IDENTIFICATION AND CHARACTERIZATION OF *Escherichia coli* FROM DRESSED CHICKEN MEAT IN DHAKA

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CERTIFICATE

*This is to certify that the thesis entitled, "**IDENTIFICATION AND CHARACTERIZATION OF Escherichia coli FROM DRESSED CHICKEN MEAT IN DHAKA**" submitted to the Department of Microbiology and Parasitology, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) in Microbiology**, embodies the result of a piece of bonafide research work carried out by **AMIT KUMAR BISWAS**, Registration No. **12-04773**, Session: **July-December/2018** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated:
Dhaka, Bangladesh

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DEDICATED TO
ASVM FAMILY

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ABSTRACT

Poultry is a major fast growing source of meat in the recent world. The consumers want a contamination free, safe and hygienic meat, which is free from any type of hazardous organism. This study has been done to find out the prevalence of *Escherichia coli* in different markets. The markets were different live bird markets and supermarkets. Also this study indicates the difference of *Escherichia coli* prevalence between live bird markets and the supermarkets in Dhaka city, Bangladesh. Samples from different markets were analyzed to determine the pathogenic bacteria *Escherichia coli.* It was isolated from the dressed meat sample of chicken through conventional cultural method. The samples were collected from twelve markets. Seven of the markets were live bird markets and other five were supermarkets. The markets were indicated as M1, M2, M3 etc for advantage while sampling. The markets were Savar Hut (M1), Shemulia Bazar (M2), Bagbari Bazar (M3), Bihari Camp (M4), Krishi Market (M5), Gigatola Bazar (M6), Taltola Bazar (M7), and Supermarkets were (M8), (M9), (M12), (M11) & (M10). From each market three samples were collected (S1, S2, S3). Result of this study demonstrated that the prevalence of *E. coli* was more in live bird market than the supermarkets. The average CFU/ml in live bird markets was 1.17534×10^{12} and the average CFU/ml of supermarkets was 3.37327×10^{11}. The *E. coli* prevalence percentage of live bird markets was 80.95% and in supermarkets it was 46.67%. So the pathogenic organism in the consumed meat of this type is alarming. This study also concluded recommendations that the personnel hygiene is crucial in processing and handling of poultry and poultry products.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATION	FULL WORD
EMB	Eosin Methylene Blue
et al.	and others
E. coli	*Escherichia coli*
H_2O_2	Hydrogen peroxide
H2S	Hydrogen Sulphide
MC	MacConkey
Mg	Milligram
MR	Methyl Red
No.	Number
PBS	Phosphate buffered solution
SAU	Sher-e-Bangla Agricultural University
Sp.	Species
TSI	Triple Sugar Iron
UTI	Urinary tract infection
VP	Voges-Proskauer
ºC	Degree Celsius
- /-ve	Negative
%	Percentage
+ / +ve	Positive
gm.	Gram
ml	Milliliter
M	Market
S	Sample
pH	Potential of Hydrogen
lbs.	pounds
sq.	Square
h	Hour
G	Glucose
L	Lactose
M	Maltose

LIST OF ABBREVIATIONS AND SYMBOLS (CONT'D)

ABBREVIATION	FULL WORD
S	Sucrose
D	Dextrose
A	Acid
G	Gas

CHAPTER 1

INTRODUCTION

Food is considered the most important energy source for humans and animals. Most of the foods contain viable bacteria unless heated or made sterile. Otherwise, it serves as an important medium for transmission of pathogenic organisms to the consumers. Contamination of food products with pathogenic organisms may influence considerably their harmlessness, endanger the health of consumers and decrease shelf quality resulting in food-borne infections, intoxications and economic losses from food spoilage. Meat may be easily contaminated with different pathogens if not handled appropriately (Mead *et al.*, 1999).

During 1980s, poultry industry was started as an excellent agribusiness in Bangladesh (Huque, 2001) and a tremendous development of this sector has been occurred since last decades (1996-2006) in the country (Rahman, 2003). Bangladesh is lacking in animal protein. The World Health Organization (WHO) prescribes 56 kg of meat and 365 eggs for every head per annum at the same time, in Bangladesh utilization of meat is just 14.57 kg. To defeat this tremendous meat deficiency in the nation poultry can assume a vital job. This is why the number of inhabitants in poultry has been believed to develop rapidly (from 198.0 million of every 2002-03 to 304.17 million of every 2016-2017) and all the more quickly contrasted with other domesticated animal species for instance, dairy animals (from 22.5 million out of 2002-30 to 25.7 million out of 2016-17). Almost certainly, generation of poultry has expanded further in the last a few years. This is believed to be because of that poultry requires less speculation to begin with the cultivating and people from low pay gathering may also begin the business on a small scale. Poultry cultivating also offers open doors for low maintenance business especially for town ladies, kids and old people on the ranch activity. Meanwhile, the division has been a method for potential pay age and neediness alleviation, just as improving human food through the stockpile of meat to their day by day life (Akond *et al.*, 2009).

In spite of the fact that poultry meat gives nutritionally beneficial food containing protein of top notch, contamination of poultry meat can prompt food contamination in people through preparing, taking care of, marketing and capacity preceding cooking.

The fundamental causative operators of human intestinal contaminations from this source are microbes, essentially *Salmonella* spp., *E. coli*, *Staphylococcus* spp. what's more, *Campylobacter* spp. (FAO, 2013).

Escherichia coli is one of the common microbial flora that is found in the gastrointestinal tract of poultry and human being including other animals. It may become pathogenic to both poultry and human (Levine, 1987) although most isolates of *E. coli* are nonpathogenic. About 10 to 15% of intestinal coliforms are opportunistic, pathogenic serospecies. They are related with regularly serious illnesses and sometimes with lethal diseases, for example, meningitis, endocarditis, urinary tract contamination, septicemia, pestilence looseness of the bowels of grown-ups and youngsters (Akond *et al.*, 2009). What's more, yolk sac disease, omphalitis, cellulitis, swollen head disorder, coligranuloma, and colibacillosis are brought about by *Escherichia coli* (Gross, 1994).

In many creating nations, food borne illnesses episode, for example, *Escherichia coli* force a substantial weight on health care frameworks and can especially decrease the monetary profitability of the nations. Food borne illnesses, brought by the operators that enter the body through the admission of contaminated food materials are one of the essential general health concerns (Tan *et al.*, 2013). It influences the individual's prosperity, and forces financial effects (Akbar and Anal, 2013). Countless intense diarrheal cases revealed each year (Hanson *et al.*, 2002; Minami *et al.*, 2010). Among the food borne pathogens, *E. coli* and *Salmonella* are the most widely recognized and visit pathogens answerable for food contamination and food related diseases. *Escherichia coli* is liable for 25% of the baby loose bowels in creating nations (WHO, 2000). Enteropathogenic, enteroinvasive and enterotoxigenic sorts of *E. coli* can be a main source of food borne loose bowels (Akbar and Anal, 2011). It is difficult to distinguish the pathogen and food vehicle liable for most of food borne infection. Poultry meat can contain the pathogens like *E. coli* and (Hughes *et al.*, 2007). Unhygienic rehearses utilization of sullied instruments and materials in food preparing are principally connected with food borne maladies (Wilfred *et al.*, 2012; Akbar and Anal, 2014). *Escherichia coli* is known to be a marker of fecal pollution, and its essence in food show the conceivable similarity of other enteric pathogens. Some of the *E. coli* strains itself are exceptionally pathogenic in human and animals.

Individuals with low resistance are the ideal objective of the pathogenic strains of *E. coli* (Akbar and Anal, 2011).

These food borne bacterial pathogens are found in foods when the food sources are presented to a constrained warmth treatment or served crude to the customer (Wu *et al.*, 2000). Generally, the individuals living in urban network of Bangladesh depend on the local live winged animal market and supermarkets for poultry meat completed an examination on prepared chicken meat from live feathered creature market and pre-dressed crude chicken meat from various supermarkets of Dhaka city, Bangladesh where they recognized *E. coli* in the chicken meat (Alam *et al.*, 2015). In any case, there are generally not many reports on food borne microorganisms in chicken meat from the live winged creature market/supermarkets of Dhaka city.

Considering the above realities that (i) there is an absence of data on the prevalence of *E. coli* in prepared/dressed chicken meat, (ii) absence of data about the better handled or dressed chicken meat of local live market and supermarkets in Dhaka city market the present investigation was intended to satisfy the accompanying objectives.

Objectives of the study

- To determine the prevalence of *E. coli* in retail broiler (chicken) meat in Dhaka city market (Bangladesh).
- To differentiate the *E. coli* load between the live bird market and the supermarket dressed chicken meat.

CHAPTER 2

REVIEW OF LITERATURE

Food-borne diseases, caused by agents that enter the body through the intake of contaminated food materials are one of the primary public health concerns (Tan *et al.*, 2013). It affects the people's well-being, and imposes economic impacts (Akbar and Anal, 2013). In developing countries, food-borne diseases outbreak from bacteria, such as *Escherichia coli*. *Escherichia* spp. imposes a substantial burden on health care systems and can markedly reduce the economic productivity of the countries. A huge number of acute diarrheal cases reported each year, while the reported cases of food poisoning are more than 120,000 per year in Thailand (Hanson *et al.*, 2002; Minami *et al.*, 2010). Amongst the food-borne pathogens, *E. coli* and *Salmonella* spp. are the most common and frequent pathogens responsible for food poisoning and food related infections. *Escherichia coli* is responsible for 25% of the infant diarrhoea in developing countries (WHO, 2000). Enteropathogenic, enteroinvasive and enterotoxigenic types of *E. coli* can be a leading cause of food- borne diarrhoea (Akbar and Anal, 2011). It is hard to identify the pathogen and food vehicle responsible for the majority of food-borne infection. Poultry meat, red meat, desserts and egg can transfer the pathogens like *Salmonella*, *E. coli* and *Campylobacter* (Hughes *et al.*, 2007). Unhygienic practices, use of contaminated instruments and materials in food processing are mainly associated with food-borne diseases (Wilfred *et al.*, 2012; Akbar and Anal, 2014). *Escherichia coli* O157:H7 are mostly associated to food materials. The low infectious dose and life-threatening complication has made this organism an important pathogen and serious threat to public health (Akkaya *et al.*, 2006). The outbreaks associated to this organism are mostly associated to food of bovine origin, ground meat and raw milk (Bachrouri, 2002).

2.1 Food borne disease challenge

Today there is an increasing concern over food borne pathogens spreading from farm animals to human populations. Epidemiological data have demonstrated that a significant source of drug-resistant food borne infections in humans is the acquisition of resistant bacteria originating from animals. This source of infection has been demonstrated through several different types of food borne disease follow-up

investigations, including laboratory surveillance, molecular subtyping, and outbreak investigations (Holmberg *et al.*, 1984).

More studies have confirmed that using antimicrobial drugs in poultry increases the risk of selecting for resistant food borne pathogens, and that these pathogens can then be transferred to humans through direct contact with either contaminated food or animals (Van den Bogaard *et al.,* 2001). Due to the lack of alternative strategies, most attempts to control gastrointestinal tract microflora in chickens have so far relied on the use of broad-spectrum antibiotics. However, the recent and widening concern over disseminating antibiotic resistance genes has led to bans on the prophylactic use of many antibiotics in a number of countries. In indigenous chicken, the diet and the environment affect the microbial status of the gastrointestinal tract.

Dirty litter and other animal management parameters affect microbial composition of the chicken gastrointestinal tract by providing a continuous source of bacteria through ingestion (Apajalahti *et al.,* 2004). Raw/dressed retail chicken meats are potential vehicles for transmitting food borne diseases. Additionally, these retail chicken meats are often associated with direct hand-to-mouth exposure to enteric pathogens and cross-contamination of the kitchen environment and ready-to-eat foods (Zhao *et al.*, 2001).

Many infections are transmitted through food and cause illness ranging from mild gastroenteritis to severe illness requiring hospitalization. The task of providing accurate information on trends in specific food borne pathogens capable of causing syndromes is at the hands of researchers (Pinner *et al.*, 2003). *E. coli* is prominent food pathogen. Factors influencing the occurrence of food borne illnesses are complex and include human population increase, poverty, changing life-styles-including more adventurous eating, more convenience foods, less time devoted to food preparation; ever-evolving technologies for food production, processing, distribution, and emergence of newly recognized microbial pathogens (Jianghong *et al.*, 2002).

2.2 *Escherichia Coli (E. coli)*

2.2.1 Historical background

Escherichia coli (*E. coli*) originally called "*Bacterium coli*", first isolated from the stool of a 2-3 days old new-born baby and subsequently from young calves in 1885 by Theodore Escherich (Buxton and Fraser, 1977; Sousa, 2006). The name of bacteria was later changed to honor its discoverer (Feng *et.al.,* 2002).

2.2.2 Outbreaks of *Escherichia coli*

A number of outbreaks causing HC (Hemorrhagic or Gangrenous Colitis), HUS (Hemolytic Uremic Syndrome) and diarrhoeic illness due to pathogenic *E. coli* have been reported from all the corners of the world. Richard (1998) reported 52 cases of HC and HUS caused by O157:H7 *E. coli* in people consuming undercooked beef and pasteurized milk. The investigation of idiopathic HUS in children revealed 75.00 percent patients infected with *E. coli* and a similar study of patients with diarrhoeic illness showed the presence of *E. coli* in 14.80 percent with bloody diarrhoea but failed to recover *E. coli* from 45 patients with non-bloody diarrhoea (Waltner, 1990).

In Japan between 1984 and 1993 four outbreaks were reported due to non- O157:H7 and five due to O157: H7 *E. coli*. Mortality rate due to VTEC infection reported to be varied from 2 to 10 per cent (Griffin and Tauxe 1991; Pickering *et al.*, 1994). In certain geographic areas, new sero-types of non-O157 including O26, O55,O68, O103, O111, O128 and O145 were associated with HC and HUS (Ritchie *et al.*,1992; Kudoh *et al.*, 1994). Non-O157 *E. coli* were also found frequently in sporadic HUS cases in India (Kishore *et al.*, 1992), but the true frequency of outbreaks still remains unknown as poor surveillance system.

The recent study conducted in the US reported 20,000 annual cases of pathogenic *E. coli* infection and 250 deaths due to vero-cytotoxigenic *E. coli* (Griffin and Tauxe, 1991; Boyce *et al.*, 1995). Bell *et al.* (1994) reported a multistate outbreak in the USA during 1992-1993 where the consumption of hamburger was responsible for 583 cases. The first outbreak of non-O157: H7 *E. coli* associated with HC and HUS in the US was reported in 1994 where *E. coli* O104: H21 was also confirmed in 11 cases. Several outbreaks were also reported from UK, Italy, Germany and New-Zealand. The prevalence of non-O157:H7 *E. coli* among HUS patients increased from 68.0 per

-cent (1987 to 1990) to 86.0 percent (1994-1995) in Australia (Goldwater and Bettelheim 1994; Elliot *et al.,* 1995; Johnson *et al.,* 1996).

The largest human outbreak of *E. coli* O157 infection in Japan occurred in 1996 (Sekiya, 1997) as well as outbreaks of *E. coli* O26 and O111 have also been reported increasingly from Japan but the true frequency still remain unknown (Ritchie *et al.,* 1992; Caprioli *et al.,* 1994; Kudoh *et al.,* 1994).

More recently there have been a number of reports from around the world of human cases of both bloody diarrhoea (BD) and HUS caused by EHEC 0103:H2 including reports from Germany (Beutin *et al.,* 1998), the USA (Tarr *et al.,* 1996 and Meng Doyle, 1998) as well as outbreaks in France.

2.2.3 Sources of contamination during process in retail market

Escherichia coli in 6.6 percent samples of equipment's and working surfaces and 11.6 per cent samples of hands. He reported the presence of *E. coli* and other organisms in large numbers from employee's hands in food processing units. Kendereski (1970) reported the worker's hands as potential source of contamination. Worker's hands were found to be frequently contaminated by nose, mouth and other secretions. Pohja (1971) reported that during dressing the carcass, contamination was brought by bacteria in soil and manure carried with the animals. The main sources were knives and worker's hands.

Presence of *E. coli* in all the samples from the equipment in slaughter house were reported by Ismail *et al.* (1972) from swabs collected during slaughter. They also stated the food processing equipment must be protected from contamination. The entire surface in contact with food must be inert smooth and non-porous. Wit and Kampelmacher (1981) reported that worker's hands in different food establishments (including slaughter houses) frequently carried pathogenic *E. coli*. They found 4-100 percent workers carried *E. coli* on their hands. They also isolated *E. coli* even after washing the hands but with considerable reduction in numbers. Workers and co-workers carried out a microbiological survey of fresh meat in supermarket in 1989. They revealed no consistency in the contamination level of different parts of carcasses of different supermarkets, although they found fore quarters to be more contaminated than hind quarters and hands, clothes and equipment like saws and mincers play vital

role in the contamination at the retail market. Pathak (1992) reported that sources of microbes in meat and meat products could be animal itself or the environment as air, floor, water, soil and handlers themselves. Deeper parts of meat are generally sterile, unless the animals are suffering from infectious diseases or the organisms are transferred from intestine via blood or lymphatics before slaughter. (Gill *et al.,* 1994) examined 150 swabs from butchers' hands and their equipment (chopping blocks and chopping knives), floors of slaughter houses and meat shops for the detection of microorganisms. *E. coli* was isolated from 17 swabs with maximum isolation from chopping blocks (18.00 %) and minimum was from butchers' hands (2.50 %). All swab samples from chopping blocks and floor were positive for bacterial growth indicating their potential role in meat contamination. (Tessi *et al.,* 2002) reported *E. coli* on knives, plastic lugs and on the surface of wood cutting boards after they were used in Argentina during study to evaluate the microbiological and sensory quality as well as the safety of ready to eat cooked food.

2.2.4 *Escherichia coli* and public health significance

Certain strains of *Escherichia coli* produce toxins, which leads to haemorrhagic enteritis (Ramachandran and Varghese, 1987). Kapoor and co-workers (1982) recovered a total of 240 *E. coli* isolates from faecal samples from four hospitals in 1995. It was noted that *E. coli* isolates produced LT (heat labile) and 34 ST (heat stable) and 28 produced both LT and ST enterotoxins. In that study, *E. coli* sero-groups O2, O7, O17, O35, O127 and O128 produced verotoxin, which were involved in the haemorrhagic enteritis. Serotype O9 has earlier been isolated from human peritoneum (most often appendicitic peritonitis), human urine, urinary infection, mastitis, coli granuloma disease (Anitakumari *et al.*, 2002).Serotype O9 was found highly enterotoxigenic and produce enterotoxin (Moon and Whip, 1970; Harnet and Gyles, 1985). Serotypes O155, O156 and O109 have earlier been isolated from fecal samples of human being (Orskov *et al.*, 1977), serotype O156 has been reported to be enterotoxigenic. Serotypes O2, O4, O6, O18 and O75 are considered common in urinary tract infection (Cooke, 1985). In a detailed study, McGeachie (1965) recorded O1, O4 and O6 to be commonest serotypes. Similarly Kenny *et al.* (1966) reported, O1, O2, O4 in severe outbreaks at two hospitals. Serotypes O6, O8, O20, O13, O18, O26, O55, O69, O85 and O11 have been recovered from cases of urinary tract infection in various parts of India viz., Amritsar (Arora and Chitkura, 1972),

Visakhapatnum (Suri and Bhaskaran, 1972), Rohtak (Chugh *et al.*, 1978) and Bareilly (Kulshrestha *et al.*, 1978 and Kapoor *et al.*, 1982). *E. coli* is considered to be responsible for 70-95 per cent of urinary tract infection (Delisle and Ley, 1989). Kapoor and Kulshrestha (1998) isolated *E. coli* from 70 samples (60.68 %) out of 115 patients of urinary tract infection at different hospitals nearby IVRI (UP). It has been shown that *E. coli* can be isolated from as many as 77.00 per cent cases of UTI (Mabeck, 1971). Only 57 isolates of *E. coli* (81.42 %) could be serotyped. The frequency of serotypes isolated in descending order was O6 (six isolates),O1, O2, O4 (five each), O8 (4), O20, O131 (three each) and O78, O26, O55, O69, O85,O11, O140 (two each) while the remaining 12 serotypes (O5, O11, O23, O24, O35, O41,O44, O53, O71, O75, O86 and O128) were one each (Kapoor and Kulshrestha, 1998). Singh *et al.* (1996) isolated serotypes O8, O77, O112, O147 and O165 from 200 attendants in Bihar. Serotype O139 found involved in oedema disease as first case of oedema was diagnosed in Denmark in 1994 caused by *E. coli* strain of serogroup O139 (Aarestrup *et al.*, 2001). Yasuoka *et al.* (2002) obtained vaginal *E. coli* (VEC) from Japanese women were distributed into 31 serotypes, including common serotypes O1, O4, O6, O18, O25 and O75 that were identified in three or more isolates, supporting the concept VEC are a reservoir along the ‒faecal-vaginal-urinary/neonatal‖ course of transmission in the extra intestinal *E. coli* infection.Most outbreaks and sporadic cases of HC and HUS have been attributed to strains of enterohaemorrhagic serotype O157:H7 (Karmali, 1989; Boerlin *et al.*, 1999; Blanco *et al.*, 2006; Mora, 2002). However, infections with some non-O157 such as O26, O91,O103, O111, O113, O117, O118, O121, O128, O145 and O146 are frequently associated with severe illness in human (Beutin, 1999; Blanco *et al.*, 2005; Mora, 2002).

2.2.5 Prevalence, isolation and identification of *Escherichia coli*

Foods of animal origin is considered as one of the important sources of human *E. coli* infection directed to various studies which confirmed that sheep harbor many serotypes in gastrointestinal tract and sheep have above 40.00 per cent carriage rate of pathogenic *E. coli* in their faeces (Sidjabat and Bensink, 1997). The information available on the prevalence of *E. coli* particularly in mutton is very limited. A total of 876 samples were tested for isolation and identification of *E. coli* from different retail outlets of meat. *E. coli* O157:H7 was isolated from 6 (3.7%) of 164 beef, 4(1.5%) of

264 pork, 4 (1.5%) of 263 poultry, and 4 (2.0%) of 205 lamb samples. Read *et al.* (1990) revealed that VTEC prevalence in meat processing plants of South-western Ontario, Canada was 36.4% in beef, 10.6% in pork and zero in chicken. Samadpour *et al.* (1990) found that 17% food samples including beef, pork, lamb, chicken, turkey, fish find shellfish were VT -probe positive. Investigation conducted by many workers revealed *E. coli* 026:H11 to be similar from human as well as animal with diarrhoea suggested the animals as a possible source of infection for human beings. Fliss *et al.* (1991) screened 270 fresh meats of poultry, cattle, sheep and horses and reported the mean level of contamination (C.F.U./cm2) for all the meat samples varied from 5×104 to 3×105 for total aerobic mesophilic microflora with highest contamination of sheep and poultry. Dutta *et al*., (2011) isolated four strains of shiga toxin producing *E. coli* belonging to the serotype O157:H7 in foods and animals sources from Kolkatta. Banerjee *et al.* (2011) investigated vero cell cytotoxicity assay of *E. coli* strains isolated from different meat and meat products revealed 15.90 per cent isolates to be verotoxigenic. Blanco *et al.* (2001) reported 52.00 per cent ovine serotypes from humans including 24 serotypes associated with HUS. Brooks *et al.* (2001) isolated STEC from 12.1% beef samples, 17.1% lamb/mutton samples, 4.0% pork samples and none of the chicken samples. Chattopadhyay *et al.* (2001) carried out survey in Calcutta to isolate O157:H7 serotype from animal, human sources and some food products. The isolation rate was higher in diarrhoeic animals (6.02%) followed by diarrhoeic handler (3.12%) and raw meat (1.78%) samples. Zhao *et al.* (2001) tested 212 chicken samples, 82 (38.7%) yielded *E. coli* ,while 19.0% of the beef samples, 16.3% of the pork samples, and 11.9% of the turkey samples were found positive for *E. coli*. D'Incau *et al.* (2006) isolated 105 *E. coli* strains during the period 2000-2004 in Lombardia and Emilia Romagna (North Italy) from avian species (poultry and turkeys).

Lee *et al.* (2009) examined three thousand meat samples for the presence of *E. coli* (2004-2006) in Korea. 273 *E. coli* isolates were obtained from beef, poultry and pork, resulting in an overall isolation rate of 9.1%. Of these isolates, 201 were obtained from 1350 pork samples (14.9%), followed by 41 of 900 poultry samples (4.6%) and 31of 750 beef samples (4.1%). Nzouankeu *et al.* (2010) collected 150 chickens from eight retail markets in Yaounde and examined for the presence of *Campylobacter*, *Escherichia coli*, and *Salmonella* using standard bacteriological procedures. Of the

150 chickens collected, 135(90%) were contaminated with *Campylobacter* (68.9% *C. coli* and 31.1% *C. jejuni*). All the chickens were positive for *E. coli*. Among the 150 isolates, 17 (11.3%) were enteropathogenic *E. coli* (EPEC). Vincent *et al.* (2010) carried out isolation of *E. coli* from clinical UTI samples, retail meat (chicken, raw beef and pork) and restaurant/ready to eat foods (chicken, beef, pork, sea foods and other meat) during 2005-2007. They isolated about 20% *E. coli* from retail beef meat and 18% from restaurant/ready to eat foods of beef origin. Dutta *et al.* (2011) isolated and identified *E. coli* from rectal swabs, intestinal contents, heart blood and spleen of 19 poultry birds that died due to acute diarrhea during the outbreak. Silva *et al.* (2011) isolated *Escherichia coli* from chicken livers from two slaughter houses were genotypically characterized in 62 samples. Thirty samples were macroscopically unchanged and 32 demonstrated alterations that led to the disposal of carcass for sanitary inspection. Thirty *Escherichia coli* strains from 21 unchanged and 9from carcasses that were rejected were isolated through the classical method.

2.2.6 Growth and inactivation

Escherichia coli is a facultative anaerobe that can grow from 7ºC to 50ºC with an optimum temperature of 37ºC, although there have been reports of some ETEC strains growing at temperatures as low as 4ºC (Adams and Moss, 2008). A near neutral pH is optimal for its growth but growth is possible down to pH 4.4 under otherwise optimal conditions. The minimum water activity for growth is 0.95 (Adams and Moss, 2008).

2.2.7 Biochemical properties

E. coli can be differentiated from other members of the Enterobacteriaceae on the basis of a number of sugar-fermentation and other biochemical tests. Classically an important group of tests used for this purpose are known by the acronym IMViC. These tested for the ability to produce: indole from tryptophan (I); sufficient acid to reduce the medium pH below 4.4, the break point of the indicator methyl red (M); acetoin (acetylmethylcarbinol) (V); and the ability to utilize citrate (C) (Adams and Moss, 2008). Despite *E. coli* can be identified with a variety of biochemical reactions, the indole test remains the most useful method to differentiate lack of production of β-glucuronidase. Sorbitol non fermenting strains of *E. coli* O157:H7 have been associated with colitis and hemolytic uremic syndrome (HUS) (Besser *et al.*, 1999).

2.2.8 Acid and salt tolerance

Escherichia coli is an acid resistant food borne pathogen that survives in the acidic environment of stomach and colonies the gastrointestinal tract (Price *et al.,* 2004). Furthermore, it also increases the survival of *E. coli* particularly STEC O157:H7 in acidic foods, enabling survival for extended periods, especially at refrigeration temperature (Meng *et al.,* 2007). Hence, contaminated cultured and fermented foods such as yoghurt and cheese have also been implicated in sporadic cases and the disease outbreaks (Baylis, 2009; Farrokh *et al.,* 2013).

2.2.9 Reservoir hosts

Domestic and wild animals are sources of *E. coli* but ruminants primarily sheep, goats and cattle have been identified as major reservoirs and source for human infection (Kiranmayi *et al.,* 2010; Rahimi *et al.,* 2012; Rehman *et al.,* 2013). Many serovars of EHEC associated with human infection like O91, O157 and O146 have been isolated from sheep (Ramachandran *et al.,* 2001; Urdhal *et al.,* 2003). O157 has also been isolated from goats (Pritchard *et al.,*2000).

2.2.10 Mode of transmission

E. coli is transmitted by ingestion of contaminated food and water, direct contact with animals, feces, contaminated soil and cross contamination directly from one person to another. Consumption of raw milk has been found to be of high risk for *E. coli* O157:H7 infections (CDC 2008; Denny *et al.,* 2008). Fermented sausage containing sheep meat was reported as a source of an STEC O103:H25 outbreak in Norway, while fermented sausage containing beef was the cause of an STEC O26:H11 outbreak in Denmark (Sekse *et al.,* 2009; Ethelberg *et al.,* 2009). VTEC O157 infection was associated with ready-to-eat foods, such as lemon-and-coriander chicken wraps (Whittaker *et al.,* 2009). The dispersion of untreated manure in the environment can cause the contamination of different items which could act as secondary source for human infection (McDowell and Sheridan, 2001).

CHAPTER 3

MATERIALS AND METHODS

3.1 Sampling

Table 1. Number of meat samples collected from different areas of Dhaka Zone

Sl. No.	Name of markets	No of samples
01.	Savar Hut (M1)	3
02.	Shemulia Bazar (M2)	3
03.	Bagbari Bazar (M3)	3
04.	Bihari Camp (M4)	3
05.	Krishi Market (M5)	3
06.	Gigatola Bazar(M6)	3
07.	Taltola Bazar (M7)	3
08.	Supermarket(M8)	3
09.	Supermarket (M9)	3
10.	Supermarket (M10)	3
11.	Supermarket (M11)	3
12.	Supermarket (M12)	3
Total	**12**	**36**

A total number of 36 poultry meat samples were collected (Table 1) from totally different open and super markets of Dhaka town in sterile polyethene baggage and kept in priorly disinfected sampling box. The samples were delivered to the Bangladesh Livestock Research Institute laboratory during a sampling box maintaining low temperature (≤4°C) using ice pads. The collected samples were dressed within six hours of its assortment. The samples were collected at random and each collected sample was marked with identification code with reference to the date and time of assortment. Sampling criteria was restricted to 150 gm. of one sample in open market and one packet in supermarkets.

3.2 Experimental design

The whole experimental design included isolation of the microorganism from chicken meat and identification of *E. coli* based on cultural properties, morphological characteristics and biochemical analysis. Motility check with hanging drop preparation and was conjointly done to confirm the isolated organism as *E. coli*.

Dressed meat samples were collected from the various areas of Dhaka (Table 1). Then they're cut into little pieces and taken into PBS solution. Further go for spread plate technique to calculate the CFU of *E. coli* in each individual sample to find out the microbial load. Then subcultures of *E. coli* were fully grown on NB media for obtaining pure culture. Pure cultures of the organism were subjected to morphological, cultural properties and biochemical analysis.

The following is a flow chart of representing design of the experiment

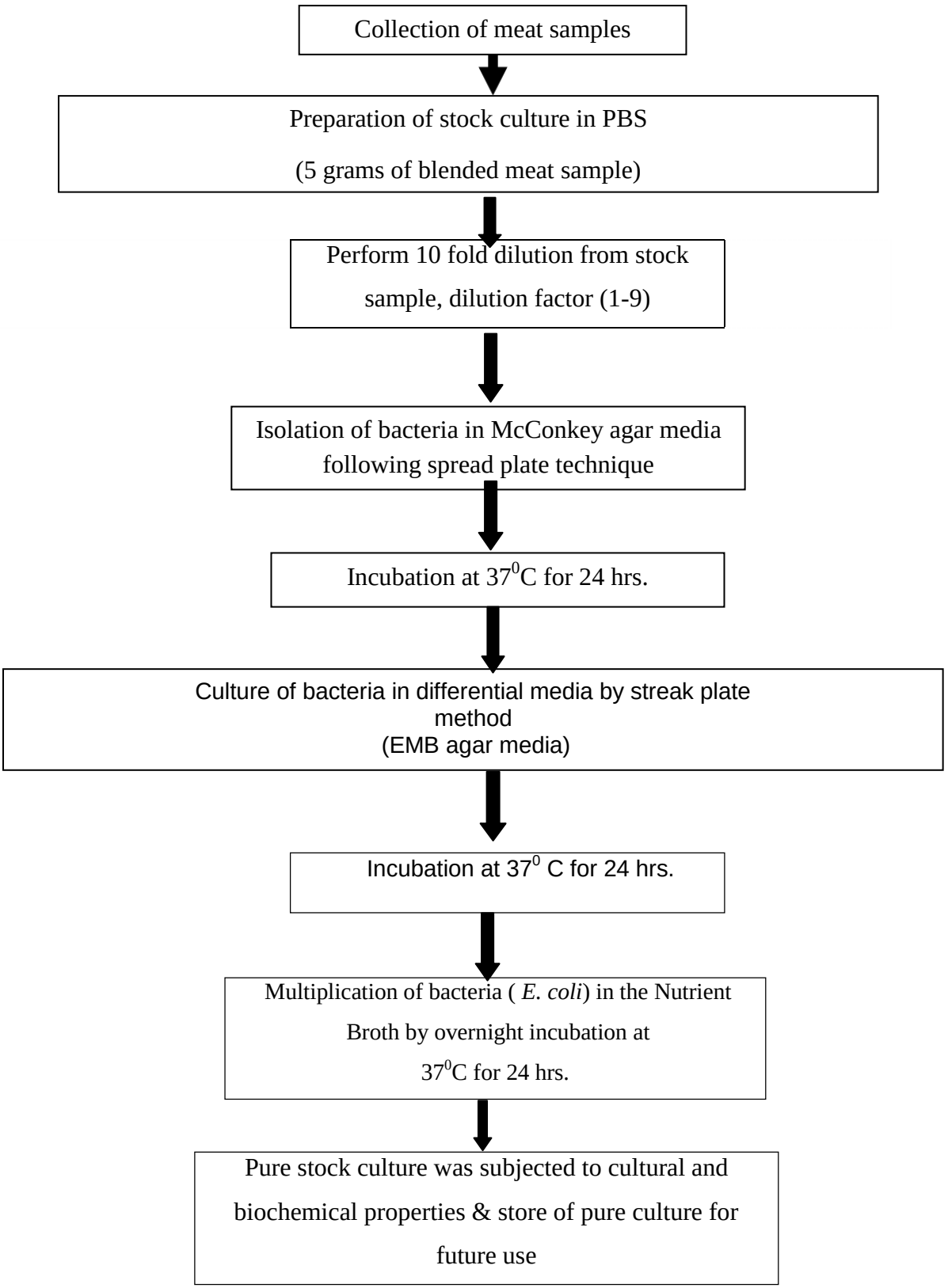


Figure 1: Schematic illustration of the experimental design.

3.3 Statistical analysis

Prevalence of each organism was obtained by dividing the number of positive samples with the total number of samples in each lot. Prevalence was expressed in percentage.

3.4 Materials

3.4.1 Media and reagents

3.4.1.1 Solid culture media

The media used for bacteriological analysis were, Eosin-Methylene-Blue (EMB), MacConkey agar (MC).

3.4.1.2 Liquid culture media (broth)

The liquid media used for this study were Nutrient broth (NB), Methyl-red and Voges-Proskauer broth (MR-VP).

3.4.1.3 Chemicals, reagents and solutions

The following reagent used during bacteriological study were phosphate buffered saline (PBS), reagents for Gram's staining (Crystal violet, Gram's iodine, Safranine, Acetone alcohol etc). Alcohol solution (100 mL bottle)

Reagents for methylene blue staining like (Methylene blue, ethyl alcohol, distilled water), Xylene, mineral oil, normal physiological saline solution and other common laboratory chemicals and reagents.

3.4.1.4 Media used for biochemical test

In order to identify bacterial species sugar media (dextrose, lactose, maltose, mannitol, and sucrose), Methyl Red and Voges-Proskauer broth (MR-VP broth). Motility indole urease (MIU) medium base (Himedia, India) was used for motility test.

3.4.2 Glass ware and other appliance

The different types of glass wares and appliances used during the course of the experiment were as follows:

Test tubes (with or without Durham's fermentation tubes and stopper), petridishes, conical flask (100 ml, 500 ml, and 1000 ml), cotton, slides and coverslips, Eppendorf tube, test tube stand, pipette, micropipette, incubator, refrigerator, sterilizing

instruments, hot air oven, autoclave machine, electronic machine, glass bit, compound microscope, whirly mixture machine.

3.5 Methods

3.5.1 Collection and transportation of sample

All meat samples of this study were collected aseptically using sterile instruments and transferred carefully to appropriate containers. The samples were carefully handled and kept in box. Due aseptic care was taken during transportation and the samples were kept in sterile container of ice box until these are prepared for bacteriological analysis.

3.5.2 Cleaning and Sterilization of glass wares and other appliances

New and previously used glass wares and plastic wares were dipped in 2% sodium hypochlorite solution and left there until cleaned. After overnight soaking in a household dish washing detergent solution ('Trix', Reckitt and Colman Bangladesh Ltd.), the glass wares were cleaned by brushing and washed thoroughly in running tap water and rinsed four times in distilled water. The cleaned glass wares were then dried by keeping on a bench at room temperature or in an oven at 50-70°C. The Petri dishes were wrapped with brown paper. This glassware was usually sterilized by dry heat at 160^0C for 1/2 hour in an oven. However, the bottles with plastic caps or rubber lined aluminum caps were sterilized by autoclaving at 121^0C for 15 minutes less than 15 pounds pressure per square inch (1 kg/cm^2). During autoclaving the caps were loosely fitted on the bottles. After autoclaving the glasses were immediately dried in an oven at 50-70^0C and the caps of the bottles were tightened after cooling.

3.6 Preparation of culture media

The commercial media were prepared according to the direction of the manufacturers and the non-commercial media were prepared in the laboratory. The composition and the procedures for the preparation of media are presented in the appendix.

3.6.1 Nutrient broth media

Nutrient broth was used as primary growth media of *Salmonella*, *E. coli* and *Staphylococcus* from the collected egg sample. Thirteen gm. of dehydrated nutrient broth (NB) base (Himeda, India) was dissolved in 1000 ml of distilled water, heated gently by an electric heater and then sterilized by autoclaving at 121°C under 15 lbs. pressure per square inch (1kg/cm^2) for 15 minutes (1 kg/cm^2). 10 ml broth was transferred in sterile tubes and then stored at 4°C in the refrigerator until use.

3.6.2 Eosin Methylene Blue (EMB) agar media

Eosin Thirty six 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 in water. The medium was autoclaved for 30 minutes less than 15 lbs. pressure per square inch (1 kg/ cm^2) to make it sterile. After autoclaving the medium was put into water bath maintaining 45°C and 10-20 ml of medium was poured into small and medium size sterile petridishes to make EMB agar plates. After solidifying the medium, the plates were kept in the incubator at 37°C for overnight to check their sterility.

3.6.3 MacConkey (MC) agar

51 grams of dehydrated Bacto-MacConkey agar (Himedia, India) was suspended in 1000 mL of cold distilled water taken in a conical flask and heated up to boiling to dissolve the medium completely. On sterilization by autoclaving, the medium was poured in 10 ml quantities in sterile glass petridishes (medium sized) and in 15 ml quantities in sterile glass petridishes (large sized) to form a thick layer there in. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours and then the covers of the petridishes partially removed. The sterility of the medium was judged and used for cultural characterization or stored at 4ºC in refrigerator for future use (Cowan, 1985).

3.7 Preparation of sugar media

Peptone water was prepared by adding 1 gm. of Bacto peptone (Difco, USA) and 0.5 gm. of sodium chloride in 100 ml distilled water. The medium was boiled for 5 minutes, adjusted to pH 7.0 cooled and then filtered through filter paper Phenol red, an indicator at the strength of 0.2 percent solution was added to peptone water and

then dispensed in 5 ml amount into cotton plugged test tubes containing a Durhum's fermentation tubes, placed inverse position. These were then sterilized by autoclaving at 121°C maintaining a pressure of 15 lb. pounds/sq. inch for 15 minutes. The sugar used for fermentation was prepared separately as 10 percent solutions in distilled water (10 gm. sugar was dissolved in 100 ml of distilled water). A gentle heat was applied to dissolve the sugar completely. The carbohydrate fermentation test was performed by inoculating a loop full of nutrient broth culture of the organisms into the tubes containing different sugar media (five basic sugars such as dextrose, sucrose, lactose, maltose and mannitol) and incubated for 24 h at 37°C. Acid production was indicated by the color change from reddish to yellow in the medium and the gas production was noted by the appearance of gas bubbles in the inverted Durham's tubes.

3.7.1 Preparation of Methyl-Red Voges-Proskauer (MR-VP) broth

A quantity of 3.4 gm. of Bacto MR-VP medium was dissolved in 250 ml of distilled water dispensed in 2 ml amount in each test tube and then the tubes were autoclaved at 121^0C maintaining a pressure of 15 lbs./sq. inch for 15 minutes. After autoclaving, the tubes containing medium were incubated at 37^0C for overnight to check their sterility and then stored in a refrigerator for future use.

3.7.2 Preparation of 50% Buffered Glycerol Saline

8.3 grams of Buffered Glycerol Saline Base was suspended in 700mL distilled water. Then 300mL of glycerol was added. Heat was given to dissolve the medium completely and then mixed well and dispensed in screw capped tubes or suitable containers. Sterilization was done by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.

3.7.3 Preparation of phosphate buffered saline (PBS)

For preparation of Phosphate buffered saline (PBS) solution, 8 gm. of sodium chloride, 2.89 gm. of disodium phosphate, 0.2 gm. of potassium chloride and 0.2 gm. of potassium hydrogen phosphate were suspended in 1000 ml of distilled water. The solution was heated to dissolve completely. The solution was then sterilized by autoclave at 121ºC maintaining a pressure of 15 pounds per square inch for 15 minutes and stored at refrigerator until use. The pH of the solution was measured by a

pH meter and maintained at 7.0-7.2 (Cheesbrough, 1985).

3.8 Processing of meat samples

a. Collection of 5 gram of meat.
b. Blended the meat samples carefully.
c. Mixed well properly with PBS.

3.9 Methodology followed for isolation and identification of *Escherichia coli*

3.9.1 Isolation of bacteria by culturing of sample into different bacteriological media

3.9.1.1 Primary stock

Primary stock was made by mixing 5 gm. of meat in 995 ml PBS.

3.9.1.2 Ten-fold dilution (Spread Plate Technique)

Principle

The spread plate technique involves using a sterilized spreader with a smooth surface made of metal or glass to apply a small amount of bacteria suspended in a solution over a plate. The plate needs to be dry and at room temperature so that the agar can absorb the bacteria more readily. A successful spread plate will have a countable number of isolated bacterial colonies evenly distributed on the plate.

Procedure

1. At first a series of test tubes, each containing 9 ml of PBS were taken.

2. From the original sample, 1 ml was transferred in the test tube no. 1 and mixed thoroughly.

3. Then 1 ml from first tube is transferred to second tube and this way dilution was made up to last tube and finally 1 ml is discarded from the last tube.

4. For each tube, 3 petridishes were taken containing EMB agar media.

5. 0.1 ml of mixture was transferred from each test tube to the center of the corresponding petridishes separately (one pipette or tip was used for each tube).

6. The samples were spreaded over the surface of the media using glass spreader (Before using glass spreader on each petridish it is sterilized by dipping into 70% alcohol and burning it in bunsen burner)

7. Incubated each plate over night for 37^0C.

8. The plate containing 30-300 colonies were counted and others are discarded.

9.The colonies found in all 3 petridishes were made an average.

10. Number of organisms is expressed as colony forming unit (CFU) per ml of sample.

3.9.1.3 Method for obtaining pure culture

Enriched culture from EMB was taken into selective NB media and incubated at 37ºC for 24 hours. Single colony appeared on the selective media was further streaked onto selective media to obtain pure cultures**.**

3.9.1.4 Colony characteristics

Colony characteristics such as shape, size, surface texture, edge and elevation, color and opacity developed on selective media after 24 hours of incubation at 37°C was recorded.

3.9.1.5 Morphological identification of bacteria by Gram's staining

Gram's staining of the pure culture was performed according to method described by (Cheesbrough, 2006). Briefly a single colony was picked up with a bacteriological loop, smeared on a glass slide and fixed by gentle heating. Crystal violate was then applied onto smear to stain for two minutes and then washed with running tap water. Few drops of Gram's iodine were then added for few seconds. After washing with water, Safranin was added for counter staining and allowed to stain for 2 minutes. The slides were then washed with water, blotted and dried with air and then examined under light microscope (400X) using immersion oil.

3.9.1.6 Motility test

The motility test was performed to differentiate motile bacteria from the non-motile one (Cheesbough 1985). This test was performed in Motility Indole Urea (MIU) medium where a sterile straight wire were used to inoculate into 5ml of sterile MIU

medium taken earlier in a screw caped test tube with a smooth pure colony of test organism. When inoculating the MIU medium a stab was made with a sterile straight wire and stoppered the tube followed by incubation at 37^0C overnight. With this a control tube of MIU also maintained. Motility was shown by a spreading turbidity from the stab line or turbidity throughout the medium (Compared with a un-inoculated tube).

3.9.2 Biochemical tests

3.9.2.1 Catalase test

This test was used to differentiate those bacteria that produced the enzyme catalase, such as *staphylococci*, from non-catalase producing bacteria such as *streptococci*. To perform the test an amount of 2-3 ml of 3% hydrogen peroxide solution was poured into a test tube. Using a sterile wooden stick or a glass rod, a good growth of the test organism was immersed into the solution. If the organisms are catalase producer, bubbles of oxygen are released.

3.9.2.2 Coagulase test

A simple slide coagulase test was performed as required. In this case, 1-2 drop of diluted plasma was mixed with an equal volume of freshly cultured broth of a particular organism on a slide and examined under microscope for the occurrence of any coagulation.

3.9.2.3 Sugar fermentation test

The sugar fermentation test was performed by inoculating a loop full of overnight NB culture of the organisms into each tube containing five basic sugars (e.g. dextrose, sucrose, lactose, maltose and mannitol) separately and incubated for 24 hours at 37^0C. Acid production was indicated by the color change from reddish to yellow in the medium and the gas production was noted by the appearance of gas bubbles in the inverted Durham's tube.

3.9.2.4 Indole test

Two milliliter of peptone water was inoculated with the 5ml of bacterial culture and incubated at 37^0C for 48 hours. Kovac's reagent (0.5 ml) was added, shaked well and examined after one minute. A red color in the reagent layer indicated indole. In negative case there is no development of red color (Cheesbrough, 2006).

3.9.2.5 Voges-Proskauer test

Two milliliter of sterile glucose peptone water was inoculated with the 5 ml of test organisms. It was incubated at 37^0C for 48 hours. A very small amount of creatine was added and mixed. Three milliliter of sodium hydroxide was added and shacked well. The bottle cap was removed and left for an hour at room temperature. It was observed closely for the slow development of a pink color for positive cases. In case of negative reaction there was no development of pink color (Cheesbrough, 2006).

3.9.2.6 Methyl-red test

The test was conducted by inoculating a colony of the test organism in 0.5 ml sterile glucose phosphate broth. After overnight incubation at 37^0C, a drop of methyl red solution was added. A red coloration was positive and indicates an acid pH resulting from the fermentation of glucose. A yellow coloration indicated negative result (Cheesbrough, 2006).

3.10 Maintenance of stock culture

Stock culture was mixed with a medium prepared by adding one ml of 50% sterilized glycerol in one ml of pure culture in nutrient broth and this was stored at -20°C for further use. 50% Buffered Glycerol Saline is used to store it for a long time (above 50 yrs.) for further research or banking.

CHAPTER 4

RESULTS AND DISCUSSION

The results presented below demonstrated the isolation and identification of bacteria isolates from dressed meat samples of chicken from different live bird market and supermarket around Dhaka district. The results also show the prevalence and the prevalence difference between live bird markets and the supermarkets.

Table 2: Prevalence of coliform in different markets of Dhaka-

Serial No	Source and Location	Total number of sample	Number of +ve sample Coliform	Prevalence of Coliform (%)
01.	Savar Hut (M1)	3	3	100
02.	Shemulia Bazar (M2)	3	3	100
03.	Bagbari Bazar (M3)	3	2	66.67
04.	Bihari Camp (M4)	3	3	100
05.	Krishi Market (M5)	3	2	66.67
06.	Gigatola Bazar (M6)	3	1	33.33
07.	Taltola Bazar (M7)	3	2	66.67
08.	Supermarket (M8)	3	1	33.33
09.	Supermarket (M9)	3	1	33.33
10.	Supermarket (M10)	3	2	66.67
11.	Supermarket (M11)	3	2	66.67
12.	Supermarket (M12)	3	1	33.33
Total	12	36	23	63.89

A total number of 36 dressed chicken meat samples were collected from different markets from Dhaka city. Samples were collected from twelve markets. Among them seven of the markets were open or local live bird markets and five of the markets were from different supermarkets. From each market 3 samples were collected (Table 2). The twelve markets were indicated as Savar Hut (M1), Shemulia Bazar (M2), Bagbari Bazar (M3), Bihari Camp (M4), Krishi Market (M5), Gigatola Bazar(M6), Taltola Bazar (M7), Supermarket (M8), Supermarket (M9), Supermarket (M10), Supermarket (M11) and Supermarket (M12) (Table 1, Table 2). Samples of each market were indicated as S1, S2 and S3. The meat sample were taken from the market in ice box and taken to the laboratory. The sample was taken in the selective Eosin Methylin Blue (For *E. coli*) media and incubated for 24 hours in 37^0C. Then from the EMB agar *E. coli* was taken to NB for stock of the bacteria, so that it could be used in further examination (Figure 1).

Among 36 samples, 23 samples were *E. coli* positive. In most of the live bird markets the positive results was found. But in the supermarkets the *E. coli* load was negative in some sample. Here the M1, M2 and M4 market samples were 100% *E. coli* contaminated or positive. And the M3, M5, M7, M10 and M11 markets were 66.67% *E. coli* positive. And other remaining markets like M4, M6, M8, M9 and M12 were of 33.33% positive result (Table 2 & Figure 2, 3, 3, 4, 5). Figure 2 and figure 3 showed the prevalence percentage along with *E. coli* positive sample of different live bird markets. And the Figure 4 was for the supermarkets. Figure 5 showing the overall condition of all the markets. So comparatively the supermarkets showed less *E. coli* load than the live bird markets. The average prevalence of *E. coli* in live bird markets was 80.95% and in supermarkets was 46.67% (Figure 6).

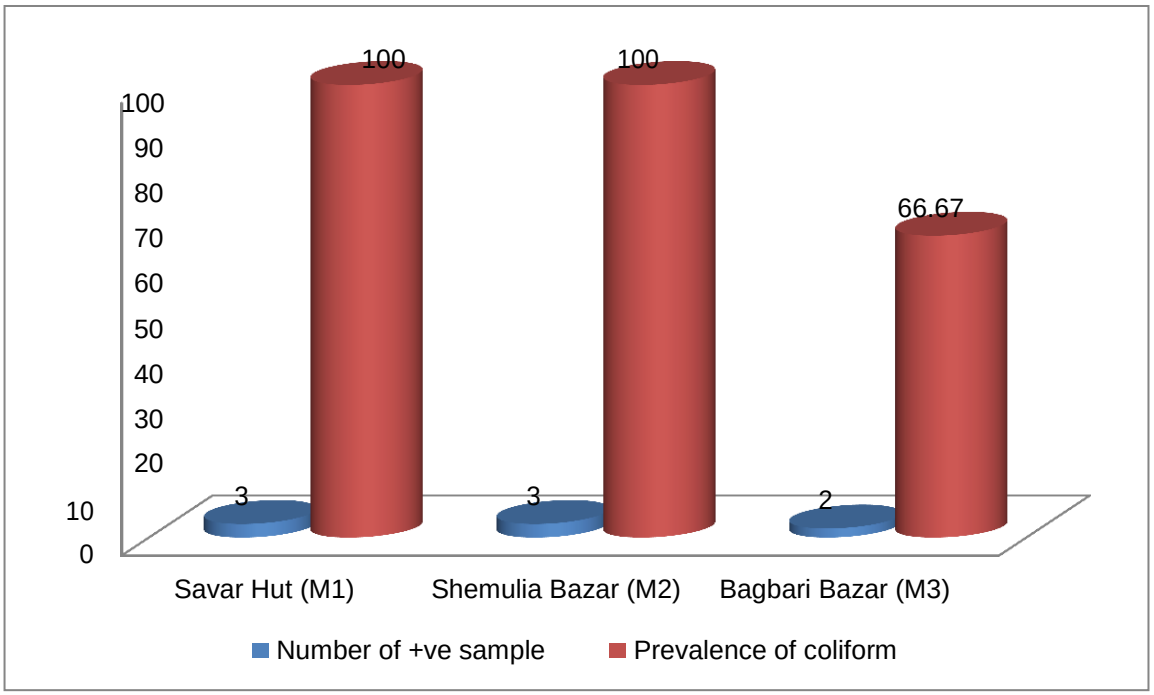


Figure 2: Prevalence of coliform in live bird markets of Savar, Dhaka

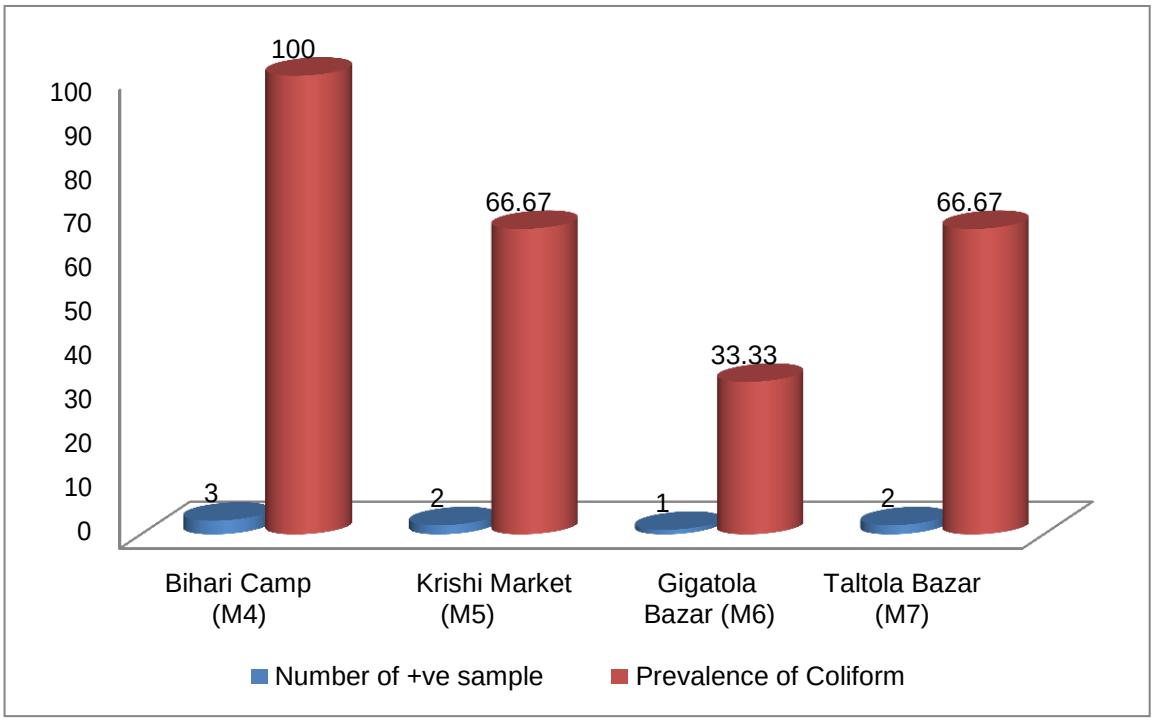


Figure 3: Prevalence of coliform in live bird markets of Mohammadpur, Dhaka

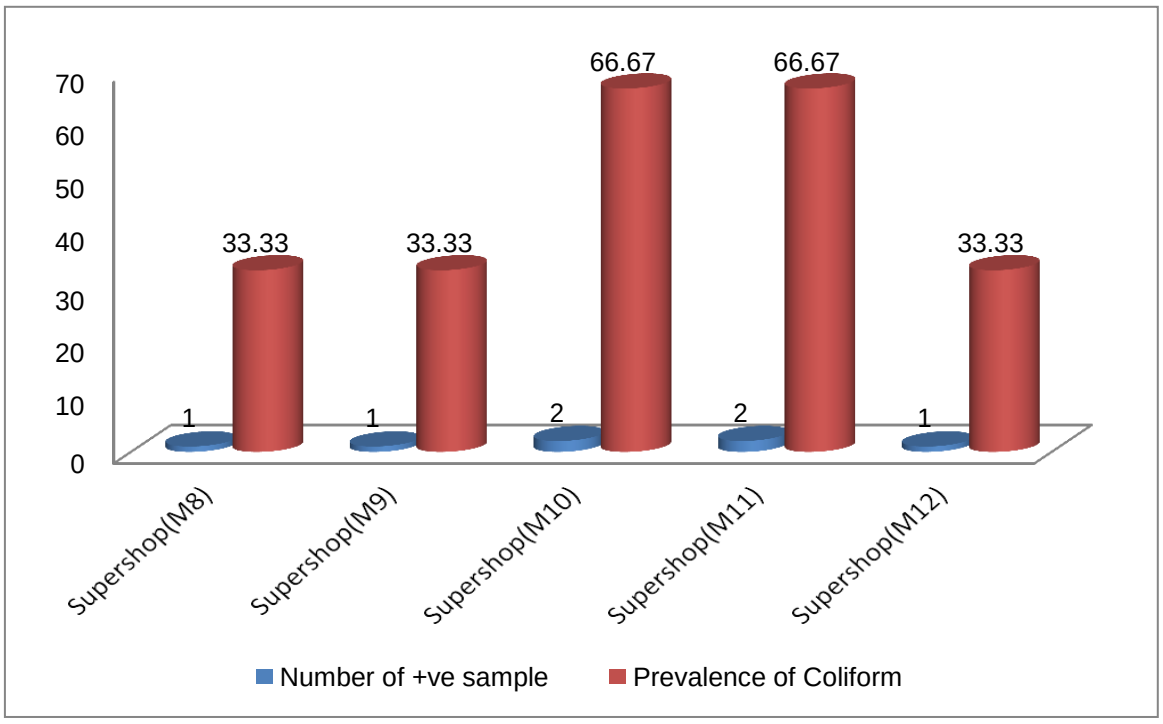


Figure 4: Prevalence of coliform in supermarkets of Mohammadpur and Mirpur

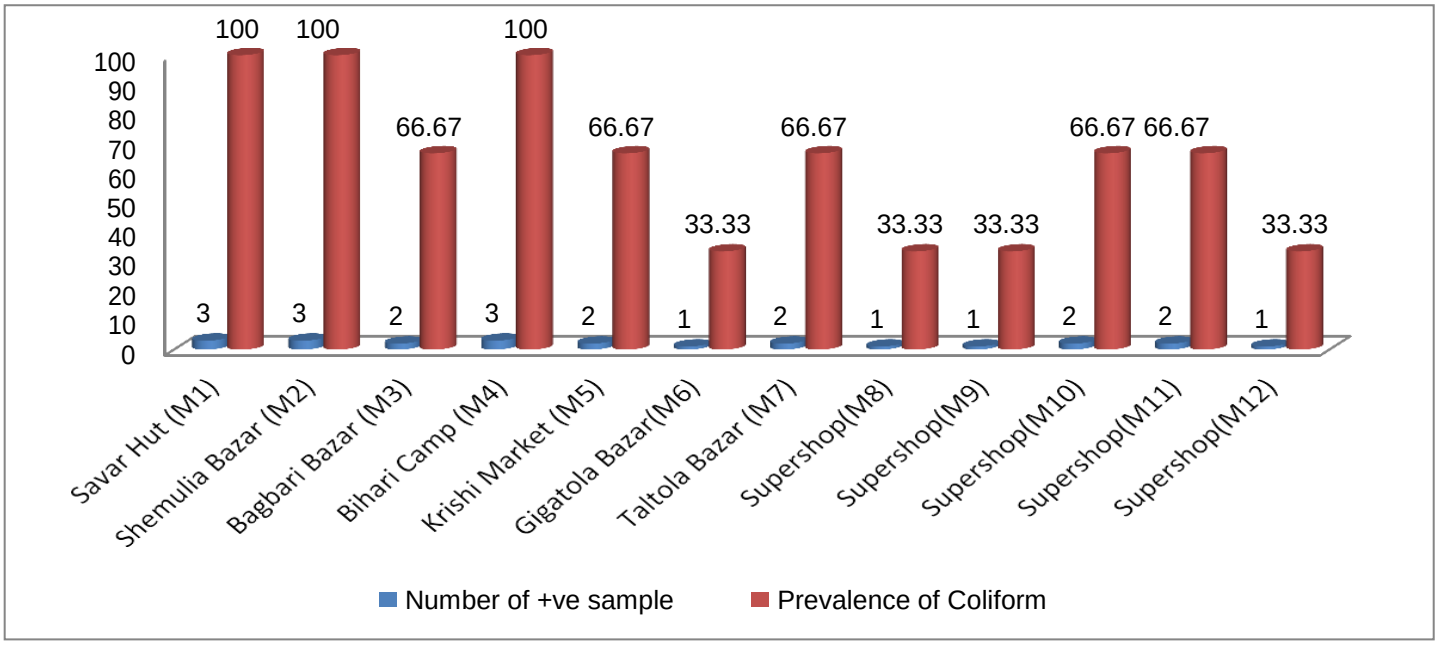


Figure 5: Prevalence of coliform in live bird markets and supermarkets of Dhaka

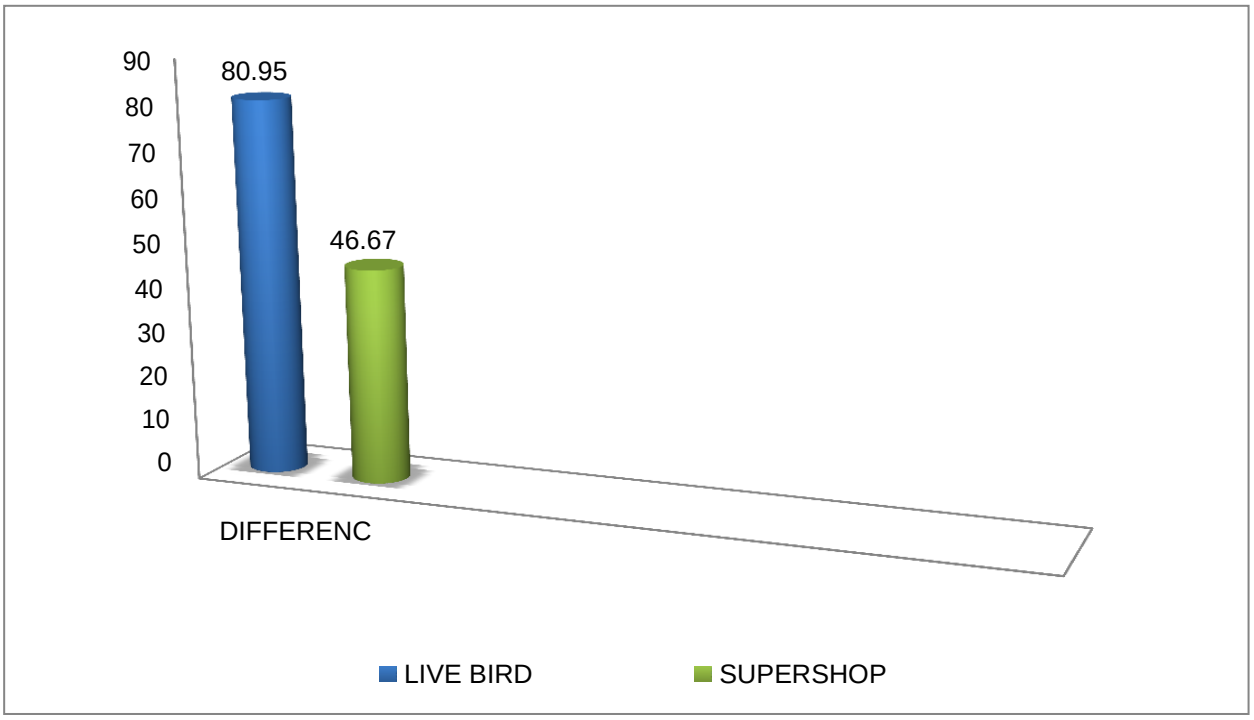


Figure 6: Comparison in the prevalence of coliform count between live bird markets and supermarkets in Dhaka

Table 3: Average colony count with Colony Forming Unit (CFU)-

Market Name	Sample	Average colony count	CFU
Savar Hut (M1)	**S1**	144.33	$1.4433X\ 10^{12}$
	S2	200	$2.00\ X\ 10^{12}$
	S3	203.33	$2.0333\ X\ 10^{12}$
Shemulia Bazar (M2)	**S1**	137.67	$1.3767\ X\ 10^{12}$
	S2	199.33	$1.9933\ X\ 10^{12}$
	S3	189.33	$1.8933\ X\ 10^{12}$
Bagbari Bazar (M3)	**S1**	134.67	$1.3467\ X\ 10^{12}$
	S2	-	-
	S3	88.67	$8.867\ X\ 10^{11}$
Bihari Camp (M4)	**S1**	211	$2.11\ X\ 10^{12}$
	S2	164.67	$1.6467\ X\ 10^{12}$
	S3	234.67	$2.3467\ X\ 10^{12}$
Krishi Market (M5)	**S1**	159	$1.59\ X\ 10^{12}$
	S2	-	-
	S3	112.33	$1.1233\ X\ 10^{12}$
Gigatola Bazar (M6)	**S1**	75.67	$7.567\ X\ 10^{11}$
	S2	-	-
	S3	-	-
Taltola Bazar (M7)	**S1**	140.67	$1.4067\ X\ 10^{12}$
	S2	-	-
	S3	99.67	$9.967\ X\ 10^{12}$

Supermarket (M8)	**S1**	-	-
	S2	183	1.83×10^{12}
	S3	-	-
Supermarket (M9)	**S1**	-	-
	S2	61.33	6.133×10^{11}
	S3	-	-
Supermarket (M10)	**S1**	79.67	7.967×10^{11}
	S2	-	-
	S3	44.33	4.433×10^{11}
Supermarket (M11)	**S1**	46.33	4.633×10^{11}
	S2	-	-
	S3	47	4.7×10^{12}
Supermarket (M12)	**S1**	-	-
	S2	44.33	4.433×10^{11}
	S3	-	-

Table 4: Side by side CFU/ml difference between live bird market and supermarkets-

Live Bird market Samples		CFU/ml	Super Market Samples		CFU/ml
Savar Hut (M1)	S1	1.4433×10^{12}	Supermarket (M8)	S1	1.83×10^{12}
	S2	2.00×10^{12}		S2	-
	S3	2.0333×10^{12}		S3	-
Shemulia Baza (M2)	S1	1.3767×10^{12}	Supermarket (M9)	S1	6.133×10^{11}
	S2	1.9933×10^{12}		S2	-
	S3	1.8933×10^{12}		S3	7.967×10^{11}
Bagbari Bazar (M3)	S1	1.3467×10^{12}	Supermarket (M10)	S1	-
	S2	-		S2	4.433×10^{11}
	S3	8.867×10^{11}		S3	4.633×10^{11}
Bihari Camp (M4)	S1	2.11×10^{12}	Supermarket (M11)	S1	-
	S2	1.6467×10^{12}		S2	4.7×10^{11}
	S3	2.3467×10^{12}		S3	-
Krishi Market (M5)	S1	1.59×10^{12}	Supermarket (M12)	S1	4.433×10^{11}
	S2	-		S2	-
	S3	1.1233×10^{12}		S3	1.83×10^{12}
Gigatola Bazar (M6)	S1	7.567×10^{11}			
	S2	-			
	S3	-			
Taltola Bazar (M7)	S1	1.4067×10^{12}			
	S2	-			
	S3	9.967×10^{11}			

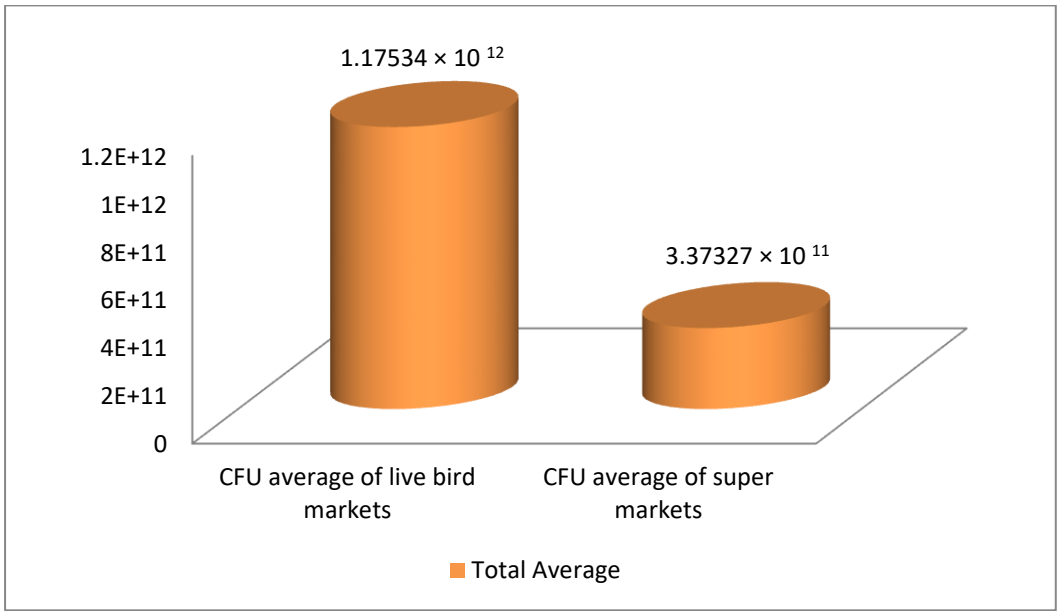
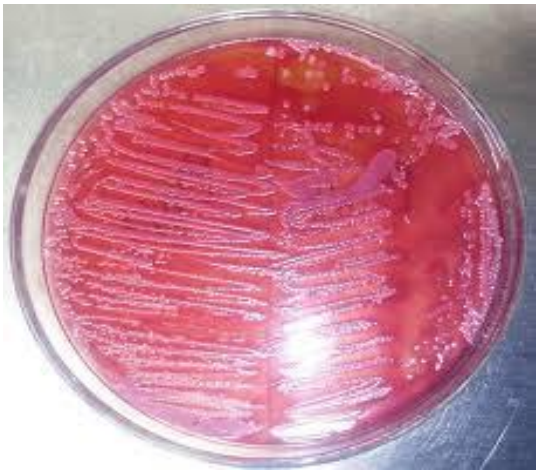


Figure 7: Comparison of CFU/ml (Coliform load) between live bird markets and supermarkets of Dhaka

4.1 Cultural characteristics of the isolated bacteria recovered from chicken meat

Table 5: Cultural characteristics of isolated bacteria on specific media

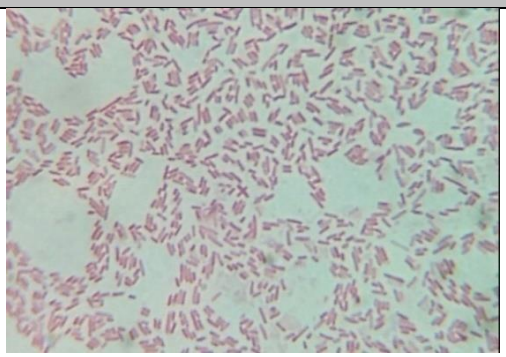
Serial No.	Name of bacteria	Bacteria on specific media	Colony characteristics
1	*E. coli*		*E. coli* on EMB agar was indicated by smooth, circular, black or green color colonies with metallic sheen

			*E. coli* on MacConkeygar was indicated by rose pink lactose fermenter colonies
2	*Enterobacter* spp.		*Enterobacter sp* on MacConkey agar was indicated by very small pink colonies.
3	*Klebsiella* spp.		*Klebsiella* spp. on MacConkey agar was indicated by mucoid pink colonies.

4.2 Gram's staining

Morphological and staining characteristics of bacteria recorded from meat by Gram's staining presented in Table 6

Table 6: Morphological and staining properties of the bacterial isolates

Characteristics			**Microscopic characteristics**
Organism	**Gram's staining**	**Motility**	**Interpretation**
E. coli		Motile	Gram negative (-ve) single or paired short plump rods of *E. coli* (100X)

4.3 Bio-chemical properties

Table 7: Results of sugar fermentation and biochemical tests

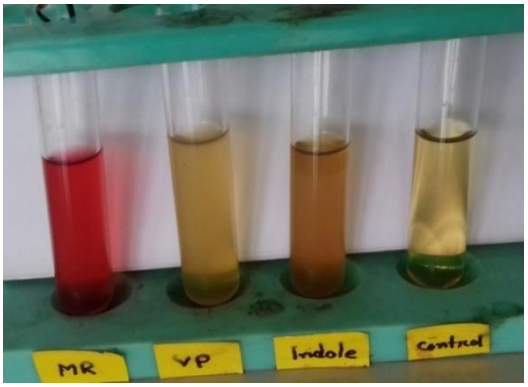
Name of bacteria	Sugar fermentation and biochemical tests	Interpretation
E. coli		*E. coli* fermented dextrose, maltose, lactose, sucrose and mannitol with acid and gas production
		For *E. coli* MR and Indole were positive but VP test was negative

Table 8: Demonstration of the biochemical reactivity pattern of *E. coli*

Organisms	Sugar Fermentation					Cata-lase Test	Indole Test	MR Test	VP Test	TSI
	DX	**ML**	**L**	**S**	**MN**					
Escherichia coli	AG	AG	A±/-	AG	AG±	-ve	+ve	+ve	-ve	+ve

Table 9: Biochemical test of *E. coli* and other coliform from chicken meat-

Sample	Suger test					Catalase	Indole	MR	VP	Citrate Utilization	TSI
	DX	ML	L	S	MN						
M1S1	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M1S2	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M1S3	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M2S1	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M2S2	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M2S3	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M3S1	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M3S2	AG	AG	A↓G↓	A↓G↓	A↓G↓	+	+	+	+	+	+
M3S3	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M4S1	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M4S2	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M4S3	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M5S1	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M5S2	AG	AG	-	AG	AG	+	-	-		+	+
M5S3	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+

M6S1	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M6S2	AG	AG	-	AG	AG	+	-	-	+	+	+
M6S3	AG	AG	-	AG	AG	+	-	-	+	+	+
M7S1	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M7S2	AG	AG	-	AG	AG	+	-	-	+	+	+
M7S3	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M8S1	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M8S2	AG	AG	-	AG	AG	+	-	-	+	+	+
M8S3	AG	AG	-	AG	AG	+	-	-	+	+	+
M9S1	AG	AG	A↓G↓	A↓G↓	A↓G↓	+	+	+	+	+	+
M9S2	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M9S3	AG	AG	A↓G↓	A↓G↓	A↓G↓	+	+	+	+	+	+
M10S1	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M10S2	AG	AG	A↓G↓	A↓G↓	A↓G↓	+	-	+	+	+	+
M10S3	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M11S1	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+
M11S2	AG	AG	A↓G↓	A↓G↓	A↓G↓	+	+	+	+	+	+
M11S3	AG	AG	AG	A↓G↓	AG	+	+	+	-	-	+

M12S1	AG	AG	-	AG	AG	+	-	-	+	+	+
M12S2	+	+	+	+	+	+	+	+	-	-	+
M12S3	AG	AG	-	AG	AG	+	-	-	+	+	+

Legends: DX = Dextrose; ML = Maltose; L = Lactose; S = Sucrose; MN=Mannitol; A = Acid production; G = Gas production; A↓ = Less acid production; NF = No Fermentation; G = Gas production; + = Positive reaction; - = Negative reaction.

From Table 9 we get different coliform along with *E. coli*

Table 10: Various coliform according to biochemical test character

Samples	Coliform
M1S1, M1S2, M1S3, M2S1, M2S2, M2S3, M3S1, M3S3, M4S1, M4S 2, M4S3, M5S1, M5S3, M6S1, M7S1, M7S3, M8S1, M9S2, M10S1, M10S3, M11S1, M11S3, M12S2	*E. coli*
M5S2, M6S2, M6S3, M7S2, M8S2, M8S3, M12S1, M12S3	*Enterobacter* spp.
M3S2, M9S1, M9S3, M10S2, M11S2	*Klebsiella* spp.

Legends: M = Market; S = Sample

4.4 Discussion

Food-borne pathogens are very diverse in their nature and are of major concern to public health worldwide. Many high-risk pathogens that cause diseases in humans are transmitted through various food items or water. Therefore, the microbiological safety of food has become an important issue for consumers and industry and regulatory agencies (Bai *et al.*, 2016).

The present study was conducted primarily for the isolation and identification of the *E. coli* isolated from dressed meat of chicken in Dhaka and around Dhaka city and also to determine the current status of prevalence and prevalence difference between live bird market and supermarket. The isolates were identified by cultural and staining characteristics, motility, and biochemical test.

Isolation and identification results of the study indicated that the selected samples contained Gram negative (-ve) and motile organisms (*E. coli*). Colony characteristics of *E. coli* in two 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 metallic sheen colony on the EMB agar, bright/rose pink colony on MacConkey agar which agreed with the findings of others (Kabir *et al.*, 2017; Parvej *et al.,* 2018). The media used in this study were selected considering the experience of the past researcher worked in various fields relevant to the present study by (Hassan *et al.,* 2014). In Gram's staining, the morphology of the isolated bacteria exhibited pink, small rod shaped Gram negative bacilli and in the hanging drop technique all the isolates revealed motile. These findings were supported by several authors such as (Buxton and Fraser, 1977); (Freeman, 1985) and (Jones *et al.*, 1987). Also in Gram's staining, the morphology of the isolated *E. coli* was exhibited as Gram negative, short plump rod arranged as single, paired or in short chain which was reported by (Tanzin *et al.* 2016; Mamun *et al.*, 2016; Kabir *et al.*, 2017; Parvej *et al.,* 2018;) previously. Another fundamental basis for the identification of *E. coli* organism was determining the ability or inability of fermentation of five basic sugars with acid and gas production. However, species identification and differentiation by fermentation reaction was difficult (Freeman, 1985) and showed similar reactions in different sugars (OIE Manual, 2000). All the *E. coli* isolates from chicken revealed a complete fermentation of five basic sugars as stated by (Mckec *et al.*, 1995), (Shandhu *et al.*, 1996) and (Beutin *et al.*, 1997). All the isolates of this study fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment sucrose and lactose which satisfied the statement of (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. (Ewing *et al.*, 1973) and (Ali *et al.,* 1998) also studied the biochemical characteristics of the different strains of *E. coli* isolated from different sources. They reported a little or no difference in these biochemical characters and stated that such similarity among the isolates might be due to presence of some common genetic materials. Also supported by (Beutin *et al.,* 1997; Sandhu and Clarke, 1996).

The results of Catalase, MR and indole test of the *E. coli* isolates were positive but V-P test was negative as reported by (Buxton and Fraser, 1977). Those similar results were reported by many investigators (Mishra *et al.*, 2002; Ali *et al.*, 1998).

Recommendations

Broiler meat is very popular in Bangladesh due to its cheap price and availability. But the presence of *E. coli* is degrading the food quality and also dangerous for health. In this work it is seen that the dressed meat of broiler is heavily contaminated with *E. coli* bacteria. On the basis of this finding the local live bird markets chicken meat got more bacterial load than the supermarkets chicken meat. Its main cause is the process of handling and maintenance of hygienic condition. The supermarkets samples are better but not out of *E. coli*. The local open market broiler vendor use same tools to dress the broiler without proper washing. Sometimes they wash and sometimes they do not. Even if they wash the tools they wash with unhygienic and the same water, that might contain the bacteria from the previous one and as it is open, it is easy to contaminate. The table they use is also not cleaned properly. And for the supermarkets the environment of the place is much clean and the dressed meat is packed in a box sealed with polythene. So that it might be out of reach from the air contact and the contamination. The *E. coli* we got entered during packaging of the dressed meat and may be from the used tools. So from these circumstances we can recommend that, proper hyginicity should be maintained. The local meat market vendor should use clean and covered water to wash the tools and for each broiler before using the tools should be washed properly. For this to be happened the government should impose rules and the market committee should also give proper surveillance. Not also that, for consciousness among the vendors and the market committee member's proper training should be given by government initiative. Also the supermarkets should know these things through training and proper surveillance. Not the least common people and consumers should be conscious about the hygiene.

CHAPTER 5

SUMMARY AND CONCLUSION

Escherichia coli is harmful for human and that they cause fever, nausea, diarrhoea, abdominal pain in human. *Escherichia coli* bacterium is usually found in broiler meat because of lack of hygienically production and process. Overall, the prevalence and load of *Escherichia coli* in broiler meat is very fearsome. Therefore, broiler meat business ought to be supplied with an immediate attention by the government to take care of strict biosecurity and sanitary managements in farm and live bird markets everywhere the country.

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APPENDIX

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 extract..... 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 extract..... 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.

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.

5. Brilliant Green Agar Medium

Peptone ..... 5.000 gm.

Tryptone ..... 5.000 gm.

Yeast extract ..... 3.000 gm.

Lactose ..... 10.000gm.

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. Methyl Red Indicator

Methyl red ..... 0.200 gm.

Ethyl alcohol ..... 60.000 ml

Distilled water ..... 40.000 ml

7. Voges–Proskauer (MR-VP) broth

Buffered peptone……7.000 gm.

Dextrose……..5.000 gm.

Dipotassium phosphate……5.000 gm.

Final pH (at 25°C) 6.9±0.2 10.

8. 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