3.8×10^{7}
42	B42	2.9×10^{7}	1.4×10^{7}
43	B43	3.4×10^{9}	Nil
44	B44	2.2×10^{8}	1.4×10^{5}
45	B45	1.4×10^{5}	2.1×10^{3}
46	B46	3.4×10^{7}	1.4×10^{5}
47	B47	1.4×10^{8}	5.2×10^{7}
48	B48	4.1×10^{9}	1.9×10^{5}
49	B49	2.1×10^{9}	5.9×10^{9}

Sl. No.	Name of sample	Total viable count (TVC) (CFU/ml)	Total coliform count (TCC) (CFU/ml)
50	B50	3.7×10^{7}	1.7×10^{6}
51	B51	4.4×10^{9}	2.8×10^{5}
52	B52	3.4×10^{9}	Nil
53	B53	1.3×10^{6}	5.2×10^{7}
54	B54	5.2×10^{7}	4.2×10^{5}
55	B55	1.4×10^{7}	1.9×10^{7}
56	B56	3.4×10^{9}	Nil
57	B57	1.4×10^{8}	1.7×10^{7}
58	B58	4.1×10^{8}	6.8×10^{7}
59	B59	2.1×10^{8}	1.4×10^{8}
60	B60	2.7×10^{9}	4.2×10^{4}
61	B61	5.4×10^{8}	1.4×10^{7}
62	B62	3.4×10^{9}	Nil
63	B63	1.3×10^{8}	1.4×10^{7}
64	B64	2.2×10^{9}	5.2×10^{5}
65	B65	1.4×10^{9}	1.9×10^{8}
66	B66	2.4×10^{9}	1.9×10^{5}
67	B67	3.4×10^{9}	Nil
68	B68	4.1×10^{9}	3.8×10^{8}
69	B69	2.1×10^{7}	1.4×10^{5}
70	B70	2.7×10^{7}	5.2×10^{8}
71	B71	3.4×10^{9}	Nil
72	B72	2.9×10^{9}	1.1×10^{5}
73	B73	1.3×10^{9}	3.2×10^{7}
74	B74	3.4×10^{9}	Nil
75	B75	1.4×10^{7}	5.9×10^{8}
76	B76	3.4×10^{9}	1.7×10^{5}
77	B77	1.4×10^{9}	3.8×10^{8}
78	B78	4.1×10^{9}	1.4×10^{7}
79	B79	3.4×10^{9}	Nil
80	B80	2.7×10^{9}	1.4×10^{7}
81	B81	6.4×10^{7}	1.1×10^{8}
82	B82	2.9×10^{7}	5.2×10^{6}
83	B83	4.3×10^{9}	1.9×10^{8}
84	B84	2.2×10^{7}	7.9×10^{6}
85	B85	1.4×10^{8}	1.7×10^{5}
86	B86	3.4×10^{9}	Nil
87	B87	1.4×10^{9}	1.4×10^{4}
88	B88	4.1×10^{8}	5.2×10^{7}
89	B89	2.1×10^{8}	1.4×10^{5}

Sl. No.	Name of sample	Total viable count (TVC) (CFU/ml)	Total coliform count (TCC) (CFU/ml)
90	B90	3.7×10^{8}	2.1×10^{5}
91	B91	3.4×10^{9}	Nil
92	B92	2.9×10^{9}	5.2×10^{7}
93	B93	1.3×10^{9}	1.9×10^{9}
94	B94	2.2×10^{6}	2.9×10^{5}
95	B95	3.4×10^{9}	Nil
96	B96	3.4×10^{7}	6.8×10^{7}
97	B97	1.4×10^{6}	1.4×10^{8}
98	B98	4.1×10^{9}	5.2×10^{6}
99	B99	2.1×10^{7}	1.4×10^{8}
100	B100	3.4×10^{9}	Nil
101	B101	5.4×10^{9}	1.4×10^{6}
102	B102	2.9×10^{9}	3.2×10^{6}
103	B103	1.3×10^{7}	1.9×10^{8}
104	B104	2.2×10^{8}	5.9×10^{6}
105	B105	3.4×10^{9}	Nil
106	B106	7.4×10^{7}	6.8×10^{8}
107	B107	2.9×10^{8}	1.4×10^{6}
108	B108	3.4×10^{9}	Nil
109	B109	2.2×10^{8}	1.4×10^{8}
110	B110	1.4×10^{8}	3.1×10^{6}
111	B111	3.4×10^{8}	1.4×10^{6}
112	B112	1.4×10^{6}	5.2×10^{6}
113	B113	4.1×10^{7}	1.9×10^{8}
114	B114	2.1×10^{8}	6.9×10^{8}
115	B115	3.4×10^{9}	Nil
116	B116	4.4×10^{6}	1.8×10^{6}
117	B117	3.4×10^{9}	1.4×10^{8}
118	B118	2.9×10^{9}	5.2×10^{5}
119	B119	1.3×10^{9}	1.4×10^{4}
120	B120	8.1×10^{7}	4.1×10^{6}
121	B121	2.9×10^{9}	1.4×10^{7}
122	B122	1.3×10^{8}	3.2×10^{4}
123	B123	2.2×10^{7}	1.9×10^{8}
124	B124	3.4×10^{9}	Nil
125	B125	3.4×10^{9}	1.7×10^{6}
126	B126	1.4×10^{7}	6.8×10^{6}
127	B127	4.1×10^{9}	1.4×10^{5}
128	B128	7.1×10^{7}	7.2×10^{6}

Sl. No.	Name of sample	Total viable count (TVC) (CFU/ml)	Total coliform count (TCC) (CFU/ml)
129	B129	2.7×10^{7}	1.4×10^{7}
130	B130	8.4×10^{8}	1.1×10^{3}
131	B131	2.9×10^{8}	1.4×10^{5}
132	B132	3.4×10^{9}	Nil
133	B133	2.2×10^{8}	5.9×10^{7}
134	B134	1.4×10^{8}	8.9×10^{4}
135	B135	3.4×10^{9}	1.7×10^{5}
136	B136	1.4×10^{9}	3.8×10^{7}
137	B137	8.5×10^{8}	1.4×10^{5}
138	B138	2.9×10^{9}	5.2×10^{9}
139	B139	1.3×10^{8}	1.4×10^{7}
140	B140	5.2×10^{9}	6.2×10^{8}
141	B141	3.4×10^{9}	Nil
142	B142	3.4×10^{8}	2.9×10^{5}
143	B143	1.4×10^{9}	4.2×10^{7}
144	B144	4.1×10^{9}	1.9×10^{8}
145	B145	3.1×10^{7}	7.9×10^{7}
146	B146	2.7×10^{8}	1.7×10^{4}
147	B147	6.4×10^{9}	6.8×10^{8}
148	B148	2.9×10^{8}	4.4×10^{9}
149	B149	3.3×10^{6}	3.2×10^{6}
150	B150	2.2×10^{5}	1.4×10^{8}

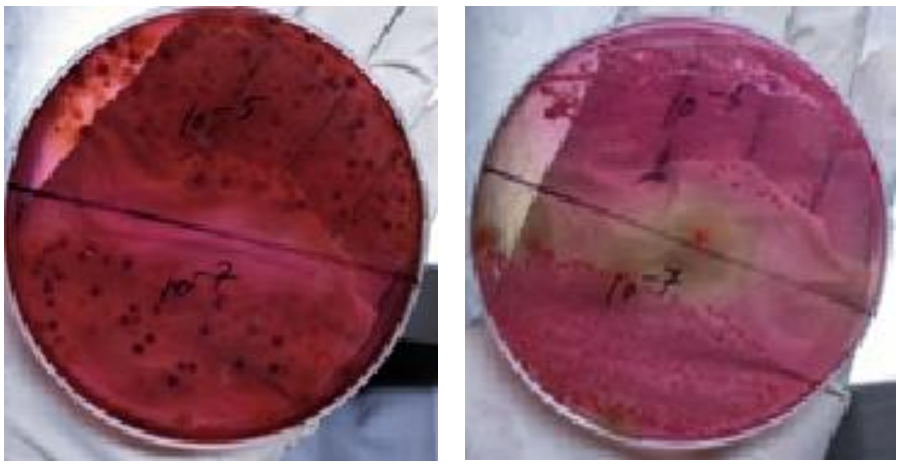

Fig 2. Total Viable count and Total Coliform count by 10 folds dilution method

4.2 Prevalence of microorganism in broiler

About 150 broilers were examined for identification of *E. coli* and *Salmonella* from liver and heart samples (Table 4). Among them 67 were diagnosed as *E. coli* positive and the prevalence rate was 44.67%. Among 150 broilers, 59 were diagnosed as *Salmonella* positive and the prevalence rate was 39.33% whereas 24 (16%) broiler samples showed no presence of *E. coli* and *Salmonella* spp.

Table 4. Prevalence of microorganism in broiler - identification of disease based on clinical signs (Percentage of prevalence with specific organism from sample)

Name	Number	Percent (%)
E. coli	67	44.67
Salmonella	59	39.33
Non identified	24	16.00
Total	150	100

4.3 Identification of *E. coli* and *Salmonella* on post mortem (PM) findings

There were 67 samples were confirmed as *E. coli* positive based on post mortem (PM) findings (Table 5). Among them 44 (34.33%) samples were identified by observing the sign of Airsacculitis whereas 23 (44.07%) samples were identified by observing the sign of Fibrinous coat.

There were 59 samples were confirmed as *Salmonella* positive based on post mortem (PM) findings (Table 5). Among them 26 (44.07%) samples were identified by observing the sign of Pin point hemorrhage, 14 (23.73%) samples were identified by observing the sign of Necrotic foci, 11 (18.64%) samples were identified by observing the sign of Pericarditis and 8 (13.56%) samples were identified by observing the sign of Perihepatitis.

Table 5. Identification of diseases based on post mortem (PM) findings

Name	PM findings	Number	Percent (%)
E. coli	• Airsacculitis • Fibrinous coat	44 23	34.33 44.07
Salmonella	• Pin point hemorrhage • Necrotic foci • Pericarditis • Perihepatitis	26 14 11 08	44.07 23.73 18.64 13.56

4.4 Prevalence (%) of *E. coli* and *Salmonella* spp. in liver and heart sample

Among 150 broiler samples, isolation of liver samples were 75 for determining the Prevalence percentage *E. coli* and *Salmonella* where isolation of *E. coli* were 34 (45.33%) and isolation of *Salmonella* were 30 (40%) (Table 6). Similarly, Among 150 broiler samples, isolation of heart samples were 75 for determining the Prevalence percentage *E. coli* and *Salmonella* where isolation of *E. coli* were 33 (44%) and isolation of *Salmonella* were 29 (38.67%) (Table 6).

Table 6. Prevalence percentage of *E. coli* and *Salmonella* spp. in liver (75) and heart (75) samples

Total samples	Isolation of organism from liver and heart samples	Isolated organism	Prevalence percentage (%)
Liver sample - 75	34	*E. coli*	45.33
	30	*Salmonella*	40.00
Heart sample - 75	33	*E. coli*	44.00
	29	*Salmonella*	38.67
Total - 150	126		84.00

4.5 Isolation and identification of *E. coli*

E. coli is identified and isolated through cultural examination performed in SAU Medicine and Public Health Laboratory.

4.5.1 Cultural examination

E. coli is cultured in Nutrient broth and then sub-cultured in EMB agar media, MacConkey (MC) agar media and BG agar media for isolation of pure bacteria.

4.5.1.1 Culture in nutrient broth

All the *E. coli* isolates produced turbidity in nutrient broth.

4.5.1.2 Culture on Eosine Methylene Blue (EMB) agar

Greenish colonies with metallic sheen produced by the organisms on EMB agar after overnight incubation were tentatively confirmed as *E. coli.* (Table 7, Fig. 3).

4.5.1.3 Culture on MacConkey (MC) agar

Bright pink colored colonies on MacConkey agar produced by the organisms after overnight incubation were presumptively selected as *E. coli* (Table 7, Fig. 4).

4.5.1.4 Culture on Brilliant green (BG) agar

Yellowish green colonies surrounded by an intense yellow green zone on BG agar produced by the organisms after overnight incubation were tentatively chosen as *E. coli* (Table 7, Fig. 5).

4.5.1.5 Culture on Salmonella-Shigella (SS) agar

Negative reaction was found by the organisms on SS agar after overnight incubation was tentatively confirmed as *E. coli* (Table 7).

Table 7. Demonstration of the cultural characteristics of *E. coli* isolated from liver and heart samples of broiler in different agar media

Sources of *E. coli*	Colony characteristics in different agar media				
	EMB agar	MC agar	BG agar	SS agar	Nutrient agar
Liver sample-34	Greenish colonies with metallic sheen	Bright pink colored colonies	Yellowish green colored colonies	--	--
Heart sample-33	Greenish colonies with metallic sheen	Bright pink colored colonies	Yellowish green colored colonies	--	--

Legends: -- = Negative reaction

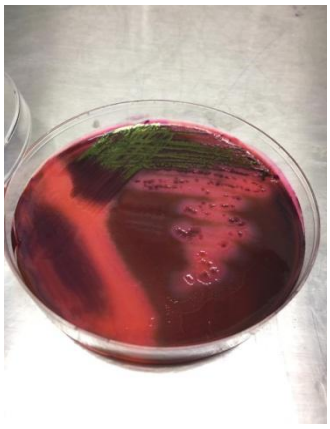

Fig. 3. *E. coli* on EMB agar media

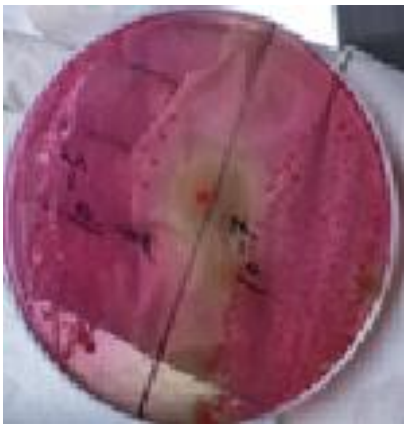

Fig. 4. *E. coli* on MC agar media

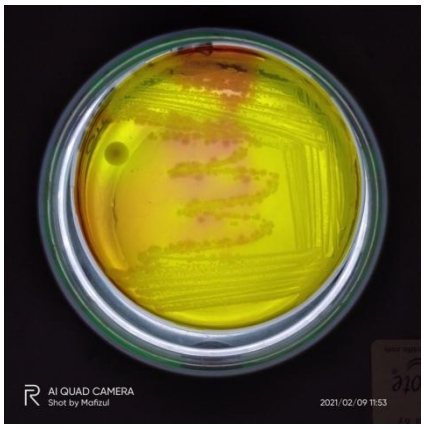

Fig. 5. *E. coli* on BG agar media

4.5.1.6 Culture on nutrient agar

Negative reaction was found by the organisms on nutrient agar after overnight incubation was tentatively confirmed as *E. coli* (Table 7).

4.5.1.7 Sugar fermentation test

All the *E. coli* isolates fermented five basic sugars with the production of acid and gas. Decreased production of acid and gas was observed in broiler liver and heart isolates during sucrose fermentation.

Table 8. Demonstration of the biochemical reactivity pattern of *E. coli* isolated from liver and heart samples of broiler

Sources of *E. coli*	Fermentation properties with five basic sugars					M R test	V-P Test	Catalase test
	DX	ML	L	S	MN			
Liver sample	AG	AG	AG	A↓G↓	AG	+	-	+
Heart sample	AG	AG	AG	A↓G↓	AG	+	-	+

Legends: DX = Dextrose; ML = Maltose; L = Lactose; S= Sucrose; MN=Mannitol; A = Acid production; G = Gas production; A↓ = Less acid production; G↓ = Less Gas production; + = Positive reaction; - = Negative reaction.

Acid production was indicated by the color change of the sugar media from reddish to yellow and the gas production was noted by the accumulation of gas bubbles in the inverted Durham's tube (Table 8, Fig. 6).

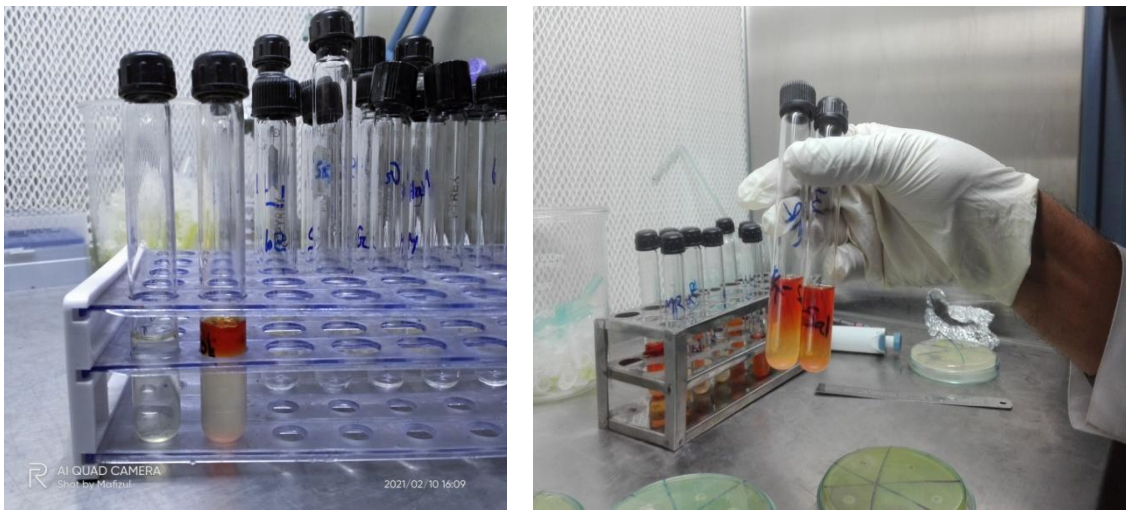

Fig. 6. Production of acid and gas in sugar fermentation test for *E. coli*

4.6 Isolation and identification of *Salmonella* spp.

For isolation and identification of *Salmonella*, different cultural examination was performed in Medicine and Public Health Laboratory, SAU.

4.6.1 Cultural examination

Salmonella is cultured in nutrient broth and then sub-cultured in EMB agar media, MacConkey (MC) agar media, Salmonella-Shigella (SS) agar media, Brilliant green (BG) media and nutrient agar media for isolation of pure bacteria.

4.6.1.1 Culture on nutrient broth

All *Salmonella* spp. isolates produced turbidity in nutrient broth.

4.6.1.2 Culture on Eosine Methylene Blue (EMB) agar

Grey color colonies produced by the organisms on EMB agar after overnight incubation were tentatively confirmed as *Salmonella* spp. (Table 9).

4.6.1.3 Culture on MacConkey (MC) agar

Red to pink-white colonies surrounded by brilliant red zones after overnight incubation was presumptively selected as *Salmonella* spp. (Table 9, Fig. 7).

4.6.1.4 Culture on Brilliant green (BG) agar

Pink colour colonies surrounded by an intense pink colour zone on BG agar produced by the organisms after overnight incubation were tentatively chosen as *Salmonella* spp. (Table 9, Fig. 8).

4.6.1.5 Culture on Salmonella-Shigella (SS) agar

Colonies with black centers produced by the organisms on **SS** agar after overnight incubation were tentatively confirmed as *Salmonella* spp. (Table 9, Fig. 9).

5.6.1.6 Culture on nutrient agar

Whitish colour colony produced by the organisms on nutrient agar after overnight incubation was tentatively confirmed as *Salmonella* spp. (Table 9, Fig. 10).

Table 9. Demonstration of the cultural characteristics of *Salmonella* isolated from liver and heart samples of broiler in different agar media

Sources of *Salmonella*	Colony characteristics in different agar media				
	EMB agar	MC agar	BG agar	SS agar	Nutrient agar
Liver sample-30	Grey colour colony	Red to pink-white colonies surrounded by brilliant red zones	Pink colour colony	Colonies with black centers	Whitish colour colony
Heart sample-29	Grey colour colony	Red to pink-white colonies surrounded by brilliant red zones	Pink colour colony	Colonies with black centers	Whitish colour colony

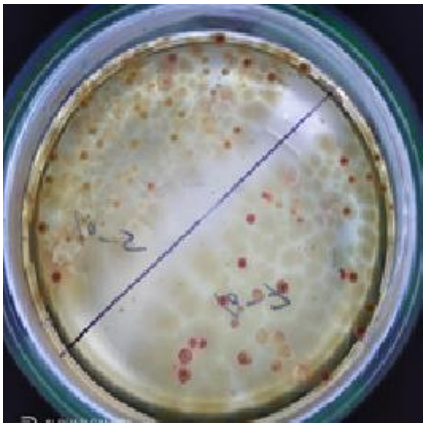

Fig. 7. *Salmonella* spp. on MC agar media

Fig. 8. *Salmonella* spp. on BG agar media

Fig. 9. *Salmonella* spp. on SS agar media

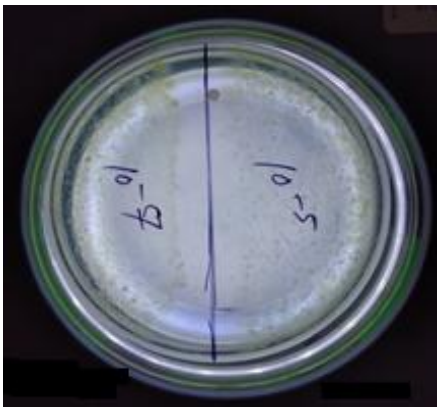

Fig. 10. *Salmonella* spp. on Nutrient agar media

4.6.1.7 Sugar fermentation test

The result carbohydrate fermentation test of *Salmonella* spp. was performed by inoculating a loopful of thick bacterial culture into the tubes containing five basic sugars (dextrose, maltose, sucrose, lactose, and mannitol) and incubated at 37°C for 24 hours. Acid production was indicated by the change of media from pink to yellow color and gas production was indicated by the appearance of gas bubbles in the inverted Durham's fermentation tubes (Table 10).

Table 10. Demonstration of the biochemical reactivity pattern of *Salmonella* isolated from liver and heart samples of broiler

Sources of *Salmonella*	Fermentation properties with five basic sugars					M R test	V-P Test	Catalase test
	DX	ML	L	S	MN			
Liver sample	AG	AG	NF	NF	A↓G	+	-	+
Heart sample	AG	AG	NF	NF	AG	+	-	+

Legends: DX =Dextrose; ML = Maltose; L = Lactose; S = Sucrose; MN=Mannitol;
A = Acid production; G = Gas production; A↓ = Less acid production; G↓ = Less Gas production;
+ = Positive reaction; - = Negative reaction

Acid production was indicated by the color change of the sugar media from reddish to yellow and the gas production was noted by the accumulation of gas bubbles in the inverted Durham's tube (Table 10, Fig. 11).

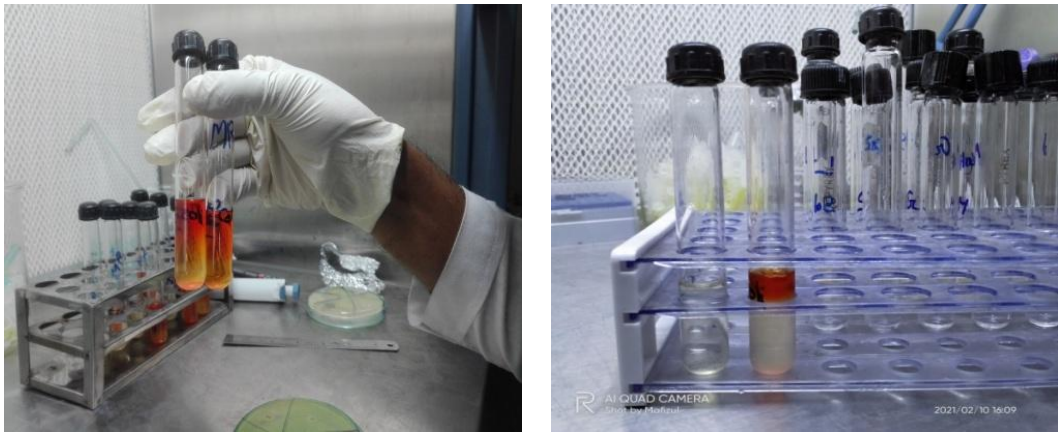

Fig. 11. Production of acid and gas in sugar fermentation test for *E. coli*

4.7 Drug sensitivity and resistance pattern of *E. coli* and *Salmonella* spp. isolated from broiler samples

A total of 67 different *E. coli* isolates collected from 150 different samples from broiler (liver and heart) were used for drug sensitivity testing. Ten different drugs were used for disc diffusion method test (Table 11a).

Table 11a. Demonstration of the sensitivity and resistance pattern of different *E. coli* isolates to different drugs in percentage

Sample	Antibiotic Disc									
	GEN-10	S-10	TE-30	CXM-30	CFM-5	CTR-30	CL-10	AMX-30	AMP-25	COT-25
B1	S	S	R	R	R	S	I	R	R	R
B2	S	I	S	R	S	R	I	I	R	S
B5	R	S	I	S	I	I	R	S	I	R
B7	S	S	R	I	S	S	S	R	S	I
B9	I	R	S	R	R	S	R	I	R	R
B11	S	S	R	S	I	I	I	R	I	R
B14	R	I	I	I	R	S	I	S	R	S
B16	R	S	R	R	S	R	S	I	S	I
B18	S	R	S	I	R	I	I	R	I	R
B20	S	S	I	S	I	I	R	R	R	R
B23	I	I	R	I	S	S	I	I	R	I
B24	S	S	S	R	R	I	S	S	I	R
B26	R	I	R	I	S	R	R	R	R	S
B28	S	S	I	R	I	S	S	R	R	I
B30	S	R	R	S	R	I	I	I	I	R
B32	I	I	I	I	S	S	S	R	R	R
B35	R	S	S	R	I	R	R	S	I	I
B38	S	S	R	R	R	S	I	I	S	R
B39	I	I	I	I	R	I	S	R	R	I
B41	R	S	R	S	S	S	R	R	I	S
B44	S	I	R	R	I	R	I	S	R	R
B48	R	S	I	I	S	S	S	I	R	I
B49	I	I	R	R	R	I	I	R	I	R
B53	S	R	S	I	I	S	R	R	R	R
B55	S	S	R	S	S	S	S	I	R	I
B58	S	I	I	R	R	R	I	R	I	R
B59	I	R	R	S	R	I	S	S	S	S
B61	I	S	R	R	I	S	R	I	I	I
B63	S	R	R	I	S	I	I	R	R	R

Sample	Antibiotic Disc									
	GEN-10	S-10	TE-30	CXM-30	CFM-5	CTR-30	CL-10	AMX-30	AMP-25	COT-25
B66	S	R	S	R	I	S	S	R	R	R
B68	I	S	R	S	R	R	I	I	I	I
B70	S	I	R	R	I	S	R	S	R	S
B75	R	R	R	I	R	I	S	R	I	R
B78	I	S	I	S	S	S	I	I	S	I
B80	I	I	I	R	R	S	S	R	R	R
B82	S	S	R	S	S	R	I	I	I	I
B85	I	R	S	I	I	S	R	R	S	R
B87	S	S	R	R	R	I	I	I	R	I
B89	S	S	I	I	S	S	S	S	I	R
B90	S	I	R	R	R	R	I	I	R	R
B92	I	R	I	R	I	I	R	R	I	S
B94	R	S	R	S	R	S	I	R	R	I
B97	S	I	R	I	S	S	I	I	I	R
B99	I	R	I	R	I	I	S	S	S	S
B101	S	S	R	R	R	S	R	R	R	I
B103	I	I	S	I	S	R	I	I	I	R
B106	R	S	R	S	I	I	I	R	R	I
B109	S	R	R	R	R	S	S	R	R	S
B111	S	S	I	I	R	S	I	I	I	R
B113	S	I	R	R	I	I	R	S	S	I
B116	S	R	R	S	S	S	I	R	R	R
B117	I	S	S	I	R	S	S	I	I	S
B119	R	I	I	R	I	I	I	R	R	I
B122	I	R	R	R	R	R	I	S	R	R
B123	S	S	I	I	R	S	R	I	I	R
B125	I	I	R	R	I	S	S	R	R	S
B128	S	R	I	R	S	I	I	R	R	I
B130	I	S	R	S	R	S	I	S	S	R
B134	S	I	S	R	I	S	S	I	R	I
B136	R	R	R	I	R	S	I	R	I	I
B137	I	S	I	R	R	I	I	S	R	R
B140	S	R	R	R	I	R	R	I	R	R
B142	S	S	I	R	R	S	S	R	S	I
B144	S	I	S	I	S	S	I	R	I	S
B146	I	S	R	R	R	I	I	S	R	I
B148	R	S	I	R	I	S	S	I	I	R
B150	I	I	R	I	R	S	I	R	R	I

R= Resistance; I= Intermediate; S= Sensitive; GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 =Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

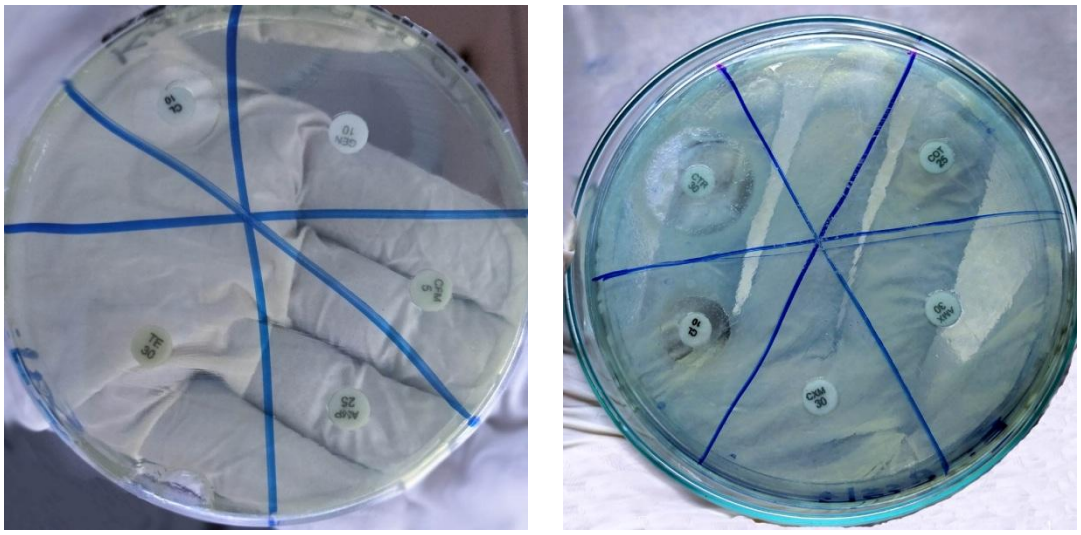

Fig. 12. Antibiotic sensitivity test for *E. coli* organism

Table 11b. Prevalence of antimicrobial resistance pattern against *E. coli* isolates

Antibiotics	Percentage (%) of resistance to different drugs		
	Resistance (R)	Intermediate (I)	Sensitive (S)
GEN-10	19.40	31.34	49.25
S-10	25.37	29.85	44.78
TE-30	52.24	29.85	17.91
CXM-30	47.76	31.34	20.90
CFM-5	43.28	29.85	26.87
CTR-30	17.91	29.85	52.24
CL-10	22.39	47.76	29.85
AMX-30	46.27	32.84	20.90
AMP-25	50.75	34.33	14.93
COT-25	46.27	35.82	17.91

GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

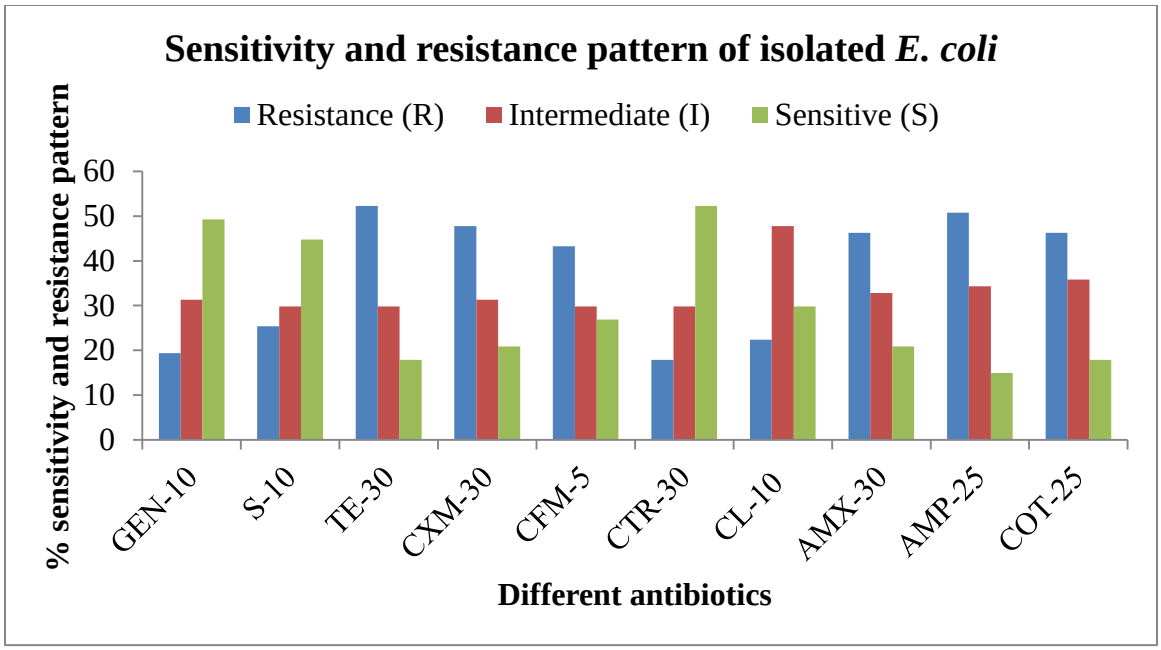

Fig. 13. Prevalence of antimicrobial resistance pattern against *E. coli* isolates

GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

A large number of *E. coli* isolates from broiler samples were found sensitive to CTR-30 (52.24%), GEN-10 (49.25%) andS-10(44.78%) (Table 11b and Fig. 13). A little number was sensitive to CXM-30 and AMX-30 (20.90%), CXM-30 andCOT-25 (17.91%) and AMP-25(14.93%). The highest resistance of *E. coli* was against TE-30(50.75%). They showed comparatively higher resistance against AMP-25(50.75%), CXM-30(47.76%), AMX-30 and COT-25 (46.27%). The lowest resistance of *E. coli* was against CTR-30 (17.91%). Comparatively lower resistance was showed by them against GEN-10, S-10, and CL-10 19.40%, 25.37%, and 22.39%, respectively. Most of the isolates showed intermediate sensitivity against CL-10(47.76%). Against GEN-10andCXM-30they have shown the equal intermediate sensitivity (31.34%) and S-10, TE-30, CFM-5 and CTR-30 (29.85%) also showed equal intermediate sensitivity which was lowest (Table 11b and Fig. 13).

Table 12(a). Demonstration of the sensitivity and resistance pattern of different *E. coli* isolates to different drugs in percentage obtained from liver

Sample	Antibiotic Disc									
	GEN-10	S-10	TE-30	CXM-30	CFM-5	CTR-30	CL-10	AMX-30	AMP-25	COT-25
B1	S	S	R	R	R	S	I	R	R	R
B2	S	I	S	R	S	R	I	I	R	S
B5	R	S	I	S	I	I	R	S	I	R
B7	S	S	R	I	S	S	S	R	S	I
B9	I	R	S	R	R	S	R	I	R	R
B11	S	S	R	S	I	I	I	R	I	R
B14	R	I	I	I	R	S	I	S	R	S
B16	R	S	R	R	S	R	S	I	S	I
B18	S	R	S	I	R	I	I	R	I	R
B20	S	S	I	S	I	I	R	R	R	R
B23	I	I	R	I	S	S	I	I	R	I
B24	S	S	S	R	R	I	S	S	I	R
B26	R	I	R	I	S	R	R	R	R	S
B28	S	S	I	R	I	S	S	R	R	I
B30	S	R	R	S	R	I	I	I	I	R
B32	I	I	I	I	S	S	S	R	R	R
B35	R	S	S	R	I	R	R	S	I	I
B38	S	S	R	R	R	S	I	I	S	R
B39	I	I	I	I	R	I	S	R	R	I
B41	R	S	R	S	S	S	R	R	I	S
B44	S	I	R	R	I	R	I	S	R	R
B48	R	S	I	I	S	S	S	I	R	I
B49	I	I	R	R	R	I	I	R	I	R
B53	S	R	S	I	I	S	R	R	R	R
B55	S	S	R	S	S	S	S	I	R	I
B58	S	I	I	R	R	R	I	R	I	R
B59	I	R	R	S	R	I	S	S	S	S
B61	I	S	R	R	I	S	R	I	I	I
B63	S	R	R	I	S	I	I	R	R	R
B66	S	R	S	R	I	S	S	R	R	R
B68	I	S	R	S	R	R	I	I	I	I
B70	S	I	R	R	I	S	R	S	R	S
B75	R	R	R	I	R	I	S	R	I	R
B78	I	S	I	S	S	S	I	I	S	I

R= Resistance; I= Intermediate; S= Sensitive; GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

Table 12(b). Prevalence of antimicrobial resistance pattern against *E. coli* isolates obtained from liver

Antibiotics	Percentage (%) of resistance to different drugs		
	Resistance (R)	Intermediate (I)	Sensitive (S)
GEN-10	23.53	26.47	50.00
S-10	23.53	29.41	47.06
TE-30	55.88	23.53	20.59
CXM-30	41.18	32.35	26.47
CFM-5	38.24	29.41	32.35
CTR-30	20.59	32.35	47.06
CL-10	26.47	41.18	32.35
AMX-30	47.06	32.35	20.59
AMP-25	52.94	32.35	14.71
COT-25	50.00	32.35	17.65

GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

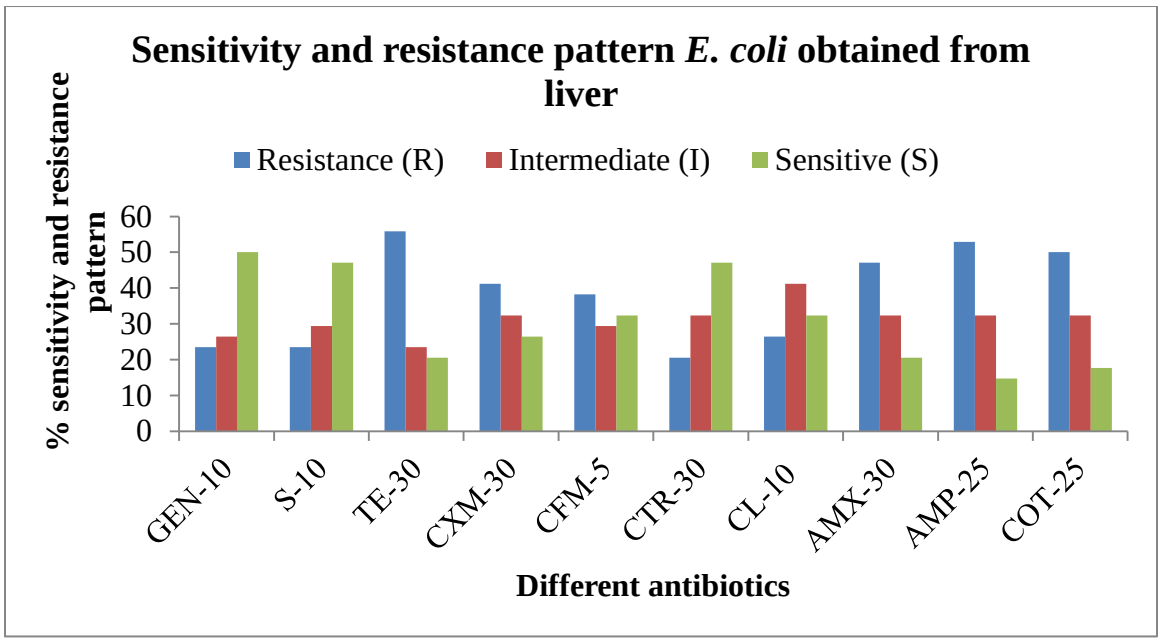

Fig. 14. Prevalence of antimicrobial resistance pattern against *E. coli* isolates obtained from liver

GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

In comparison between the *E. coli* from liver and heart samples, *E. coli* from the liver sample showed higher sensitivity against GEN-10 (50%) (Table 12b and Fig. 14) and the heart samples shows against CTR-30 (57.58%) (Table 13b). The liver isolates of *E. coli* showed comparatively higher sensitivity against S-10 and CTR-30 (47.06%) and heart isolates showed against GEN-10 (48.48%) and S-10 (42.42%) (Table 12b, 13b). Lower sensitivity found in case of liver isolates to TE-30, AMX-30, COT-25 and AMP-25 (20.59, 20.59, 17.65 and 14.71% respectively) (Table 12b).

In case of heart isolates, lower sensitivity was found against CFM-5, AMX-30, COT-25, TE-30, CXM-30 and AMP-25 (21.21, 21.21, 18.18, 15.15, 15.15 and 15.15%, respectively) (Table 13b and Fig. 15). Highest intermediate sensitivity was found against CL-10 in isolates from both (liver and heart) type of samples (41.18 and 54.55%, respectively). Liver isolated has shown highest resistance against TE-30 (55.88%) and comparatively higher against AMP-25 (52.94%), COT-25 (50%), AMX-30 (47.06) and CXM-30 (41.18%) and lower resistance was against CL-10, GEN-10, S-10 andCTR-30 (26.47, 23.53, 23.53 and 20.59%, respectively) (Table 12b and Fig. 14). On the other hand heart isolates showed higher resistance against CXM-30 (54.55%), TE-30 (48.48%), CFM-5 (48.48%), AMP-25 (48.48%), AMX-30 (45.45%) and COT-25 (42.42%). CL-10, GEN-10 and CTR-30 were relatively in lower percentage *viz*. 18.18, 15.15 and15.15%, respectively (Table 13b and Fig. 15). Highest intermediate sensitivity showed by liver isolates which was 41.18% against CL-10and comparatively higher sensitivity which was 32.35% against CXM-30, CTR-30, AMX-30, AMP-25 and COT-25 (Table 12b and Fig. 14). In case of heart samples, the highest 54.55% was intermediate sensitivity against CL-10 followed by 39.39% against COT-25 whereas 30.30% was lowest intermediate sensitivity against S-10, CXM-30 and CFM-5 (Table 13b and Fig. 15).

Table 13(a). Demonstration of the sensitivity and resistance pattern of different *E. coli* isolates to different drugs in percentage obtained from heart

Sample	Antibiotic Disc									
	GEN-10	S-10	TE-30	CXM-30	CFM-5	CTR-30	CL-10	AMX-30	AMP-25	COT-25
B80	I	I	I	R	R	S	S	R	R	R
B82	S	S	R	S	S	R	I	I	I	I
B85	I	R	S	I	I	S	R	R	S	R
B87	S	S	R	R	R	I	I	I	R	I
B89	S	S	I	I	S	S	S	S	I	R
B90	S	I	R	R	R	R	I	I	R	R
B92	I	R	I	R	I	I	R	R	I	S
B94	R	S	R	S	R	S	I	R	R	I
B97	S	I	R	I	S	S	I	I	I	R
B99	I	R	I	R	I	I	S	S	S	S
B101	S	S	R	R	R	S	R	R	R	I
B103	I	I	S	I	S	R	I	I	I	R
B106	R	S	R	S	I	I	I	R	R	I
B109	S	R	R	R	R	S	S	R	R	S
B111	S	S	I	I	R	S	I	I	I	R
B113	S	I	R	R	I	I	R	S	S	I
B116	S	R	R	S	S	S	I	R	R	R
B117	I	S	S	I	R	S	S	I	I	S
B119	R	I	I	R	I	I	I	R	R	I
B122	I	R	R	R	R	R	I	S	R	R
B123	S	S	I	I	R	S	R	I	I	R
B125	I	I	R	R	I	S	S	R	R	S
B128	S	R	I	R	S	I	I	R	R	I
B130	I	S	R	S	R	S	I	S	S	R
B134	S	I	S	R	I	S	S	I	R	I
B136	R	R	R	I	R	S	I	R	I	I
B137	I	S	I	R	R	I	I	S	R	R
B140	S	R	R	R	I	R	R	I	R	R
B142	S	S	I	R	R	S	S	R	S	I
B144	S	I	S	I	S	S	I	R	I	S
B146	I	S	R	R	R	I	I	S	R	I
B148	R	S	I	R	I	S	S	I	I	R
B150	I	I	R	I	R	S	I	R	R	I

R= Resistance; I= Intermediate; S= Sensitive; GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

Table 13(b). Prevalence of antimicrobial resistance pattern against *E. coli* isolates obtained from heart

Antibiotics	Percentage (%) of resistance to different drugs		
	Resistance (R)	Intermediate (I)	Sensitive (S)
GEN-10	15.15	36.36	48.48
S-10	27.27	30.30	42.42
TE-30	48.48	36.36	15.15
CXM-30	54.55	30.30	15.15
CFM-5	48.48	30.30	21.21
CTR-30	15.15	27.27	57.58
CL-10	18.18	54.55	27.27
AMX-30	45.45	33.33	21.21
AMP-25	48.48	36.36	15.15
COT-25	42.42	39.39	18.18

GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

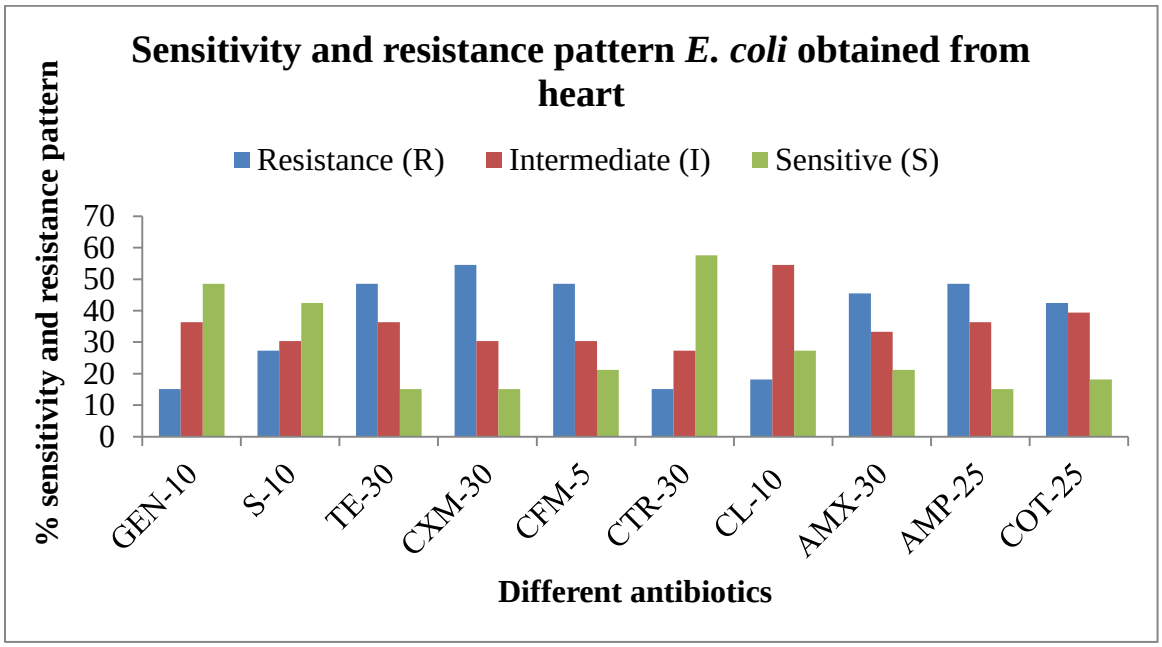

Fig.15. Prevalence of antimicrobial resistance pattern against *E. coli* isolates obtained from heart

GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

A total of 59 different *Salmonella* isolates collected from 150 different samples from broiler (liver and heart) (Table 14a).A large number of *Salmonella* isolates from broiler samples were found sensitive to CTR-30 (52.54%), GEN-10 (50.85%) and S-10 (47.46%) (Table 14b and Fig. 17).

Table 14a. Demonstration of the sensitivity and resistance pattern of different *Salmonella* spp. isolates to different drugs in percentage

Sample	Antibiotic Disc									
	GEN-10	S-10	TE-30	CXM-30	CFM-5	CTR-30	CL-10	AMX-30	AMP-25	COT-25
B3	I	S	S	R	I	S	R	S	I	R
B4	S	I	R	S	S	R	S	R	S	I
B8	R	R	I	I	R	I	I	I	R	S
B10	S	S	R	R	I	S	I	R	I	R
B12	I	R	S	R	R	I	R	S	R	R
B15	S	S	I	S	S	S	S	R	S	I
B17	R	I	R	I	R	R	I	I	R	S
B21	I	S	R	R	R	I	I	S	R	I
B22	S	R	S	R	I	S	S	R	R	R
B25	S	S	R	S	S	S	R	I	I	I
B29	R	I	I	I	R	I	I	S	R	S
B33	I	S	R	R	R	S	S	R	S	R
B34	S	S	R	R	I	S	I	I	R	R
B36	I	R	S	I	S	R	I	R	R	I
B40	R	I	R	S	R	I	R	R	I	R
B42	S	S	I	I	R	S	S	I	R	S
B45	S	S	R	R	I	S	I	S	R	I
B46	I	R	S	R	S	I	I	I	S	R
B47	R	I	R	S	R	S	I	R	R	R
B50	S	S	I	I	R	S	R	I	I	I
B51	I	S	R	R	I	R	S	R	R	S
B54	S	R	S	S	R	I	I	S	R	R
B57	I	I	R	R	S	S	I	I	I	R
B60	R	S	I	I	I	I	I	R	S	I
B64	S	S	S	S	R	S	R	R	R	R
B65	S	R	R	I	R	I	S	I	R	S
B69	R	I	S	R	I	R	I	S	I	I
B72	S	S	R	R	S	S	I	R	R	R
B73	S	R	I	I	R	S	S	R	S	R
B76	I	I	R	S	R	I	R	I	R	I
B77	R	S	S	R	I	S	I	S	I	S
B81	S	S	R	R	S	S	I	R	R	R

Sample	Antibiotic Disc									
	GEN-10	S-10	TE-30	CXM-30	CFM-5	CTR-30	CL-10	AMX-30	AMP-25	COT-25
B83	I	R	I	R	R	R	S	R	S	I
B84	S	I	R	I	R	I	R	R	R	R
B88	I	S	S	R	I	S	I	I	I	S
B93	S	S	R	S	S	S	S	S	R	I
B96	R	R	I	R	R	I	R	R	R	R
B98	R	I	R	I	R	R	I	R	S	S
B102	S	S	S	R	S	I	I	I	I	R
B104	I	R	I	R	I	S	R	S	R	I
B107	S	S	R	R	R	S	S	R	R	R
B110	S	I	R	S	R	I	I	R	S	S
B112	R	R	S	R	S	S	R	I	I	I
B114	S	S	I	R	I	S	I	S	R	R
B118	I	S	R	I	R	R	R	R	R	I
B120	S	I	R	R	R	S	S	R	S	S
B121	R	S	S	R	S	I	I	I	I	R
B126	S	R	I	S	I	S	I	R	R	R
B127	S	S	R	R	R	I	S	S	R	I
B129	S	I	R	I	I	S	R	R	S	R
B131	I	S	S	R	R	R	I	I	I	S
B133	S	S	I	R	R	S	S	R	R	R
B135	R	R	R	R	S	I	I	R	R	I
B138	S	I	S	I	I	S	R	S	S	R
B139	S	S	R	S	R	S	S	I	I	S
B143	I	R	I	R	S	S	I	R	R	R
B145	S	I	S	R	R	I	S	R	S	I
B147	R	S	R	I	I	R	R	S	I	R
B149	S	I	R	R	R	S	S	I	R	S

R= Resistance; I= Intermediate; S= Sensitive; GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

A little number was sensitive to CXM-30 (20.34%). The highest resistance was against CXM-30 (54.24%) followed by AMP-25 (52.54%), CFM-5 (50.85%), TE-30 (49.15%), AMX-30 (47.46%) and COT-25 (45.76%). Comparatively lower resistance was found against S-10 (25.42%), CL-10 (25.42%), GEN-10 (23.73%) and CTR-30 (16.95%). Most of the isolates showed intermediate sensitivity against CL-10(45.76%) followed by CTR-30 (30.51%) and COT-25 (30.51%).

The equal intermediate sensitivity was found against S-10 and CFM-5 which was 27.12%. The equal intermediate sensitivity was also found against GEN-10, CXM-30 and AMP-25 which were 25.42% and this was the lowest intermediate sensitivity against *Salmonella* (Table 14b and Fig. 17).

Table 14b. Prevalence of antimicrobial resistance pattern against *Salmonella* isolates

Antibiotics	Percentage (%) of resistance to different drugs		
	Resistance (R)	Intermediate (I)	Sensitive (S)
GEN-10	23.73	25.42	50.85
S-10	25.42	27.12	47.46
TE-30	49.15	23.73	27.12
CXM-30	54.24	25.42	20.34
CFM-5	50.85	27.12	22.03
CTR-30	16.95	30.51	52.54
CL-10	25.42	45.76	28.81
AMX-30	47.46	28.81	23.73
AMP-25	52.54	25.42	22.03
COT-25	45.76	30.51	23.73

GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

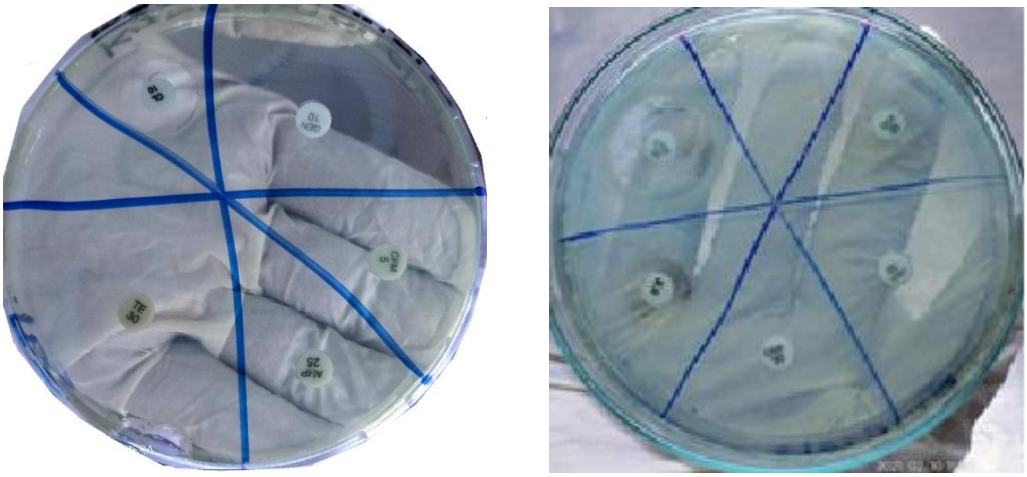

Fig. 16. Antibiotic sensitivity test for *Salmonella* spp. organism

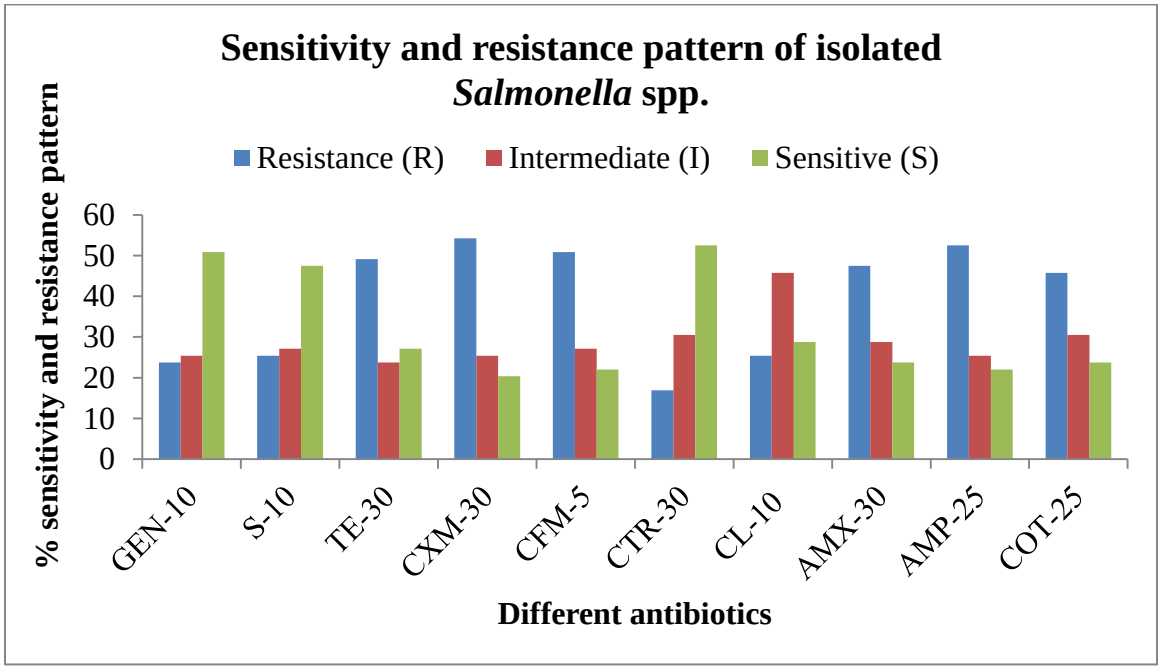

Fig. 17. Prevalence of antimicrobial resistance pattern against *Salmonella* isolates

GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

In comparison between the *Salmonella* spp. from liver and heart samples, *Salmonella* sp. from the liver sample showed higher sensitivity to CTR-30 (50%) and the heart samples shows against GEN-10 andCTR-30 (55.17%). The liver isolates of *Salmonella* sp. showed comparatively higher sensitivity against GEN-10 (46.67%) and S-10 (46.67%). The lowest sensitivity against AMP-25 and COT-25was 20% in liver isolates whereas 13.79% sensitivity against CXM-30 in heart isolates (Table 15b and Fig. 18).Liver isolates showed highest resistance against AMP-25 (56.67%) followed by TE-30 (50%) and CFM-5 (50%) whereas lowest resistance was found against CTR-30 (16.67%). Liver isolates showed highest intermediate sensitivity against CL-10 (50%) and lowest was against TE-30 (23.33%) and AMP-25 (23.33%) (Table 15b and Fig. 18).

Table 15a. Demonstration of the sensitivity and resistance pattern of different *Salmonella* isolates to different drugs in percentage obtained from liver

Sample	Antibiotic Disc									
	GEN-10	S-10	TE-30	CXM-30	CFM-5	CTR-30	CL-10	AMX-30	AMP-25	COT-25
B3	I	S	S	R	I	S	R	S	I	R
B4	S	I	R	S	S	R	S	R	S	I
B8	R	R	I	I	R	I	I	I	R	S
B10	S	S	R	R	I	S	I	R	I	R
B12	I	R	S	R	R	I	R	S	R	R
B15	S	S	I	S	S	S	S	R	S	I
B17	R	I	R	I	R	R	I	I	R	S
B21	I	S	R	R	R	I	I	S	R	I
B22	S	R	S	R	I	S	S	R	R	R
B25	S	S	R	S	S	S	R	I	I	I
B29	R	I	I	I	R	I	I	S	R	S
B33	I	S	R	R	R	S	S	R	S	R
B34	S	S	R	R	I	S	I	I	R	R
B36	I	R	S	I	S	R	I	R	R	I
B40	R	I	R	S	R	I	R	R	I	R
B42	S	S	I	I	R	S	S	I	R	S
B45	S	S	R	R	I	S	I	S	R	I
B46	I	R	S	R	S	I	I	I	S	R
B47	R	I	R	S	R	S	I	R	R	R
B50	S	S	I	I	R	S	R	I	I	I
B51	I	S	R	R	I	R	S	R	R	S
B54	S	R	S	S	R	I	I	S	R	R
B57	I	I	R	R	S	S	I	I	I	R
B60	R	S	I	I	I	I	I	R	S	I
B64	S	S	S	S	R	S	R	R	R	R
B65	S	R	R	I	R	I	S	I	R	S
B69	R	I	S	R	I	R	I	S	I	I
B72	S	S	R	R	S	S	I	R	R	R
B73	S	R	I	I	R	S	S	R	S	R
B76	I	I	R	S	R	I	R	I	R	I

R= Resistance; I= Intermediate; S= Sensitive; GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

Table 15b. Prevalence of antimicrobial resistance pattern against *Salmonella* spp. isolates obtained from liver

Antibiotics	Percentage (%) of resistance to different drugs		
	Resistance (R)	Intermediate (I)	Sensitive (S)
GEN-10	23.33	30.00	46.67
S-10	26.67	26.67	46.67
TE-30	50.00	23.33	26.67
CXM-30	43.33	30.00	26.67
CFM-5	50.00	26.67	23.33
CTR-30	16.67	33.33	50.00
CL-10	23.33	50.00	26.67
AMX-30	43.33	33.33	23.33
AMP-25	56.67	23.33	20.00
COT-25	50.00	30.00	20.00

GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

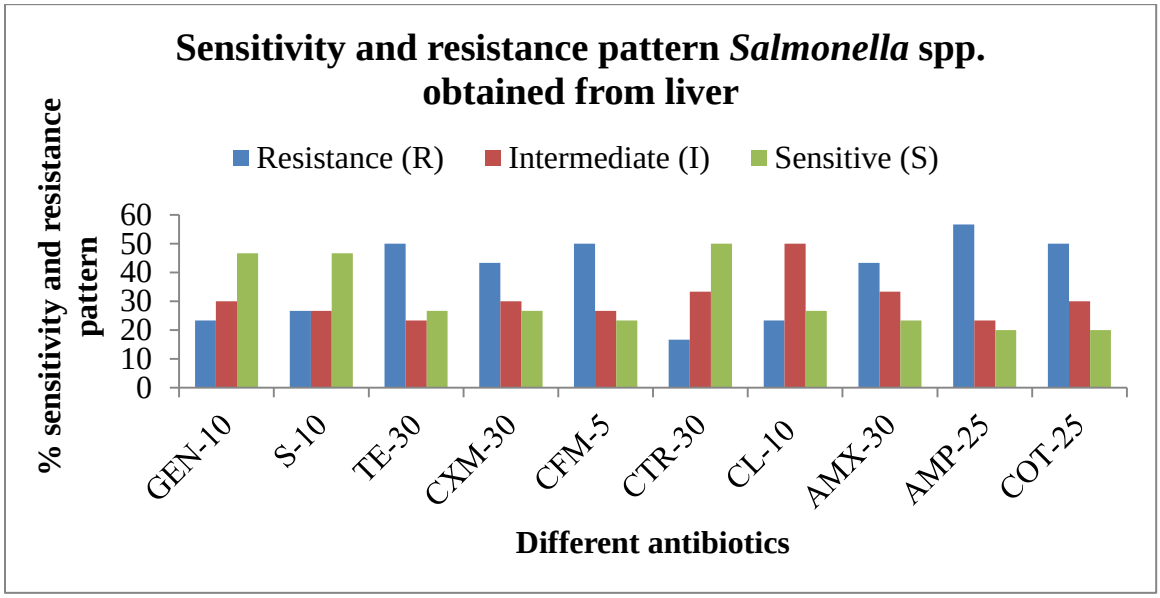

Fig. 18. Prevalence of antimicrobial resistance pattern against *Salmonella* isolates obtained from liver

GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

Similarly, the heart isolates showed higher resistance against CXM-30 (65.52%) followed by CFM-5 (51.72%), AMX-30 (51.72%), TE-30 (48.28%) andAMP-25 (48.28%). Relatively lower resistances of heart sample against GEN-10, S-10,CTR-30 was 24.14, 24.14, 17.24%,respectively (Table 16b and Fig. 19).Heart isolates showed highest intermediate sensitivity against CL-10 (41.38%) and lowest was against GEN-10 (20.69%) (Table 16b and Fig. 19).

Table 16a. Demonstration of the sensitivity and resistance pattern of different *Salmonella* isolates to different drugs in percentage obtained from heart

Sample	Antibiotic Disc									
	GEN-10	S-10	TE-30	CXM-30	CFM-5	CTR-30	CL-10	AMX-30	AMP-25	COT-25
B77	R	S	S	R	I	S	I	S	I	S
B81	S	S	R	R	S	S	I	R	R	R
B83	I	R	I	R	R	R	S	R	S	I
B84	S	I	R	I	R	I	R	R	R	R
B88	I	S	S	R	I	S	I	I	I	S
B93	S	S	R	S	S	S	S	S	R	I
B96	R	R	I	R	R	I	R	R	R	R
B98	R	I	R	I	R	R	I	R	S	S
B102	S	S	S	R	S	I	I	I	I	R
B104	I	R	I	R	I	S	R	S	R	I
B107	S	S	R	R	R	S	S	R	R	R
B110	S	I	R	S	R	I	I	R	S	S
B112	R	R	S	R	S	S	R	I	I	I
B114	S	S	I	R	I	S	I	S	R	R
B118	I	S	R	I	R	R	R	R	R	I
B120	S	I	R	R	R	S	S	R	S	S
B121	R	S	S	R	S	I	I	I	I	R
B126	S	R	I	S	I	S	I	R	R	R
B127	S	S	R	R	R	I	S	S	R	I
B129	S	I	R	I	I	S	R	R	S	R
B131	I	S	S	R	R	R	I	I	I	S
B133	S	S	I	R	R	S	S	R	R	R
B135	R	R	R	R	S	I	I	R	R	I
B138	S	I	S	I	I	S	R	S	S	R
B139	S	S	R	S	R	S	S	I	I	S
B143	I	R	I	R	S	S	I	R	R	R
B145	S	I	S	R	R	I	S	R	S	I
B147	R	S	R	I	I	R	R	S	I	R
B149	S	I	R	R	R	S	S	I	R	S

R= Resistance; I= Intermediate; S= Sensitive; GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

Table 16b. Prevalence of antimicrobial resistance pattern against *Salmonella* spp. isolates obtained from heart

Antibiotics	Percentage (%) of resistance to different drugs		
	Resistance (R)	Intermediate (I)	Sensitive (S)
GEN-10	24.14	20.69	55.17
S-10	24.14	27.59	48.28
TE-30	48.28	24.14	27.59
CXM-30	65.52	20.69	13.79
CFM-5	51.72	27.59	20.69
CTR-30	17.24	27.59	55.17
CL-10	27.59	41.38	31.03
AMX-30	51.72	24.14	24.14
AMP-25	48.28	27.59	24.14
COT-25	41.38	31.03	27.59

GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

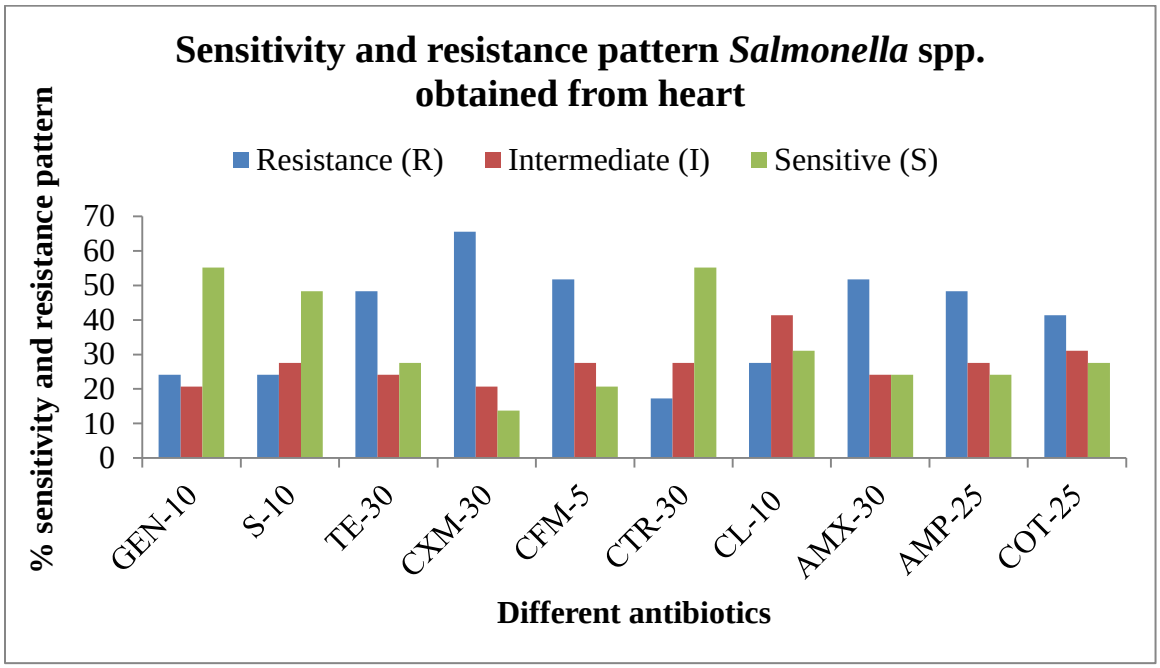

Fig. 19. Prevalence of antimicrobial resistance pattern against *Salmonella* spp. isolates obtained from heart

GEN-10 = Gentamicin, S-10 = Streptomicin, TE-30 = Tetracycline, CXM-30 = Cefuroxime, CFM-5 = Cefixime, CTR-30 = Ceftriaxone, CL-10 = Colistin, AMX-30 = Amoxycillin, AMP-25 = Ampicillin, COT-25 = Co-Trimoxazole

4.8 Discussion

The present study was conducted primarily for the isolation and identification of the *E. coli* and *Salmonella* spp. isolated from liver and heart samples of dead broiler in Dhaka and around Dhaka city and also to determine the current status of drug sensitivity and resistance pattern of the isolates to determine the drug of choice for therapeutic use against infection caused by these organisms.

Colony characteristics of *E. coli* in five different agar media and fermentation ability with five basic sugars were similar with a bit exception. Interesting findings of the colony characteristics of the isolates were also observed. All the *E. coli* isolates were able to produce characteristic greenish metallic sheen colony on the EMB agar, bright pink colony on MacConkey agar, yellowish green colonies surrounded by an intense yellow green zone on BG agar. All the *Salmonella* isolates were able to produce grey color colonies in EMB agar, red to pink-white colonies surrounded by brilliant red zones in MacConkey agar, Pink colour colony in BG agar, Colonies with black centers in SS agar and Whitish colour colony in Nutrient agar.

Another fundamental basis for the identification of *E. coli* and *Salmonella* organism was determining the ability or inability of fermentation of five basic sugars with acid and gas production. All the *E. coli* isolates from broiler revealed a complete fermentation of five basic sugars as stated by (Mckec *et al*., 1995), (Shandhu et al., and 1996) and (Beutin *et al*., 1997). However, differentiation of *Salmonella* species was difficult based on their sugar fermentation pattern. All the *Salmonella* isolates of this study fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment sucrose and lactose which supported the statement of Buxton and Fraser (1977), Hossain (2002) and Han *et al*. (2011).

In the present study, the isolated *E. coli* organisms fermented dextrose, maltose, lactose, sucrose and mannitol with the production of both acid and gas. Less

production of acid and gas during sucrose fermentation was found by *E. coli* organisms. Ewing *et al*. (1973) and Ali *et al*. (1998) also studied the biochemical characteristics of *E. coli* isolated from different sources. They reported a little or no difference in these biochemical characters.

The results of MR and Catalase test of the *E. coli* isolates were positive but V-P test was negative as reported by Buxton and Fraser (1977).The result of MR test was positive and result of V-P test was negative which satisfy the statement of Buxton and Fraser (1977).

In the present study, prevalence of microorganism in broiler was 100%. Among 150 samples (liver = 75 and heart = 75) all were infected with different microorganisms. Among 150 samples, 67 samples were infected with *E. coli*, 59 samples were infected with *Salmonella* and rest 24 samples were unidentified. The infection with *E. coli* was (44.67%). Infection with *E. coli* for liver and heart sample was respectively 45.33% and 44%. This result was similar with the finding of Hossain *et al*. (2008) who reported the prevalence was 63.6%, whereas Jakaria *et al*. (2012) found 82% prevalence and Bashar *et al*. (2011) found 100% prevalence of *E. coli* in poultry.

The prevalence of *Salmonella* in broiler was (39.33%) and Infection with *Salmonella* for liver and heart sample was respectively 40% and 38%. This finding is supported with some of the previous study; the prevalence of *Salmonella* was 30% in broiler (Cardinale *et al*., 2003). Generally low prevalence of *Salmonella* was found in poultry reported by Nhung *et al*. (2017), Jajere *et al*. (2015) and Geidam *et al*. (2012).

Bacteria can overcome the effect of drugs used for the treatment purpose by producing various enzymes and metabolites that either degrade the antimicrobial agents or help the bacteria to survive through various mechanisms. So, the current status of sensitivity and resistance pattern of the *E. coli* and *Salmonella* isolates to

different drugs should be determined to choose the best drug for treatment purpose.

To perform this studies a total of 67 *E. coli* isolates from 150 broiler (liver + heart) samples, were subjected to antimicrobial sensitivity test which was carried out by disc diffusion method. Ten different drugs were used for this study. The sensitivity test revealed that most of the *E. coli* isolates, from broiler liver and heart samples were sensitive to CTR-30 followed by GEN-10 and S-10.

In terms of resistance, most of the isolates were resistant to TE-30 and AMP-25 followed by CXM-30, AMX-30, COT-25 and CFM-5. In the present study a high percentage of *E. coli* isolates, from broiler samples were sensitive to CTR-30 (52.24%) and GEN-10 (49.25%) followed by S-10 showing 44.78% sensitivity. On the other hand, 52.24% of the isolates were resistant to TE-30 and 50.75% to AMP-25 followed by CXM-30 (47.76%), AMX-30 (46.27%), COT-25 (46.27%) and CFM-5 (43.28%). A similar result was reported by Azad *et al*. (2017), who observed 100% resistance in *E. coli* isolates to ampicillin, tetracycline and trimethoprim-sulfamethoxazole isolated from broiler colacal swab samples in Rajshahi area, Bangladesh Sarker *et al*. (2019) revealed that ceftriaxone, gentamicin, and erythromycin were sensitive to 56.76%, 56.76%, and 43.24% isolates, respectively, while Azad *et al*. (2017) reported 36% to gentamicin and 100% to erythromycin. Hassan *et al*. (2008) found the most strains were frequently highly resistance to trimethoprim-sulphmethoxazol (89.5%) followed by ampicillin (84.2%), tetracycline (78.9%), erythromycin (78.9%) and doxycycline (73.6%). Zhang *et al*., (2014) from Hubei of China reported high rate of resistance toward (N=111) gentamicin (95%), amikacin (46%), which is contrast to this study.

In this study a number of isolates also showed intermediate reaction to CL-10 (47.76%) followed by COT-25 (35.82%), AMP-25 (34.33%), AMX-30 (32.84%),

GEN-10 and CXM-30 (31.34%), S-10, TE-30, CFM-5 and CTR-30 (29.85%). Intermediate Sensitivity drugs could not be compared due to lack of relevant literature.

In the present study, all the *E. coli* isolates from broiler liver found to be sensitive to GEN-10 (50%), S-10 and CTR-30 (47.06%) followed by CFM-5 and CL-10 (32.35%), CXM-30 (26.47%), TE-30 and AMX-30 (20.59%), COT-25 (17.65%) and AMP-25 (14.71%) showing marked resistance to TE-30 (55.88%), AMP-25 (52.94%), COT-25 (50%) and AMX-30 (47.06%). Specific sensitivity pattern of liver sample cannot be compared due to lack of relevant literature.

In the present study, all the *E. coli* isolates from broiler heart found to be sensitive to CTR-30 (57.58%), GEN-10 (48.48%) and S-10 (42.42%) followed by CL-10 (27.27%), CFM-5 and AMX-30 (21.21%), COT-25 (18.18%), TE-30, CXM-30 and AMP-25 (21.21%) showing marked resistance to CXM-30 (54.55%), TE-30, CFM-5 and AMP-25 (48.48%), AMX-30 (45.45%) and COT-25 (42.42%) followed by S-10 (27.27%), CL-10 (18.18%), GEN-10 and CTR-30 (15.15%). This finding was similar with the findings of Karczmarczyk, *et al*., (2011) where resistance to Tetracycline was the highest followed by Cephalothin (cephalosporin) and Amoxicillin. This results were also similar with a previous study by Zinnah *et al*. (2008) where *E. coli* was sensitive to LVX (90%) and CIP (80%) followed by GM (60%) and AZM (50%) but highly resistant to other drugs such as MET (100%), NA (90%), TET (80%), AMX (80%), ERY (90%) and AMP (70%).

The intermediate sensitivity of *E. coli* isolates from heart of broiler was highest against CL-10 (54.55%) followed by COT-25 (39.39%), GEN-10, TE-30 and AMP-25 (36.36%), AMX-30 (33.33%), S-10, CXM-30 and CFM-5 (30.30%) and CTR-30 (27.27%). This finding is also partly supported by the findings of Saidi *et al*., (2012) where *E. coli* isolates showed moderate rates of resistance to

tetracycline and chloramphenicol. Antibiotic resistance of avian bacterial pathogens is also a common problem in poultry in Bangladesh. Hasan *et al*. (2011) noted that more than 55% (N=101) of *E coli* isolated were resistant to at least one or more of the tested antibiotics, and 36% of the isolates showed multiple-drug-resistant phenotypes. The most common resistances observed were against Tetracycline. This supports our finding in the way that the resistance of *E. coli* was highest against tetracycline in laying birds. It also reported moderate resistance to gentamicin which is in similar line to our finding.

From 150 broiler (liver + heart) samples, 59 *Salmonella* isolates were subjected to antimicrobial sensitivity test which was carried out by disc diffusion method. Ten different drugs were used for this study. The sensitivity test revealed that most of the *Salmonella* isolates, from broiler liver and heart samples were sensitive to CTR-30 followed by GEN-10 andS-10. In terms of resistance, most of the isolates were resistant to CXM-30, AMP-25 and CFM-5 followed by TE-30, AMX-30 and COT-25. In the present study a high percentage of *Salmonella* isolates, from broiler samples were sensitive to CTR-30 (52.54%) followed by GEN-10 and S-10 showing 50.85% and 47.46% sensitivity respectively. On the other hand, 54.24%, 52.54% and 50.85 of the isolates were resistant to CXM-30, AMP-25 and CFM-5, respectively followed by TE-30 (49.15%), AMX-30 (47.46%) and COT-25 (45.76%). In a previous study, Saifullah *et al*. (2016) revealed multi-drug resistance *Salmonella* where the highest resistance was found against Ampicillin (88.23%) followed by Cephalexin (82.35%). The rate of sensitivity of the isolates was higher to Ciprofloxacin (100%) followed by Azithromycin (82.35%) Gentamicin (76.47%) and Nalidixic acid (76.47%). In this section, the variation was found in the sensitivity pattern of *Salmonella* isolates against CFM-5, GEN-10 and S-10. A number of isolates also showed intermediate reaction to CL-10 (45.76%), CTR-30 and COT-25 (30.51%), AMX-30 (28.81%), S-10 and CFM-5(27.12%), GEN-10, CXM-30 and AMP-25 (25.42%) and TE-30 (23.73%).This is

similar to what was reported in India in which resistance was 18% amoxicillin-clavulanic acid, 18% ampicillin, 20% cefotaxime (Arora *et al*., 2013). The frequency and extent of *Salmonella* spp. resistance to antimicrobial drugs varies based on their usage in animal production and humans as well as on ecological differences in the epidemiology of *Salmonella* spp. infections (Zhao *et al*., 2008).

In the present study, all the *Salmonella* isolates from broiler liver found to be sensitive to CTR-30 (50%) followed by GEN-10 (46.67%) and S-10 (46.67%) showing marked resistance to AMP-25 (56.67%), TE-30 (50%), CFM-5 (50%) and COT-25 (50%) followed by CXM-30 and AMX-30 (43.33%), S-10 (26.67%), GEN-10 and CL-10 (23.33%) and CTR-30 (16.67%) which were similar with the findings of Saifullah *et al*. (2016) and they showed that the isolates were 12.5%, 62.5%, 37.5%, 25% and 0% resistant to GEN, AMP, CN (Cephalexin), NA and AZM respectively. Under the present study, a number of isolates also showed intermediate reaction to CL-10 (50%) followed by CTR-30 and AMX-30 (33.33%).

In the present study, all the *Salmonella* isolates from broiler heart found to be sensitive to GEN-10 and CTR-30 (55.17%) followed by S-10 (48.28%), CL-10 (31.03%) and TE-30 and COT-25 (27.59%) showing marked resistance to CXM-30 (65.52%) followed by CFM-5 (51.72%), AMX-30 (51.72%), TE-30 (48.28%) and AMP-25 (48.25%). This finding agrees with another study conducted in Bangladesh, which reported the similar resistance trend (Gonçalves-Tenório *et al*., 2018). In a previous study Hosain *et al*. (2012) revealed that 80% of the *Salmonella* isolates were sensitive to ciprofloxacin followed by sulphamethoxazole (70%), chloramphenicol (60%), kanamycin (60%), gentamicin (60%) and nalidixic acid (60%), on the other hand 90% of the *Salmonella* isolates were found resistant to amoxicillin (90%), followed by ampicillin (80%), erythromycin (80%) and tetracycline (60%).In general, antimicrobial resistance in

bacteria occurs when the bacteria develop a mechanism to survive in the presence of antimicrobials (McManus *et al*., 1997).

The intermediate sensitivity shown by the heart isolates of broiler was CL-10 (41.38%), COT-25 (31.03%), S-10 (27.59%), CFM-5 (27.59%), CTR-30 (27.59%), and AMP-25 (27.59%). This may be an intermediate phage for the conversion of *Salmonella* isolates from sensitive to resistant form.

The significance of occurrence of antibiotic resistance in food-borne pathogens have been increased sharply and probably linked with the extensive use of antimicrobial agents in veterinary medicine and human (Bronzwaer *et al*., 2002). Several species of *Salmonella* are known to carry multi drug resistant genes (Gebreyes and Altier, 2002) which have been a matter of concern.

Based on the present study, it may be concluded that CTR-30, GEN-10 and S-10 will be the first drugs of choice CL-10 and CFM-5 will be the second drugs choice to resist the infections caused *by E. coli* and *Salmonella* in broiler and as well as human, cattle, sheep, goat, chicken and duck.

It may be noted that the determination of drug sensitivity and resistant pattern may be valuable as background information for the use of future therapeutics to control the bacterial diseases effectively. Otherwise, indiscriminate use of antimicrobial drugs may lead to the development of drug resistant mutants causing serious health hazards of different animals and birds including human being. However, routine laboratory isolation and drug sensitivity determination of the organisms is impracticable. So, periodical checking of the drug sensitivity and resistance pattern of the organisms remains more important to select the best drug of choice for the treatment of diseases caused by the infectious diseases.

CHAPTER 5

SUMMARY AND CONCLUSION

The present study was conducted for isolation and identification of *E. coli* and *Salmonella* spp. microorganisms from broiler and also to perform clinical investigation of common bacterial causes of death of broiler in small holder farming system in Bangladesh.

After collection, the samples were subjected to various tests and experiments for isolation and identification of organism in broiler. It is reported that *E. coli* and *Salmonella*. Primary isolation was performed by propagating the organisms in nutrient broth followed by culture on different agar media such as EMB agar, MacConkey agar, BG agar, SS agar and nutrient agar for the determination of their colony characteristics.

From 150 dead broiler (liver + heart) samples, 75 for *E. coli* and 75 for *Salmonella* were subjected as two different samples. Total Viable Count (TVC) and Total Coliform Count (TCC) were done by 10 fold dilution method. Among total isolates, 67 was *E. coli* and 59 were *Salmonella* and the rest 24 couldn't be identified in this study. They were identified on the basis of colony morphology. Biochemical properties of the isolates were studied by fermentation test with five basic sugars and also by MR test, V-P test and Catalase test.

The study was also extended to investigate in vitro sensitivity and resistance pattern of the *E. coli* and *Salmonella* spp. isolates to different drugs. Study revealed that there were considerable variations among the isolates of different sources in respect of drug sensitivity and resistance pattern.

A high percentage of *E. coli* isolates from the broiler were sensitive to CTR-30, GEN-10 and S-10 followed by CL-10 and CFM-5 while most of the *E. coli* isolates were resistant to TE-30, AMP-25, CXM-30, AMX-30 and COT-25.

In case of *Salmonella* isolated good sensitivity found against CTR-30 followed by GEN-10 and S-10 while most of the *Salmonella* spp. isolates were resistant to CXM-30, AMP-25, CFM-5, TE-30 and AMX-30.

It is assumed that one or more drug resistant clones have gradually acquired resistance to other drugs by conjugation with multi-drug resistant strains.

From the present study it may be concluded that

a) *E. coli* infections of different animals and birds and also of human being may be treated effectively with CTR-30, GEN-10 and S-10. Infection with *Salmonella* spp. can be treated with CTR-30 followed by GEN-10 and S-10.
b) Liver and heart samples collected from the broiler farm and markets near Dhaka are infected with *E. coli* and *Salmonella* spp. Identified bacteria from the broiler was *E. coli* and *Salmonella*.

Indiscriminate use of antimicrobial agents should be avoided in order to eliminate health hazards in man and animals caused by *E. coli* and *Salmonella* through preventing the development of multi-drug resistant mutants in nature.

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APPENDIX

Appendix I. Composition of different media

1. Nutrient broth

Peptic digest of animal tissue	:	5.0 gm.
Sodium chloride	:	5.0 gm.
Beef extract	:	1.5 gm.
Yeast extracts	:	1.5 gm.
Distilled water	:	1000 ml
Final pH (at 25ºC)	:	7.4 ± 0.2

2. Nutrient Agar

Peptone	:	5.000 gm.
Sodium chloride	:	5.000 gm.
HM peptone B#	:	1.500 gm.
Yeast extracts	:	1.500 gm.
Agar	:	15.000 gm.
Final pH (at 25°C)	:	7.4±0.2

3. MacConkey Agar

Peptones (meat and casein)	:	3.000 gm.
Pancreatic digest of gelatin	:	17.000 gm.
Lactose monohydrate	:	10.000 gm.
Bile salts	:	1.500 gm.
Sodium chloride	:	5.000 gm.
Crystal violet	:	0.001 gm.
Neutral red	:	0.030 gm.
Agar	:	13.500 gm.
pH after sterilization(at 25°C)	:	7.1±0.2

4. Eosin Methylene Blue Agar

Peptic digest of animal tissue	:	10.000 gm
Dipotassium phosphate	:	2.000 gm.
Lactose	:	5.000 gm.
Sucrose	:	5.000 gm.
Eosin - Y	:	0.400 gm.
Methylene blue	:	0.065 gm.
Agar	:	13.500 gm.
Final pH (at 25°C)	:	7.2±0.2

5. Brilliant Green Agar Medium

Peptone	:	5.000 gm.
Tryptone	:	5.000 gm.
Yeast extracts	:	3.000 gm.
Lactose	:	10.000 gm.
Sucrose	:	10.000 gm.
Sodium chloride	:	5.000 gm.
Phenol red	:	0.080 gm.
Brilliant green	:	0.0125 gm.
Agar	:	20.000 gm.
pH after sterilization (at 25°C)	:	6.9±0.2 gm.

6. Salmonella-Shigella agar

Protease peptone	:	5.000 gm.
Lactose	:	10.000 gm.
Bile salts mixture	:	8.500 gm.
Sodium citrate	:	8.500 gm.
Sodium thiosulphate	:	8.500 gm.
Ferric citrate	:	1.000 gm.

Brilliant green	:	0.00033 gm.
Neutral red	:	0.025 gm.
Agar	:	13.500 gm.
Final pH (at 25°C)	:	7.0±0.2

7. Mueller Hinton Agar

HM infusion B from	:	300.000
Acicase	:	17.500
Starch	:	1.500
Agar	:	17.000
Final pH (at 25°C)	:	7.4±0.1

8. Methyl Red Indicator

Methyl red	:	0.200 gm.
Ethyl alcohol	:	60.000 ml
Distilled water	:	40.000 ml

9. Voges–Proskauer (MR-VP) broth

Buffered peptone	:	7.000
Dextrose	:	5.000
Dipotassium phosphate	:	5.000
Final pH (at 25°C)	:	6.9±0.2

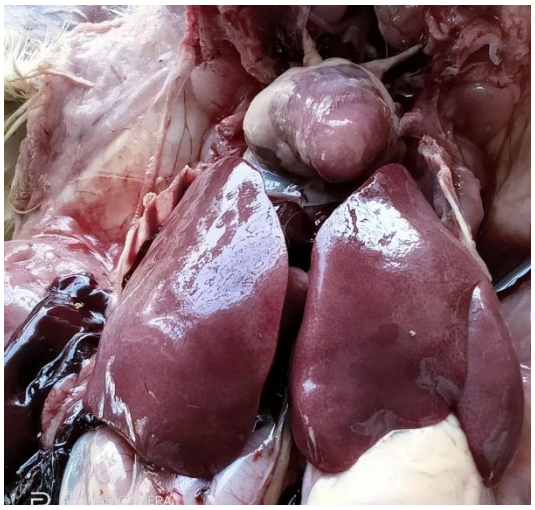
10. Phosphate buffer saline

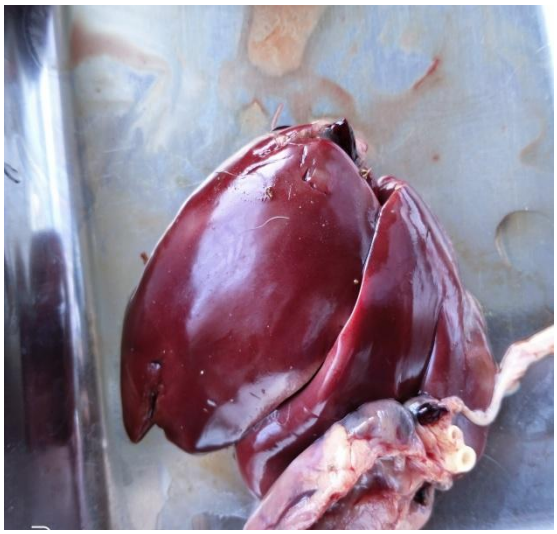
Sodium chloride	:	8.0 gm
Disodium hydrogen phosphate	:	2.8 gm
Potassium chloride	:	0.2 gm
Potassium hydrogen phosphate	:	0.2 gm
Distilled water to make	:	1000 ml

Some photographs of experiment at Medicine & Public Health lab, SAU, Dhaka

Figure: Collection of dead broilers in local market of Dhaka city

Some photographs of experiment at Medicine & Public Health lab, SAU, Dhaka





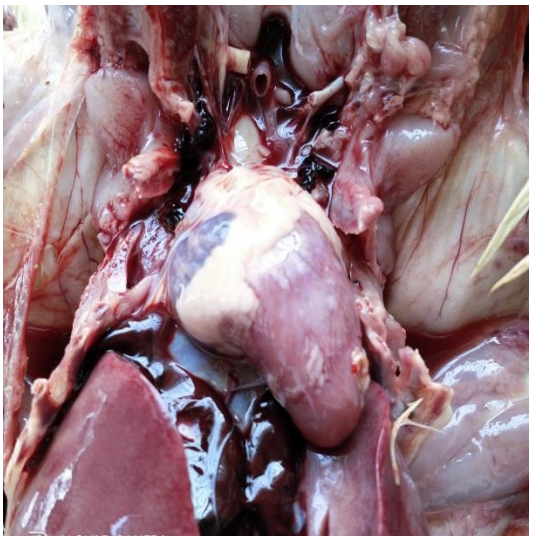


Figure: Post mortem of dead broilers

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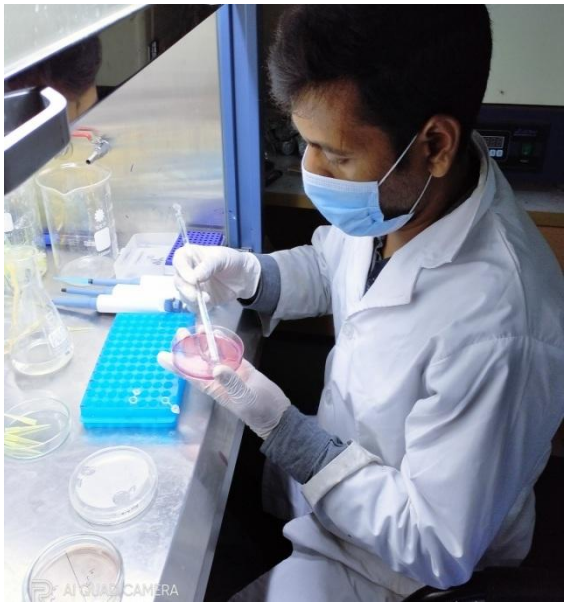
Figure: Preparation of media



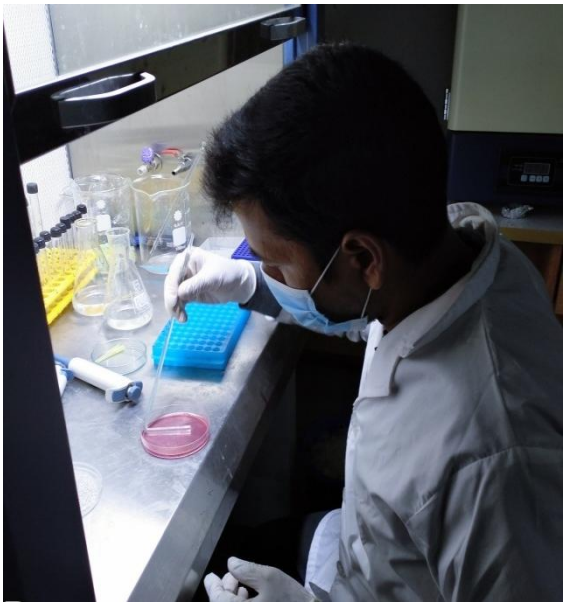


Figure: 10 fold dilution method

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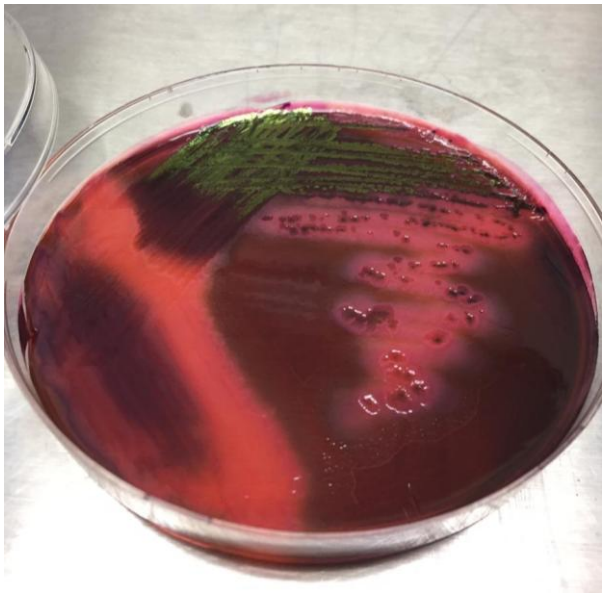

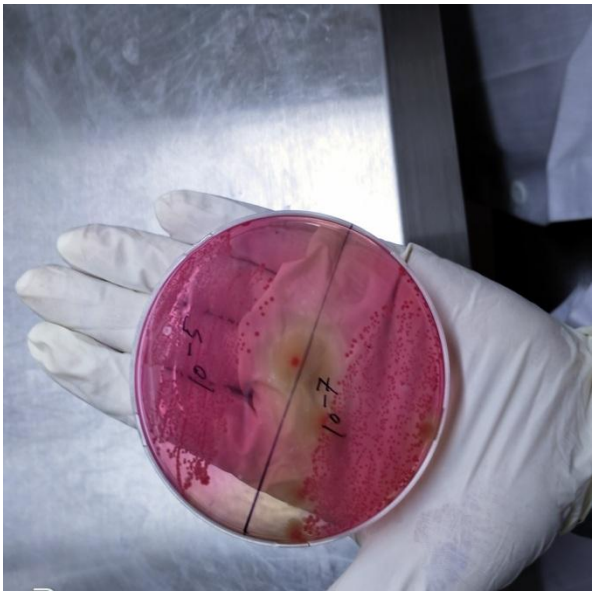

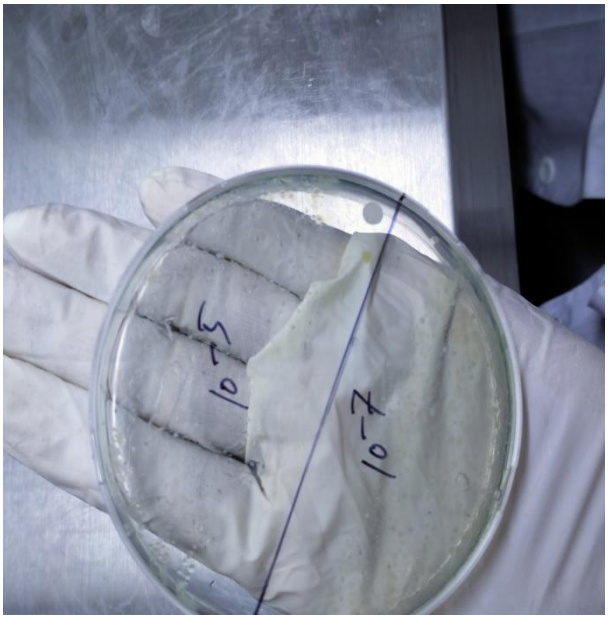

Figure: Bacteria cultured on EMB, MC, SS and NA media

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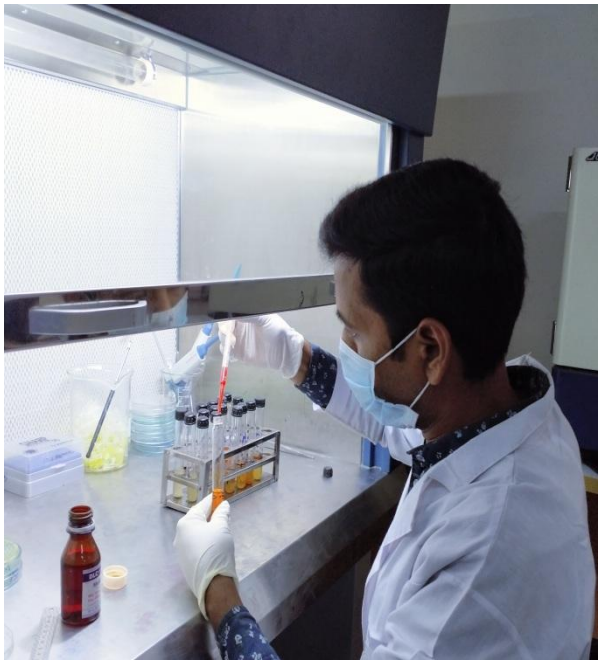

Figure: Biochemical test

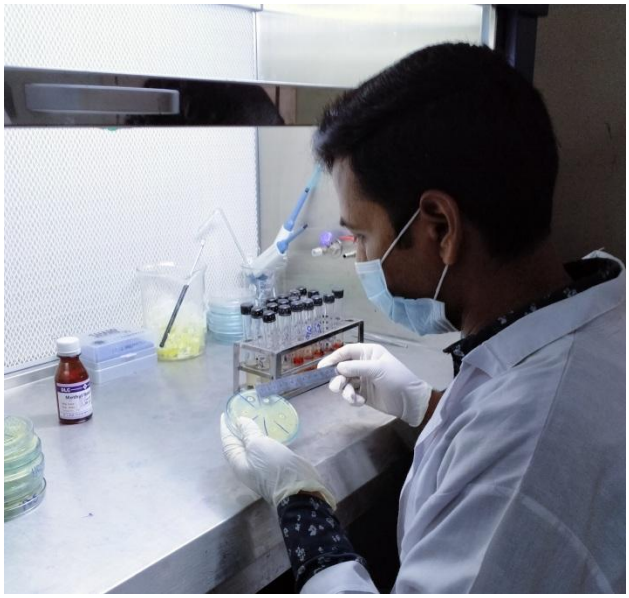

Figure: Antibiotic sensitivity test