PREVALENCE AND ANTIBIOGRAM ASSAY OF ZOONOTIC BACTERIA IN RAW MEAT

MST. TASMIM SULTANA

MASTER OF SCIENCE IN MICROBIOLOGY
DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

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BY

MST. TASMIM SULTANA
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APPROVED BY

Dr. Uday Kumar Mohanta
Supervisor
Associate Professor
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University,
Dhaka-1207

Dr. M. A. Mannan
Co-Supervisor
Assistant Professor
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University,
Dhaka-1207

Dr. Uday Kumar Mohanta
Chairman , Examination Committee
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University, Dhaka-1207

DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

Memo No: SAU/MIPA

CERTIFICATE

This is to certify that the thesis entitled "PREVALENCE AND ANTIBIOGRAM ASSAY OF ZOONOTIC BACTERIA IN RAW MEAT" submitted to the Department of Microbiology and Parasitology, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, as partial fulfillment for the requirements of the degree of Master of Science (MS) in Microbiology, embodies the result of a piece of bonafide research work carried out by MST. TASMIM SULTANA, Registration No.: 12-04913, Session: Jan June/2018 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.

Dated: June 2019
Place: Dhaka, Bangladesh

Dr. Uday Kumar Mohanta
Supervisor
Associate Professor
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University,
Dhaka-1207


DEDICATED
TO
MY BELOVED PARENTS

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LIST OF ABBREVIATIONS

MAC	MacConkey
EMB	Eosin Methylene Blue
SSA	Salmonella-Shigella Agar
MSA	Manitol Salt Agar
SAU	Sher-e-Bangla Agricultural University
CHO	Carbohydrate
BA	Blood agar
NA	Nutrient Agar
MHA	Muller Hinton Agar
µg	Micro gram
MAR	Multiple drug resistant
spp.	Species

PREVALENCE AND ANTIBIOGRAM ASSAY OF ZOONOTIC BACTERIA IN RAW MEAT

ABSTRACT

The present research work was undertaken to isolate and to investigate the prevalence of zoonotic bacteria from broiler meat samples sold in Krishi Market, Bihari Camp, Agargaon Bazar, Taltola Bazaar and SAU Mini Bazaar, Dhaka, Bangladesh from January to May 2018. A total of 45 samples were randomly collected from markets and transported to the Microbiology and Parasitology laboratory of Sher-e-Bangla Agricultural University for microbiological analysis. After processing of samples primary culture was done in nutrient broth and nutrient agar then pure culture was obtained from different selective media. The prevalence of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus* spp. were 74%, 42% and 18% respectively. All pure isolates were subjected to Antibiogram assay test by disc diffusion method against 8 different antibiotics. *E. coli* isolates were sensitive to ciprofloxacin (91.6%), gentamycin (87.5%), azithromycin (66.66%), tetracycline (58%) and resistant to penicillin (79.16%) , amoxycillin (75%), streptomycin (75%) and ampicillin (58.3%). Among all *Salmonella* spp. isolates, ciprofloxacin (81.81%) showed the highest susceptibility pattern followed by the gentamycin (72.72%) and azithromycin (63.63%). Highest resistant pattern was showed by tetracycline (58%), streptomycin(72.72%), penicillin (72.72%) and amoxycillin (63.63%). In case of *Staphylococcus* spp., ciprofloxacin (85.71%) showed the highest susceptibility pattern followed by the gentamycin (71.42%). also, ampicillin (71.42%), streptomycin (57.14%), azithromycin (57.14%) and tetracycline (57.14%) found sensitive in this study. Highest resistant pattern showed by amoxycillin (71.42%) and penicillin (71.42%). This study revealed that broiler meat sold at some local markets in Dhaka city were contaminated with multiple species of multidrug resistant bacteria which may risk for human health.

CHAPTER 1

INTRODUCTION

Food is considered as energy source for humans and animals. Most of the foods contain viable bacteria unless thoroughly 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 is most perishable of all important foods since it contains sufficient nutrients needed to support the growth of microorganisms. (Magnus, 1981). Sources for contamination of the chicken meat can be abattoir, storage at the retailer's stall or shop, heavily contaminated utensils and benches used for the handling of meat. Meat contamination occur by a variety of ways, including bowel rupture during evisceration in direct contamination with tainted water and also handling and packaging of finished meat products. Apart from these factors, meat at the point of sale may also carry disease causing bacteria whose mere presence may be of concern because the meat then become the vehicle for food poisoning outbreaks (Jackson *et al*., 2001).

Meat may be easily contaminated with different pathogens if not handled appropriately (Mead *et al.,* 1999). The World Health Organization (WHO) defines foodborne illnesses as diseases, usually either toxic or infectious in nature, caused by agents that enter the human body through the process of food ingestion. There are more than 200 known causative agents can cause foodborne diseases; these include bacteria, parasites, viruses, prions, toxins and metals. The symptoms and severity of foodborne illnesses vary, range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal syndromes (Mead *et al.,* 1999). In 2005, WHO reported that 1.8 million people died from diarrheal diseases and a high proportion of these cases due to contamination of food and drinking water (WHO, 2004). The important food meat is the fleshy material of the body obtained by slaughtering of healthy animals. It is major source of proteins, vitamins and minerals to the many people of the world. As it is important source of nutrients and due to favorable intrinsic factors, it is an ideal culture media for the growth of different types of microorganisms including the bacteria and fungi. In practice of slaughtering, the main sources of microorganisms are exterior of the animal and the intestinal tract. Meat carcasses may become contaminated from fecal material, paunch content, and from the

hide (Maharjan, 2006). Contaminated raw or undercooked red meats are particularly important in transmitting these foods borne pathogens (Meng *et al.*,1998). *Escherichia coli* are considered the most commensally living microorganism in the alimentary tract of nearly all domestic and wild animals as well as human. Enteropathogenic *E. coli* organisms usually lead to severe diarrhea in infants and it may also be the causal organisms in appendicular abscess, peritonitis and cholecystitis (Mackie and McCartney,1989). The nature and level of microbial contamination in meat have important consequence in relation to public health, storage life and the type of spoilage of meat. The most important pathogens associated with meat include *Salmonella, Staphylococcus aureus, Escherichia coli, Clostridium perfringens, Campylobacter jejuni, Listeria monocytogenes, Yersinia enterocolitica* and *Aeromonas hydrophilia* (Koutsoumanis, 2004). Meat is a chemically complex matrix, and predicting whether, or how fast, microorganisms will grow in any given food is difficult. Most foods contain sufficient nutrients to support microbial growth. Several factors encourage, prevent, or limit the growth of microorganisms in foods; the most important are water availability, pH, and temperature .

The occurrence of antimicrobial-resistant bacteria is associated with the use of antimicrobial agents in food producing animals. Human infections involving antimicrobial-resistant bacteria have increased and are now common. Animals are recognized as reservoirs for human intestinal pathogenic *Escherichia coli* and a source for human extra intestinal pathogenic *E. coli*. Because antimicrobial agents are commonly used for food-producing animals in farms, human infections involving antimicrobial resistant extra-intestinal pathogenic *E. coli* transferred from animals could be more difficult to treat. (Antibiotic Resistance in Bacterial Pathogens from Retail Raw Meats and Food-Producing Animals in Japan).

Considering the above facts, the present study was conducted to investigate the prevalence and antibiogram assay of zoonotic bacteria in raw meat in selected areas of Dhaka city.

On the above situation the present study was undertaken with the following objectives:

- To isolate and identify the *Salmonella* spp., *E. coli, Staphylococcus* spp. from raw meat samples
- To study the prevalence of zoonotic infection in raw meat
- Assessment of antibiotic resistance pattern of *E. coli, Salmonella* spp., *Staphylococcus* spp.

CHAPTER 2

REVIEW OF LITERATURE

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 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).

2.2 *Escherichia. coli (E. coli)*

2.2.1 Historical background

Escherichia coli (*E. coli*) originally called "Bacterium coli" was 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 honour its discoverer (Feng *et.al.,* 2002).

2.2.2 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).

2.2.3 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 (acetylmethyl carbinol) (V); and the ability to utilise 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).

2.2.4 Acid and salt tolerance

Escherichia coli is an acid resistant food borne pathogen that survives in the acidic environment of stomach and colonise 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).

2.2.5 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 (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).

2.2.6 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 (Mc Dowell and Sheridan, 2001).

2.3 *Salmonella* spp.

Salmonellosis is one of the most common and widely distributed food borne diseases. It constitutes a major public health burden and represents a significant cost in many countries. Millions of human cases are reported worldwide every year and the disease results in thousands of deaths (WHO, 2005). *Salmonella* infections are mainly asy mptomatic in poultry, but are associated with widespread human illness from this source. Therefore, there is continuing interest in finding ways of preventing flock infection and hence contamination of poultry products with *Salmonella* (Saeed *et al.,* 1999). Pullorum disease, (*S. pullorum*) and fowl typhoid (*S. gallinarum*) are two classic and distinctive diseases of poultry that have received considerable attention because of their economic impacts (Snoeyenbos, 1994).

Salmonella enterica-associated gastroenteritis is an important food borne human disease. Most serotypes are capable of infecting a variety of animal species, including humans. There is considerable variation with time and geographical location in serotypes commonly associated with human Salmonellosis notably *S. enterica* serovar *typhimurium*

and *S. enterica* serovar *enteritidis* (Cormican *et al.,* 2002), serotype *typhimurium* is responsible for various disease manifestations, usually in the form of mild gastroenteritis with low mortality, but it can cause septicemia with high mortality (Scalzoe *et al.,* 2004). The level of contamination of chicken and chicken products with pathogens associated with gastroenteritis such as *Salmonella* spp. is significantly increasing in many countries, for example *Salmonella* serotypes were isolated from 22.0% of broiler flocks, and from 15.3% of the layer flocks in The Netherlands (Dufrenne *et al.,* 2001). The most important cause of Salmonellosis has been attributed to broiler chickens and layer hens (Wegener *et al.,* 2003).

2.3.1 Characteristics, nomenclature and habitat of *Salmonella*

Salmonella is a Gram-negative facultative anaerobic rod-shaped bacterium in the family of Enterobacteriaceae, also known as enteric bacteria. *Salmonella* is a motile bacterium with the exception of *S. gallinarum* and *S. pullorum* and they are all non spore forming. There is a widespread occurrence of Salmonellosis in animals, especially poultry (FDA, 1998). There are over 2500 serotypes, of *Salmonella* (WHO, 2005). Different strains of *Salmonella*e have been identified, and these are placed into groupings called serovars on the basis of their antigens (Snoeyenbos, 1994). The latest nomenclature, which reflects recent advances in taxonomy (Popoff, 2001), in the genus *Salmonella* consists of only two species: *S. enterica* and *S. bongori* (Cooper, 1994). *Salmonella enterica* is divided into six subspecies, which are distinguishable by certain biochemical characteristics (Brenner *et al.,* 2000). Strains of *Salmonella* are classified into serovars on the basis of extensive diversity of lipo-polysaccharide (LPS) antigens (O) and flagellar protein antigens (H) in accordance with the Kauffmann–White scheme.

*Salmonella*e have a wide range of hosts. Although primarily intestinal bacteria of animals and birds, *Salmonella*e are widespread in the environment and commonly found in farm effluents, human sewage and in any material subject to fecal contamination and are transmitted to humans by contaminated foods of animal origin (Acheson & Hohmann, 2001). Some serovars show remarkable host specify for instance *Salmonella typhi* and *Salmonella gallinarium* are strictly found in humans and birds respectively (Jorgensen, 2001). Epidemiological and bacteriological evidence indicate that these animals may transmit the infection to human (Handeland *et al.,* 2002)

2.3.2 Isolation, pathogenesis of *Salmonella*

The most commonly used media selective for *Salmonella* are *Salmonella*-Shigella (SS) agar, bismuth sulfite agar, Hektoen enteric (HE) medium, brilliant green agar, xyloselysine-deoxycholate (XLD), and MacConkey agar. All these media contain both selective and differential ingredients (Edwards and Ewing, 1972).

Salmonella pathogenesis is initiated by oral ingestion and penetration into the intestinal epithelium; induce degeneration of enterocyte microvilli causing profuse micropinocytosis, which leads to the internalization of bacteria (Gulig, 1996).

2.3.3 Control of Salmonellosis

Salmonella enterica remains one of the most important food borne pathogens of humans and is often acquired through consumption of infected poultry meat or eggs. Control of *Salmonella* infections in chicken is therefore an important public health issue, three types of typhoid vaccines are currently available for use: (1) an oral live attenuated vaccine, (2) a parenteral heat-phenol-inactivated vaccine, (3) a newly developed capsular polysaccharide vaccine for parenteral use, a fourth vaccine, and an acetone-inactivated parenteral vaccine are available only to the armed forces in USA (Beal *et al.*, 2004).

Hazards from *Salmonella* can be prevented by heating food sufficiently to kill the bacteria, holding chilled food below 4.4ºC, preventing post-cooking cross contamination and prohibiting people who are ill or are carriers of *Salmonella* from working in food operations (Ward *et al.,* 1997). *Salmonella* surveillance and control of poultry industry at slaughter should be done to identify infected flocks as regulatory procedures for food safety and security program (Veling *et al.,* 2002).

2.3.4 Epidemiology of *Salmonella*

Salmonellosis is one of the most common and widely distributed food- borne diseases. It constitutes a major public health burden and represents a significant cost in many countries. Millions of human cases are reported worldwide every year and the disease results in thousands of deaths. In addition to acquiring infection from contaminated food, human cases have also occurred where individuals have had contact with infected animals, including domestic animals (WHO, 2005).

Non-typhoidal *Salmonella* are important food borne pathogens that cause gastroenteritis, bacteremia, and subsequent focal infection. These bacteria are especially problematic (cause opportunistic infections) in a wide variety of immune-compromised individuals,

including patients with malignancy, human immunodeficiency virus, or diabetes, and those receiving corticosteroid therapy or treatment with other immunotherapy agents. Endovascular infection and deep bone or visceral abscesses are important complications that may be difficult to treat (Acheson, 2001). During the last decade, antibiotic resistance and multi resistance of *Salmonella* spp. have increased a great deal due to increased indiscriminate use of antibiotics in the treatment of humans and animals; and the addition of growth-promoting antibiotics to the food of breeding animals (WHO, 2005). Strains of *Salmonella* which are resistant to a range of antimicrobials, including first-choice agents for the treatment of humans, have emerged and are threatening to become a serious public health concern (Holmberg *et al.*, 1984).

2.4 *Staphylococcus* spp

2.4.1 Characteristics of *Staphylococcus* spp.

The genus *Staphylococcus* comprises of different species which have been classified and differentiated on the basis of a variety of phenotypic characteristics such as morphology, and biochemical reactions. Pigment was the initial criterion used to classify staphylococcal species, and in 1885, Rosenbach recognized members of the genus *Staphylococcus* based on the color of colonies. Staphylococci forming orange-yellow colonies were named *S. aureus* (or *S. pyogenes aureus*) by Rosenbach, while staphylococci forming white colonies were named *S. albus* (or *S. pyogenes albus*) (Kloos, 1980).

Another characteristic feature which was described for differentiation between staphylococci was the coagulase test which involves the investigation of the ability of *S. aureus* to clot blood plasma (Kloos, 1980) which paved way for the separation of Staphylococci into two main groups Coagulase positive *S. aureus* (CPS) and Coagulase negative *S. aureus* (CNS). Based on different studies carried by different researchers, at present the genus *Staphylococcus* comprises of 37 species and 17 subspecies (Schleifer and Kloos, 1975).

2.4.2 Morphology and identification

S. aureus is a gram-positive, catalase-positive, usually oxidase-negative, facultative anaerobic coccus, which belongs to the family of Micrococcaceae and the group of Staphylococci. Different phenotypic methods are been proposed to identify *S. aureus* isolates from humans and animals from other species of *Staphylococcus*. These methods include anaerobic fermentation of mannitol, production of coagulase, production of heat

stable thermonuclease and production of acetoin from glucose (Devriese, 1981; Roberson *et al.,* 1992).

2.4.3 Importance of *Staphylococcus* spp.

Staphylococcal enterotoxins can cause skin, heart valve, blood and bone infections, which can lead to septic shock and death. These enterotoxins also cause food poisoning (Saiyers and Whitt, 2002) and the superantigens if present in the blood stream can cause toxic shock syndrome (Waldvogel, 2000). Nosocomial (hospital- acquired) infections are generally caused by Staphylococcal related infections. *S. aureus* is one of the most common pathogens which cause mastitis in ruminants and gangrenous dermatitis in poultry (Sasidhar*et al.,* 2002). *S. aureus* is also one of the main causative agents in gangrenous dermatitis in poultry (Sheela and Krupanidhi, 2015).

2.4.4 Development of antibiotic resistance in *Staphylococcus* spp.

The indiscriminate use of antibiotics can lead to development of resistant strains and result in the increase in the cost of treatment. The production of β–lactamases encoded by the structural blaZ gene and by the production of an altered form of penicillin binding protein 2A (PBP-2A) which is encoded by the mecA gene is responsible for β-lactam resistance in Staphylococci (Fuda*et al.,* 2005).

2.5 Prevalence and antimicrobial sensitivity pattern of *Escherichia coli, Salmonella* spp*. and Staphylococcus* spp **isolates**

Chattopadhya *et al.,* (2001) observed that all the *E. coli* (STEC) isolates (12 of animals, 1 of human and 4 of food samples) from a total of 876 samples (330 of animals,184 of humans and 362 food samples) were uniformly sensitive to common antibiotics, except tetracycline, dicloxicillin, erythromycin, cephalaxin and linomycin.

Leelaporn *et al.,* (2003) performed antimicrobial susceptibility tests of *E. coli* isolates in Bangkok, by disc diffusion method. All the isolates were found susceptible to cefaclor, ceftriaxone, imipenem, netilmicin, norfloxacin, ciptofloxacin, nalidixic acid, and forfomycin. More than 90% of the isolates were susceptible to cefdinir, gentamycin, neomycin and chlorophenicol. Resistance rates to ampicillin, co-trimoxazole and tetracycline were 17, 39, and 65 percent respectively.

Cergole-Novella *et al.,* (2006) isolated a total of 107 STEC from human infections, cattle and foods in Brazil. They observed that the highest frequencies of susceptibility to

antimicrobial agents were among food (100) and bovines (87%) strains while 47.6% of the human isolates were resistant to at least one drug. The antimicrobials to which resistance most frequently observed were tetracycline (90%) and streptomycin (75%) among human strains and sulphazotrin (88%) in animal strains.

Rashid *et al.,* (2006) isolated 21 strains of *E. coli* from 120 samples of bovine mastitic milk over a period of about two years of which 20 isolates were typed and one isolate was untypeable. Multiple drug resistance (MDR) was recorded being the highest against piperacillin (100%), Ampicillin (71%) Cephoxitin (71%), Enerofloxacin (33.33%) and Nalidixic acid (61.90%), the most commonly used antibiotics in the field. The sensitivity was highest against Chloramphenicol (80.95%), Ciprofloxacin (76.20%), Norfloxacin (76.20%) and gentamycin (71.42%).

Mora (2007) examined 722 *E. coli* (STEC) isolates recovered from humans, sheep, and food in Spain to determine antimicrobial resistance profiles. Fifty-eight (41%) out of 141 STEC O157:H7 isolates and 240 (40%) out of 581 non-O157 STEC isolates showed resistance to atleast one of the 26 antimicrobial agents tested. Shiga toxin- producing *E. coli* O157:H7 showed a higher percentage of resistant strains recovered from bovine (53%) and beef meat (57%), human (23%) and ovine (23%) sources. Sulfisoxazole (36%) had the most common antimicrobial resistance followed by tetracycline (32%), streptomycin (29%), amplicillin (10%), trimethoprim (8%), cotrimazole (8%), chloramphenicol (7%), kanamycin (7%), piperacillin (6%), and neomycin (5%). Ten (7%) STEC O157:H7 and 17 (21%) non- O157 isolates were resistant to five or more antimicrobial agents. The multiple resistance pattern most often observed was that of streptomycin, sulfisoxazole and tetracycline.

Yadav *et al.,* (2007) assessed 49 isolates of *Escherichia coli* isolated from mutton in Gujarat state of India for the drug susceptibility and the percentage of isolates resistant to antibacterial agents was recorded as sulphadiazine (93.33%), cephaloridine (80.00%), cephalexin (33.33%), penicillin G, ceftiofur and norfloxacin, carbenicillin and enrofloxacin (26.67% each), and oxytetracycline and amoxycillin (20.00% each).

Aruno *et al.,* (2007) found that *E. coli* O157: H 7 is one of the major threats to public health due to consumption of insufficient cooked meat and meat products. The microorganism is known as a foodborne pathogen evening the presence of low levels.

Shriver-lake *et al.,* (2007) mentioned that food poisoning causes untold discomfort to many people each year. One of the primary culprits in food poisoning is *E.coli* O157:H7, while most cases cause intestinal discomfort, up to 7 % of the incidence leads to a severe complication called hemolytic uremic syndrome which may be fatal.

Cebedo *et al.,* (2008) concluded that *Salmonella* are pathogenic bacteria that can contaminate food products during or after processing. Ready-to-eat food dose not undergo any treatment to ensure its safety before consumption, and therefore risk of foodborne diseases must be considered if these pathogens are present in food.

Iroha et. al. (2011) collected three hundred raw meat samples including beef (n=100), chicken (n=100), chevon (n=100) from Abakaliki abattoir and analyzed for microbiological contamination using standard microbiological methods. Also determined the antimicrobial susceptibility of isolated microbes using the Kirby and Bauer method of disc diffusion. Out of the 300 samples, 79 (29.3%) were contaminated with bacteria species including *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae* and *Staphylococcus aureus*. Of these, *E. coli* had the highest occurrence (8%), followed by *K. pneumoniae* (5.3%), *S. typhi* (5%), *S. dysenteriae* 2.6%, *P. aeruginosa* 2.0%, *B. cereus* 2.0% and *S. aureus* (1.3%).

Rigobelo *et al.,* (2010) determined the sensitivity pattern of 120 *E. coli* (STEC) isolated from an abattoir in Brazil to ten antimicrobial drugs. Resistance was seen against cephalothin (84.0%), streptomycin (45%) nalidixic acid (42%), tetracyclin (20%), and less frequently to trimethoprim (9%), cephlothin (8%), and amikacin (6%). Multidrug resistance (MDR) was seen in 38.4% of the isolates and resistance to 2 or 3 antibiotics was common.

Rashid (2011) studied Antibiogram study of *E. coli* from clinical cases and foods of animal origin in Jammu. 47 of 120 isolates revealed multidrug resistance to four or more antibiotics. Fifty percent of the isolates were resistant to amoxycillin, 44.16 % to ampicillin, 40% to cefotexime, 19.16% to amikacin, 6.66% to chloramphenicol and 13.33% to gentamycin.

Saad *et al.,* (2011) collected a total of 100 random samples of chicken (thigh and breast) and red meat cuts (mutton and beef shoulders) from different poulterer's and butcher's shops at Cairo, El- Kalyobia and El-Gharbia governorates to detect level of *Salmonella*

and *E.coli* contamination. The obtained results indicated that *Salmonella* organisms were isolated from the examined samples of chicken thigh, chicken breast, mutton and beef with percentages of 16%, 16%, 8% and 8% respectively. Moreover, the isolated *Salmonella*e could be serologically identified as *S. typhimurium* (28%), *S. enteritidis* (16%) and *S. haifa* (4%). On the other hand, the percentages of isolated *E. coli* from the examined samples of chicken thigh, chicken breast, mutton and beef were 16%, 12%, 28%and 12% respectively. Moreover, the results cleared that PCR is an ideal method for identification of *Salmonella* spp. as it was effective, less labor and more sensitive as well as reduces effort and time. Out of 10 strains of different serotypes of *Salmonella* isolated from chicken (thigh and breast), mutton and beef by traditional method, 4 strains were positive in m-PCR for *Salmonella* from which, one strain was identified as *S. typhimurium*. As well as out of 10 strains of different serotypes of E .coli isolated from chicken (thigh and breast), mutton and beef shoulders, 2 strains were positive in m-PCR. *E.coli* O55: K59 (B5) and *E.coli* O119: K69 (B14) isolated from thigh and breast, respectively, which were positive for elt gene (labile toxin).

Samaha *et.al* (2012) collected a total of 100 random samples of chicken meat, chicken nuggets, chicken paneehh and chicken luncheon (25 of each). The collected samples were subjected to bacteriological examination for detection of enteropathogens. The obtained results as following; *Salmonella* isolated from chicken meat, chicken nuggets, chicken paneehh and chicken luncheon as 56, 8, 12 and 8 %, respectively. *E.coli* was isolated by 68, 12, 12 and 8 % in chicken meat, chicken nuggets, chicken paneehh and chicken luncheon, respectively.

Aly *et al.*, (2012) isolated a total of 147 *E. coli* strains from clinical specimens and food samples. The antibiotic resistance profile of these strains was determined against 7 classes of antimicrobial agents (26 different members). Almost 90% of *E. coli* strains were resistant to at least one of the tested antibiotics. The highest antibiotic resistance was recorded against conventional Beta-lactams. The highest sensitivity of the isolates was to imipenem and polymyxin-B where all isolated *E. coli* strains were sensitive to imipenem. The resistance to tetracyclines, macrolides and sulfonamides/trimethoprim was almost in the same order of magnitude of 30-37%. The resistance to quinolones and aminoglycosides was 19 and 10 % respectively.

Hiroi *et al.*, (2012) determined the antimicrobial resistance patterns of 138 *E. coli* isolated from humans in Japan. 31 isolates showed the resistance to one or more antimicrobial

agents. 24 of the isolates were resistant to tetracycline, 23 to streptomycin, 12 to ampicillin, 7 to chloramphenicol and kanamycin, 3 to nalidixic acid, 1 to gentamycin and 1 to cefuroxime.

Moses *et al.,* (2012) studied 18 *E. coli* O157 isolates from human stool (12) from cattle faeces, unpasteurized milk (5) and water (1) using agar disk diffusion method to determine the drug resistance in Nigeria. Resistant rate among human strains was low against gentamycin (8.3%), streptomycin (8.3%), chloramphenicol (25.0%) and sulphamethoxazole-trimethoprim (25.0%). Increasing resistant pattern against tetracycline, ampicillin, cephalexin and clavulanic acid-potentiated amoxicillin was observed in about 50% to 80% of human and cattle isolates.

Sasaki *et al.,* (2012) studied the antimicrobial resistance in O157 and O26 strains of STEC. Resistance to dihydro-streptomycin was detected most frequently followed by Oxytetracycline and ampicillin. Resistance to one or more antimicrobial agents was detected in 13.3% of the O157 isolates and 54.5% (6/11) of the O26 isolates. The antimicrobial resistance rate in the O26 STEC isolates was significantly higher than in the O157 isolates.

Dey *et al.,* (2013) tested 112 samples from poultry to determine the prevalence of antimicrobial resistance. The Antibiogram study pattern showed that *E. coli* isolates were sensitive to erythromycin, ciprofloxacin, kanamycin, nalidixic acid and resistant to amoxicillin, tetracycline and sulphamethaxazole.

Mahmoud *et al.,* (2013) tested 12 isolates of EHEC which were isolated from lambs (4), calves (4) and fish (4) in Egypt to determine the frequency of resistance to commonly used antimicrobial agents in veterinary field. Results showed that among the antimicrobial discs tested, ampicillin was the most common antibiotic that the isolates were resistant to (91.6%), followed by tetracycline (83.3%).

Rajput *et al.,* (2013) tested *E. coli* isolates which were isolated from clinical cases of diarrhoea in kids of Mathura area of U.P for their sensitivity to the commonly used antibiotics. About 67% of the isolates were resistant to Oxytetracycline, Gentamicin, and Ceftriaxone. Only antibiotic groups like fluoroquinolones and the Chloramphenicol have fared well in effectively inhibiting the in vitro growth of *E. coli*. Chloramphenicol has given the lowest percentage of resistance at zero and the next best was Enrofloxacin at 34%.

Mahanti *et al.,* (2014) studied the antibiogram of 363 isolates of *E. coli* which were isolated from fecal samples of buffaloes in West Bengal, India. The antibiotics used were amikacin (30µg), gentamicin (30µg), kanamycin (30µg), neomycin (30µg), oxytetracycline (30µg), co-trimoxazole (25µg), ceftazidime (30µg), levofloxacin (5µg), cefepime (30µg), ciprofloxacin (5µg), ceftriaxone (30µg), enrofloxacin (5µg), pefloxacin (5µg), amoxycillin (25µg), chloramphenicol (30µg), cefuroxime (30µg) and norfloxacin (10µg) (Hi Media, India). The antibiotic resistance of ETEC isolates was observed most frequently towards amikacin (56%), kanamycin (44%), gentamicin (40%) and neomycin (36%).

Ferede (2014) examined 249 goat carcass swabs for the presence of *Salmonella* following the standard techniques and procedures. Out of the total of 249 carcass swab samples, 44 (17.7%) were positive to *Salmonella*. Of all isolates, 43(97.7%) were multiple antimicrobial resistant and highest level of resistance was observed for tetracycline (100%), nitrofurans (100%), streptomycin (81.8%) and kanamycin (79.5%). However, all isolates were susceptible to ciprofloxacin.

Radwan *et al.,* (2014) recovered 83 *E. coli* isolates from 200 broiler chicken suffering from colibacillosis. The disc diffusion method was used to determine antibiotic susceptibility of the isolates for 10 antibiotics comprising 6 different antimicrobial classes. Antibiogram profiles indicated maximum resistance to ampicillin (100%), high frequency of resistance to amoxicillin (97.6%), sulfamethoxazole/trimthoprim (94%), streptomycin (92.8%) and ciprofloxacin (89.2%). Conversely, the aminoglycoside amikacin was shown effective against 97.6% of the isolates.

Jarallah *et al.,* (2014) isolated two bacterial species: *Escherichia coli* (40%) and *Staphylococcus aureus* (29%) in butcher's shops, in front of *E.coli* (19%),*S.aureus* (28%) and *Klebsiella sp.* (9%) in restaurants. The antibiotics susceptibility pattern results showed all of these isolates were resist to most traditional antibiotics but in different ratios.

Mohammed *et al.,* (2014) cultured a total of 384 meat samples for detection of *E. coli* and which were also tested for antimicrobial susceptibility. Investigation revealed a 15.89% overall prevalence of *E. coli* in the meat samples. All the *E. coli* isolates were found insensitive to penicillin, ampicillin, doxicycline and erythromycin but sensitive for tetracycline.

Al-Salauddin *et al.,* (2015) isolated *E. coli* from 50 (83.33%) samples and *Salmonella* spp. from 18 (31.66%) samples by using standard bacteriological techniques. Furthermore, the

isolates were subjected to antibiogram studies by disk diffusion method using eight commonly used antibiotics. Antibiogram studies revealed that gentamycin, ciprofloxacin, and norfloxacin were highly sensitive against all the isolated bacteria, whereas most of the isolates were resistant to amoxicillin, erythromycin, and tetracycline. Out of all the isolates, 5 isolates of *E. coli* and 3 isolates of *Salmonella* were found multidrug resistant.

Gwida *et al.,* (2015) recovered *E.Coli, S. aureus* and *Salmonella* spp.from the raw chicken meat at the following percentages: (35, 22 and 5%, respectively) using conventional biochemical identification methods. Serotyping of the obtained *Salmonella* spp., revealed that *Salmonella Kentucky* presented at the highest rate of isolation followed by *Salmonella enteritidis, Salmonella infantis* and *Salmonella typhimurium*. High frequency of *S. aureus* were found to colonize the skin (40%) and the stool specimens (30%) of chicken meat handlers; whereas four out of 50 stool samples (8%) and one out of 50 hand swabs (2%) from handlers were found to be contaminated with *Salmonella* spp. *E.coli* was also detected in 40% of the stool samples and in 24% of handlers hand swabs. Serological identification of *E. coli* isolates revealed the presence of *E. coli* (O26: H11, O103:H2, O128:H2, O111:H2 and O78) in the examined raw meat, O26: H11, O2:H4 and O128:H2 in stool samples and O26: H11, O103:H2 and O125:H21 in hand swabs. All recovered isolates showed various degree of antibiotic resistance.

Begum *et al.,* (2015) tested 12 and 6 isolates of STEC from cattle and pig faecal samples respectively in Guwahati, India for in vitro susceptibility against 15 different antimicrobial agents and showed highest sensitivity towards ciprofloxacin (100%) followed by norfloxacin (91.67%), chloramphenicol (83.33%), nalidixic acid (83.33%), co- trimoxazol (75%) and cephotaxim (66.67%), gentamicin (58.33%), streptomycin (58.33%), enrofloxacin (41.67%), tetracycline (33.33%) and amoxicillin (16.67%) in case of isolates from cattle. While STEC isolates from pigs showed highest sensitivity to ciprofloxacin (100%) and norfloxacin (100%).

Jahan *et al.,* (2015) confirmed 12 (25.53%) isolates as *S. aureus* out of 47 milk samples by using bacteriological, biochemical and PCR-based identification schemes,. All the isolates showed β-hemolysis on 5% sheep blood agar. *S. aureus* specific *nuc*gene (target size 279-bp) was amplified in the cases of all isolates. The isolates were found as resistant

to penicillin (100%), erythromycin (75%) and amoxicillin (100%). On the other hand, the isolates were sensitive to ciprofloxacin (83.33%), oxacillin (100%), cloxacillin (100%) and neomycin (100%). The isolated *S. aureus* showed increased resistance to broad spectrum antibiotic (e.g., Ciprofloxacin).

Mahanti *et al.,* (2015) screened 36 *E. coli* (STEC) isolates isolated from fecal samples of healthy goats for Antibiogram study and found that resistance was observed most frequently to erythromycin (80.5%), amikacin (52.7%), cephalothin (50%), kanamycin (41.6%), neomycin (36.1%) and gentamycin (36.1%) and less frequently to norfloxacin (2.7%), enrofloxacin (2.7%), and ciprofloxacin (2.7%). Multidrug resistance was observed in 11 STEC isolates.

Noori *et al.,* (2016) concluded that among 100 broiler meat samples, 85% were bacterial positive isolates and in local broiler meats, 37 out of 49 were positive isolated while in imported broiler meat, 48 out 51 were bacterial positive isolates ,among 48 local broiler meat, it was reported 39% *Salmonella* spp, *E. coli* 29% , 6% *Pseudomonas* spp, 6% *Citrobacter* and 5% *Proteus* spp. The present study showed that the main *Salmonella* spp isolates are *S.infantis* 0.54%, *S.vichow* 0.13%, *S.enteritidis*0.21%, *S.hato* 0.08%, *S.dublin* 0.05%. It was recorded that *Salmonella infantis* was high resistant to intermediate resistant to ciproflaoxacin (CIP10) amikacine (AK10) gentamicin (CN10).

Das *et al.,* (2016) analyzed 30 samples out of 65, 17 from chicken and 13 from goat were positive for Staphylococci with the prevalence rate of 48.57% from chicken and 43.33% from goat. Staphylococcal isolates were found variably resistant to the antibiotics tested. 80% of the isolates were positive for at least one of the antibiotics used in this study. The isolates showed maximum resistance for penicillin (73.33%) which is followed by erythromycin (36.66%), tetracyclin(26.66%), oxacillin(23.33%), ciprofloxacin (16.66%), chloramphenicol(10%) , vancomycin(3.33%).

Albarri *et al.,* (2017) collected a total of 48 samples of vegetables, meat and chicken from retail stores and supermarkets. MNP with the medium Fluorocult Lauryl Sulfate Broth (FLSB) was used as the conventional method to isolate and detect *E.coli* from the samples, while Polymerase chain reaction with uidA-specific primers was used to confirm the present of *E. coli* isolates. Of the total 48 food samples analyzed *E. coli* was isolated from 34 (70.8%) out of which 11 (22.9%) had *E. coli* O157:H7. The highest percentage (93.75%) of *E. coil* was isolated from chicken, while lowest percentage (56.25%) was

isolated from meat. *E. coli* O157:H7 was found in chicken as highest percentage (31.25%), while lowest percentage (18.75%) was isolated from meat and vegetables.

Rahman *et al.,* (2017) collected a total of 169 samples including milk (n=108), chicken meat (n=51) and beef (n=10) 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.

Adugna *et al.,* (2018) found that the prevalence of *S. aureus* in the abattoir, butcher, cutting table, hook, and knife was 9.4%, 19.8%, 15%, 15%, and 22.5%, respectively. The prevalence of *S. aureus* in the knife and butcher was found to be 2.8 (OR = 2.8, CI = 1.2–6.4) and 2.4 (OR = 2.4, CI = 1.6–3.6) times that of the abattoir results ($p< 0.01$). The antimicrobial susceptibility testing was also conducted on 133 isolates of *S. aureus* using the disc diffusion susceptibility method. Bacitracin, neomycin, and methicillin were found to be 100% resistant to *S. aureus*.

CHAPTER 3

MATERIALS AND METHODS

This study was conducted at the laboratory of the Department of Microbiology & Parasitology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka 1207, during the period of January to May, 2018.

3.1 Sample collection

This study was designed to investigate the prevalence of bacteria in poultry meat at various market in Dhaka city. A total of 45 raw broiler meat samples were collected from Krishi market, Bihari camp, Agargaon bazar, Taltola bazaar and SAU mini bazaar, Dhaka from January to May 2018. A total of 45 raw broiler meat samples were collected. Collected samples were immediately transported on ice to the Microbiology & Parasitology laboratory of the Sher-e-Bangla Agricultural University for analysis. The samples were directly transferred in an icebox to the laboratory for further preparation and examination.

Table 1. No. of meat samples collected from different areas of Dhaka Zone

Sl. No.	Name of market	No of samples
01.	Krishi Market	10
02.	Bihari Camp	10
03.	Agargaon Bazar	10
04.	Taltola Bazar	10
05.	SAU Mini Bazar	5
Total		**45**

3.2 Experimental design

The whole experimental design is accomplished into two steps. The first step included isolation of the bacteria from broiler meat and identification of *Staphylococcus* spp., *Salmonella* spp., and *E. coli* by cultural and morphological characteristics. Motility test with hanging drop preparation and carbohydrate fermentation tests were also done to confirm the isolated organism as *E. coli, Staphylococcus* spp. and *Salmonella* spp. The second step included the study of response of the isolated bacteria against commercially available antibiotic discs.

Raw meat samples were collected from the different areas of Dhaka. Then they are cut into small pieces and placed into Nutrient Broth (NB). Primary growths of bacteria of each collected sample were performed in NB. Individual samples were then subjected to Gram's staining to ascertain the different types (morphologically) of organism present in the culture. Each incubated broth sample was then streaked onto NA plates separately as to obtain individual colony. From individual colony subcultures were grown on BA, NA, SS agar, EMB and MC agar media for obtaining pure culture of the isolated organisms. After determining cultural character, these pure cultures of the organisms were subjected to staining and morphological examination for identification of organisms. The samples of NB were first inoculated on Nutrient Agar by spreading method. Then the isolated organism was inoculated on Eosin Methylene Blue (EMB) Agar, McConkey Agar for selection of *E. coli.* Then they are cultured on *Salmonella*, *shigella* selective media, *Salmonella –Shigella* (SS) Agar. Later the isolates were characterized by cultural characteristics on Staphylococcal selective media, Mannitol Salt Agar (MSA). Then Gram's staining, biochemical tests and molecular characterization was performed by PCR. Finally the isolated organisms were subjected to Antibiogram study test to observe the resistant characteristics of organism on some specific antibiotic disk.

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

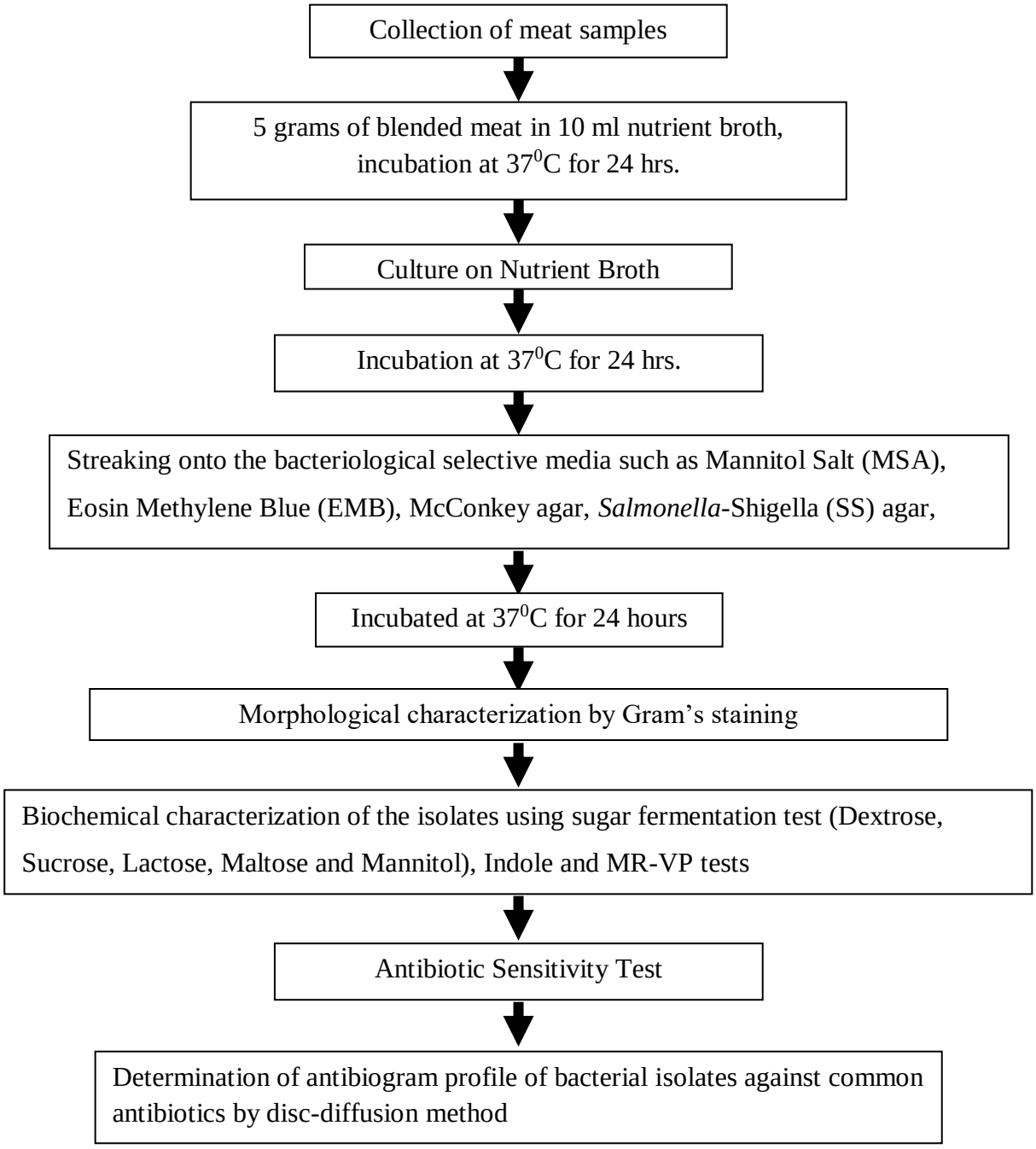


Figure 1: Schematic illustration of the experimental design

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 Nutrient agar, Eosin-Methylene-Blue (EMB), MacConkey agar (MC), *Salmonella-shigella* (SS), Manitol salt agar (MS) and Blood Agar (BA).

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 like (Crystal violet, Gram's iodine, Safranine, Acetone alcohol , Alcohol solution (100 mL bottle) etc.

Reagents for methylene blue staining like (Methylene blue, ethyl alcohol, distilled water), Xylene, 4% sodium hydroxide, 3% hydrogen peroxide, oxidase reagent, Kovac's indole reagent (4 dimethyl amino benzaldehyde, concentrate HCL), 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), peptone broth were used , Himedia. Motility indole urease (MIU) medium base (Himedia, India) was used for motility test.

3.4.2 Glassware and other appliances

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, eppendrof 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 Materials required for anti-biogram study

3.5.1 Muller Hinton Agar (MHA)

Muller Hinton Agar plates were specially used for the Antibiogram study test (Hi media, India).

3.5.2 McFarland standards

McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within the standard range.

3.5.3 Antibiotic discs

Commercially available antibiotic discs (Oxoid, England) were used to determine the drug sensitivity pattern.

Table No. 2: Antimicrobial agent with their disc concentration

Antimicrobial agent with their disc concentration are presented below

Antimicrobial agents	**Disc Concentration (µg)**
Amoxycillin (AMX)	30
Azithromycin (AZM)	30
Ciprofloxacin (CIP)	5
Streptomycin (S)	10
Ampicillin (AMP)	10
Penicillin (P)	10
Gentamycin (GEN)	10
Tetracycline (TE)	30

3.6 Methods

3.6.1 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.

3.6.2 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 glasswares were usually sterilized by dry heat at 160°C for 1/2 hour in an oven. However, the bottles with plastic caps or rubber lined aluminum caps were sterilized by autoclaving at 121°C 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°C and the caps of the bottles were tightened after cooling

3.7 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.

3.7.1 Nutrient Agar Media

Nutrient agar was prepared according to the procedure of manufacturer; 2.5gms of Bacto-Nutrient Agar (Difco) was suspended in 100 ml distilled water and boiled to dissolve completely. The solution was sterilized by autoclaving at 121°C at 15 lbs. per sq. inch (1 kg/cm2) for 15 minutes. After autoclaving, the medium was poured in 10

ml quantities in sterile petridishes (75 mm diameter) to form a thick layer and allowed to solidify. The sterility of the medium was checked by incubating overnight at 37°C and then the plates were stored at 4-8°C for future use.

3.7.2 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.

3.7.3 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 petridish 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.

3.7.4 MacConkey (MC) agar

51 gms 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).

3.7.5 Mannitol salt (MS) agar media

11.1grams powder of MS agar base (Hi-media, India) was suspended in 100 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 under 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 petridish to make MS agar plates. After solidifying the medium, the plates were kept in the incubator at 37°C for overnight to check their sterility.

3.7.6 *Salmonella-Shigella* (SS) agar media

63.03grams powder of SS agar base (Hi-media, India) was suspended in 1000 ml of autoclaved distilled water. The suspension was heated to boil for few minutes to dissolve the powder completely in water. The distilled water was autoclaved for 30 minutes less than 15 lbs pressure per square inch (1 kg/ cm^2) to make it sterile. Over heating or autoclaving may destroy the specificity of the medium. Then 10-20 ml of medium was poured into small and medium size sterile Petri dish to make SS agar plates. After solidifying the medium, the plates were kept in the incubator at 37°C for overnight to check their sterility.

3.7.7 Muller Hinton Agar (MHA)

Thirty-eighty grams of dehydrated Muller Hinton Agar Medium was suspended in 1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs. pressure for 15 minutes. The autoclaved materials were allowed to cool to a temperature of 45°C in a water bath and distributed to sterile petridishes. After solidification petridishes were placed in an incubator for 24 hours at 37°C to check sterility and then placed in a refrigerator at 4°C until use

3.7.8 Preparation of Blood Agar (BA) Media

Twenty-three grams of dehydrated blood agar base (Difco, England) was suspended in 100 ml of distilled water and boiled until dissolved completely. It was then sterilized by autoclave at 121^0C for 15 minutes less than 15 pounds pressure per square inch (kg/cm^2). After autoclaving, the medium was allowed to cool down at

45^0C in water bath and then 5% defibrinated bovine blood was added. The medium was then poured in the sterile petridishes (75mm diameter) in a volume of 10 ml quantities in each to form thick layer and was kept at room temperature for solidification. After solidification, sterility of the media was checked by incubating at 37^0C in the incubator for 2A hours and those found sterile were kept at 4-8°C until used (Cowan, 1985).

3.8 Reagents preparation

3.8.1 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.

3.8.2 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°C maintaining a pressure of 15 Ib/sq. inch for 15 minutes. After autoclaving, the tubes containing medium were incubated at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

3.8.3 Kovac's reagent

This reagent was prepared by dissolving 0.1 gm of Bacto methyl-red in 300 mL of 95% alcohol and diluted to 500 ml with the addition of distilled water.

3.8.4 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.

3.8.5 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).

3.9 Processing of meat samples

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

3.10 Methodology followed for isolation and identification of *E. coli, Salmonella* and *Staphylococcus*.

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

3.10.1.1 Primary culture

Primary growth was performed in nutrient broth followed by inoculation at 37°C for overnight.

3.10.1.2 Method for obtaining pure culture

Enriched culture from nutrient broth was streaked on to selective agar 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**.**

3.10.1.3 Identification of isolated bacteria

The cultural examination of meat samples for bacteriological analysis was done according to the standard method (ICMSF, 1985). Identification of bacteria was performed on the basis of colony morphology; Gram's staining reaction and biochemical test.

3.10.1.4 Colony characteristics

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

3.10.1.5 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 as counter stain and allowed to stain for 2 minutes. The slides were then washed with water, blotted and dried in air and then examined under light microscope (400X) using immersion oil.

3.10.1.6 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°C 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 an un-inoculated tube).

3.10.2 Biochemical tests

3.10.2.1 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.

3.10.2.2 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.

3.10.2.3 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, manitol) separately and incubated for 24 hours 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 tube.

3.10.2.4 Indole test

Two milliliter of peptone water was inoculated with the 5ml of bacterial culture and incubated at 37°C 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).

3.10.2.5 Voges-Proskauer test

Two milliliter of sterile glucose peptone water was inoculated with the 5 ml of test organisms. It was incubated at 37°C 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).

3.10.2.6 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 P^H resulting from the fermentation of glucose. A yellow coloration indicated negative result (Cheesbrough, 2006).

3.11 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.

3.12 Antibiogram study test

The disc diffusion method was used to detect antimicrobial susceptibility assay according to the recommendation of Clinical and Laboratory Standards Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards, CCLS: 2016). Antimicrobial drug susceptibility against nine commonly used antibiotics were performed by disc diffusion or Kirby–Bauer method (Bauer *et al.,* 1966). The procedure of disc diffusion method is presented below:

i. One well isolated colony was selected from the S-S, EMB and MS agar plate.

ii. Colony was touched with a sterile loop and streaked onto nutrient agar and incubated overnight at 37°C.

iii. 4 or 5 well isolated colonies were transferred into a tube of sterile physiological saline and vortex thoroughly .

iv. The bacterial suspension was compared with 0.5 McFarland standard.The comparison was made by viewing this tube against a sheet of white paper on which black lines were drawn.

v. A sterile cotton swab was dipped into the bacterial suspension. The excess fluid of swab was removed by pressing firmly against the inside of the tube just above the fluid level.

vi. The swab was streaked over the entire surface of Mueller-Hinton agar (Himedia, India) medium three times, rotating the plate approximately 60

degrees after each application to ensure an even distribution of the inoculums.

vii. The antimicrobial discs were placed individually using sterile forceps and then gently press down onto the agar.

viii. The plates were inverted and incubated at 37˚C temperature for overnight. After incubation the diameter of the zone of complete inhibition (including diameter of the discs) was measured in millimeters with a ruler.

3.12.1 Interpretation of the results

After the discs are placed on the plate, the plates were inverted and incubated at 35℃ for 8 to 12 hours following which the diameter of the zones of complete inhibition (including the diameter of the disc) was measured and recorded in millimeters. The measurements were made with a ruler on the under surface of the plate without opening the lid. The zones of growth inhibition was compared with the zone-size interpretative table provided by Clinical and Laboratory Standards Institute (CLSI, 2016) (Table No. 12, 13 & 14). Antimicrobial testing results were recorded as susceptible, intermediate and resistant according to zone diameter interpretive standards provided by CLSI (2016).

Table No. 3: The zone-size (mm) of *E. coli* interpretative table provided by Clinical and Laboratory Standards Institute (CLSI, 2016).

Antimicrobial agents	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Amoxycillin	≤ 13	14-17	≥ 18
Azithromycin	≤ 12	_	≥ 13
Ciprofloxacin	≤ 20	21-30	≥ 31
Streptomycin	≤15	16-21	≥22
Ampicillin	≤12	13-19	≥20
Penicillin	≤14	15-22	≥23
Tetracycline	≤ 11	12-14	≥ 15
Gentamycin	≤ 12	13-14	≥ 15

Table No. 4: The zone-size (mm) for *Salmonella* interpretative table provided by Clinical and Laboratory Standards Institute (CLSI, 2016).

Antimicrobial agents	**Resistant**	**Intermediate**	**Sensitive**
Amoxycillin	≤ 13	14-17	≥ 18
Azithromycin	≤ 12	_	≥ 13
Ciprofloxacin	≤ 20	21-30	≥ 31
Streptomycin	≤15	16-21	≥22
Ampicillin	≤12	13-19	≥20
Penicillin	≤14	15-22	≥23
Tetracycline	≤ 11	12-14	≥ 15
Gentamycin	≤ 12	13-14	≥ 15

Table No. 5: The zone-size (mm) for *Staphylococcus* interpretative table provided by Clinical and Laboratory Standards Institute (CLSI, 2016).

Antimicrobial agents	**Resistant**	**Intermediate**	**Sensitive**
Amoxycillin	≤ 19	_	≥ 20
Azithromycin	≤ 13	14-17	≥ 18
Ciprofloxacin	≤ 15	16-20	≥ 21
Streptomycin	≤16	17-21	≥22
Ampicillin	≤12	13-19	≥20
Penicillin	≤14	15-22	≥23
Gentamycin	≤ 12	13-14	≥ 15
Tetracycline	≤ 14	15-18	≥ 19

CHAPTER 4

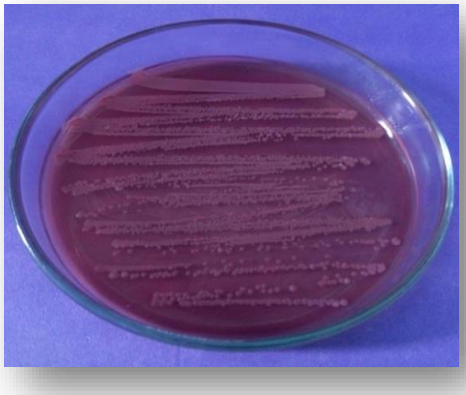
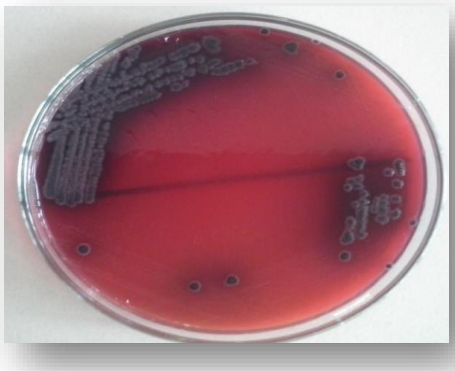
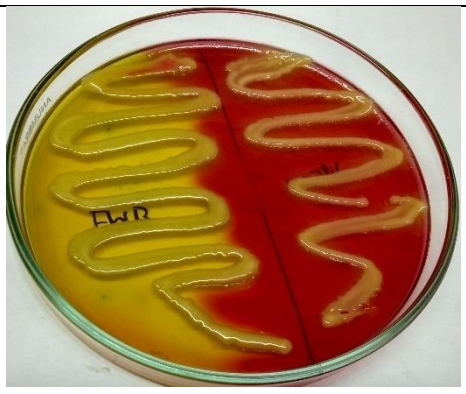
RESULTS AND DISCUSSION

A total 45 chicken meat samples were collected from various market of Dhaka city for microbiological analysis. After processing samples were incubated for 24 hours at 37°C in NB. Firstly a loop full of broth is streaked on Nutrient Agar then to different selective media and incubated for 24 hrs. at 37°C and then observed colony morphology for cultural characterstics. Three genera of bacteria such as: *Salmonella* spp., *E. coli and Staphylococcus* spp. were isolated from chicken meat samples.

4.1 Cultural characteristics of the isolated bacteria recovered from chicken meat

Table No. 6: 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 SS agar was indicated by smooth, circular, pink color colonies
2	*Salmonella* spp.		*Salmonella* spp. on SS agar was indicated by smooth, circular, black color colonies
			*Salmonella* spp. on XLD agar was indicated by smooth, circular, black center colonies
3	*Staphylococcus* spp.		*Staphylococcus* spp. on MS agar was indicated by smooth, circular, small whitish or yellowish colony

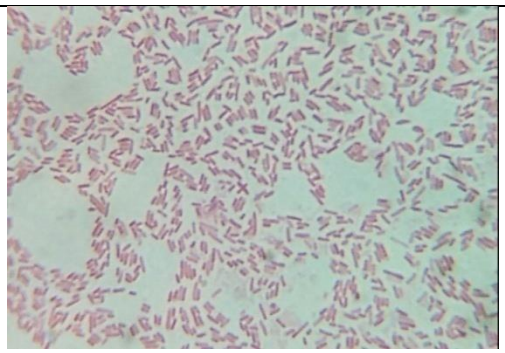
			*Staphylococcus* spp. on Blood agar was indicated by smooth, circular, small, whitish colony

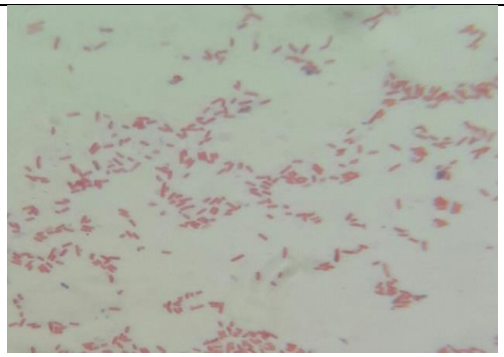
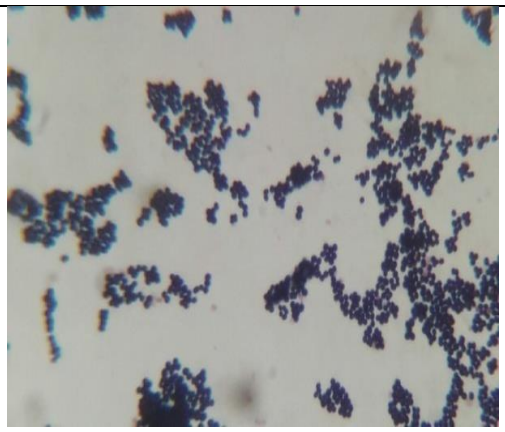
EMB= Eosin Methylene Blue; MS=Mannitol Salt; SS= *Salmonella-Shigella*

4.2 Gram's staining

Morphological and staining characteristics of bacteria recorded from meat by Gram's staining presented in Table No. 07

Table No. 7: Morphological and staining properties of the bacterial isolates by Gram's staining

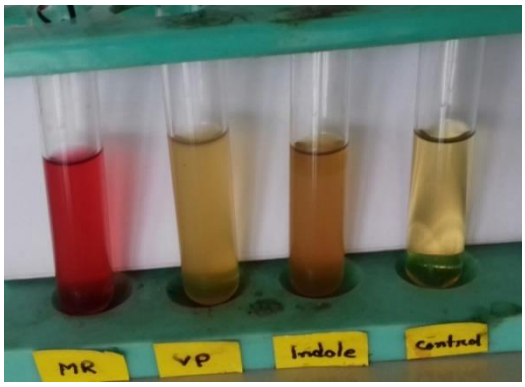
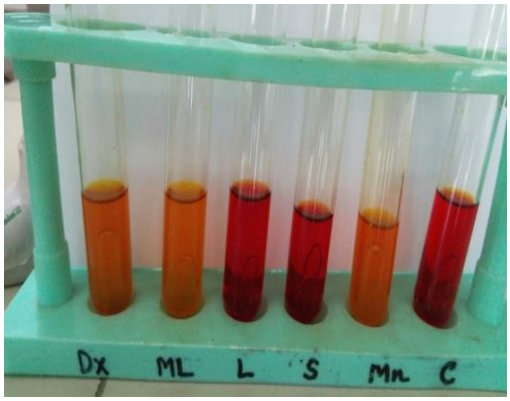
Serial No.	Name of Organisms	Characteristics	
		Gram's staining	Microscopic characteristics
1	*E. coli*		Gram negative single or pairedshort plump rods of *E. coli* (100X)

2	*Salmonella* spp..		Gram negative single or paired short plump rods of *Salmonella* spp. (100X)
3	*Staphylococcus* spp.		Gram positive cocci shaped bacteria arranged in grapes like cluster indicative of *Staphylococcus* spp. (100X)

4.3 Biochemical Test

Table No. 8 Results of sugar fermentation and biochemical tests

Sl. No.	Name of bacteria	Sugar fermentation and biochemical tests	Interpretation
1	*E. coli*		*E.coli* fermented dextrose, mannitol, lactose, maltose, and sucrose with acid and gasproduction

			For *E. coli* MR and Indole were positive but VP test was negative
2	*Salmonella* spp.		*Salmonella* spp. fermented dextrose, maltose, and mannitol with acid and gas production but did not ferment lactose and sucrose.
			*Salmonella* spp. MR was positive but Indole and VP test were negative

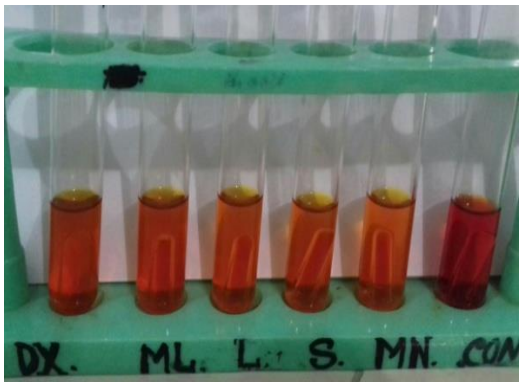
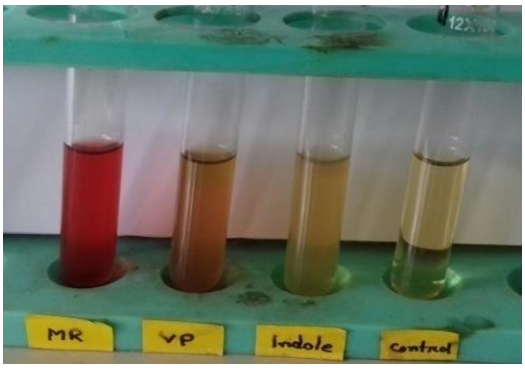
3	*Staphylococcus* spp.		*Staphylococcus* spp. fermented dextrose, mannitol, lactose, maltose, and sucrose with acid production
			*Staphylococcus* spp. MR and VP test were positive but Indole was negative
4	Catalase test of *Staphylococcus* spp.		Bubble formation (B) indicating positive reaction, no bubble formation indicating negative reaction or control (A).

Table No. 9: Biochemical test of Bacteria from chicken meat

Organisms	Gram stain	Sugar Fermentation					Cata-lase Test	Indo-le Test	MR Test	VP Test
		G	L	S	M	D				
Escherichia coli	Pinkish rod colony	AG	AG	A±/-	AG	AG±	-VE	+VE	+VE	-VE
Salmonella spp.	Pinkish cocco-bacilli	A	-	A±/-	A	AG±	+VE	-VE	+VE	-VE
Staphylococcus aureus	Purple cocci	A	A	A	A	A	++ VE	-VE	+VE	+VE

Table No. 10: Summary of prevalence of bacteria from chicken meat.

Sl. No.	Sources and Location	Total	Prevalence of *E. coli* (%)	Prevalence of *Salmonella* spp. (%)	Prevalence of *Staphylococcus aureus* (%)
1.	Krishi Market	10	60	50	30
2.	Bihari Camp	10	100	60	30
3.	Agargaon Bazar	10	70	50	20
4.	Taltola Bazar	10	80	40	10
5.	SAUMini Bazar	5	60	10	0

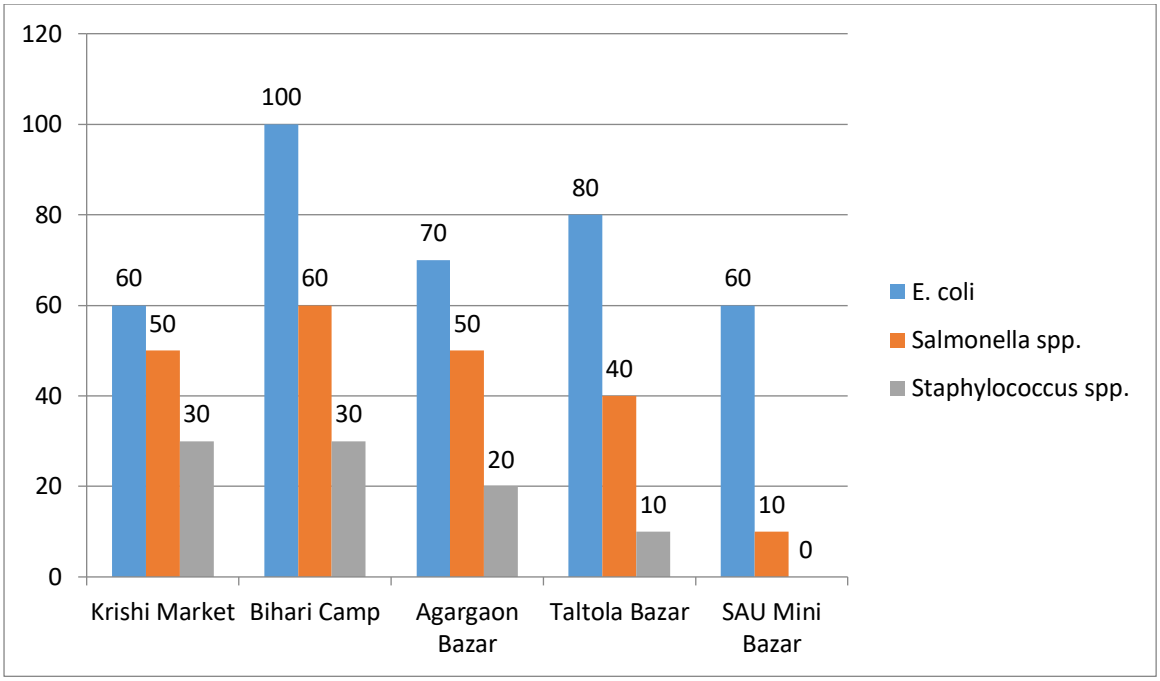


Fig. 2: Graphical representation of prevalence (%) of bacteria in chicken meat.

4.4 Results of Antibiogram assay

A total of three isolates such as. *E. coli, Salmonella* spp. and *Staphylococcus* spp. were subjected to Antibiogram assay. The results of Antibiogram assay are presented below

4.4.1 Antibiogram assay of *E. coli*

E.coli isolates were tested against eight different antibiotics. Among them Ciprofloxacin showed the highest susceptibility pattern followed by the gentamycin, azithromycin and tetracycline found sensitive in this study. Highest resistant pattern was showed by penicillin then amoxycillin, streptomycin and ampicillin.

Table No. 11: Antibiogram assay of *E. coli*

Name of antibiotic disc	Interpretation
Amoxicillin	R
Azithromycin	S
Ciprofloxacin	S
Streptomycin	R
Ampicillin	R
Penicillin	R
Tetracycline	S
Gentamycin	S

R=Resistant, S=Sensitive

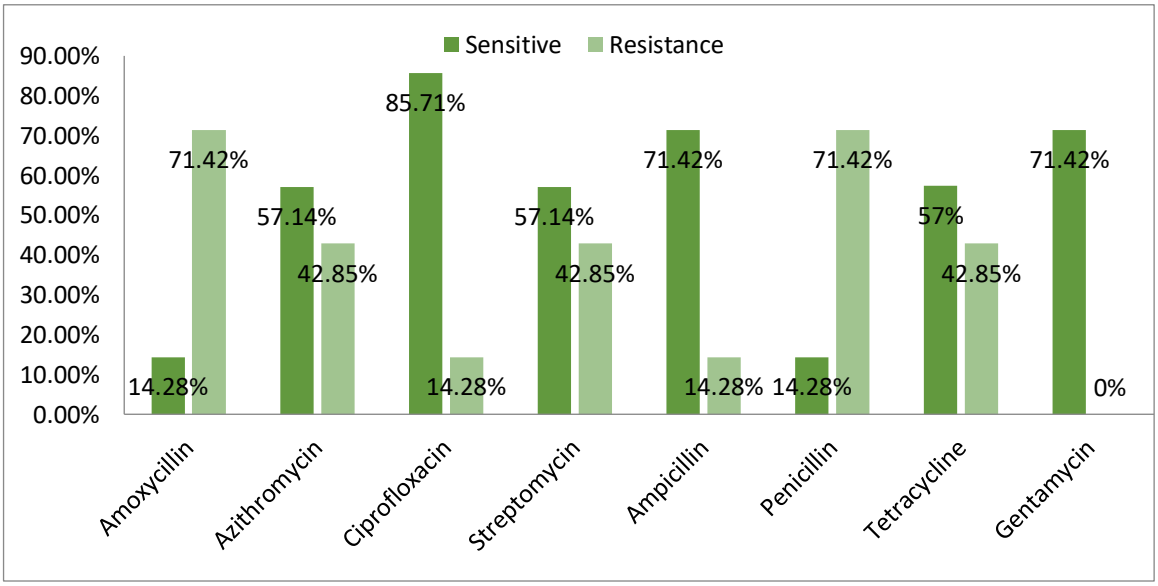


Fig. 3: Antibiogram of *E. coli*

4.4.2 Antibiogram assay of *Salmonella* spp.

Salmonella isolates were tested against eight different antibiotics. Among them Ciprofloxacin showed the highest susceptibility pattern followed by the Gentamycin

and Azithromycin. Highest resistant pattern was showed by Tetracycline, Streptomycin, Penicillin and Amoxycillin.

Table No. 12: Antibiogram assay of *Salmonella* spp.

Name of antibiotic disc	Interpretation
Amoxicillin	R
Azithromycin	S
Ciprofloxacin	S
Streptomycin	R
Ampicillin	R
Penicillin	R
Tetracycline	R
Gentamycin	S

Resistant, S=Sensitive

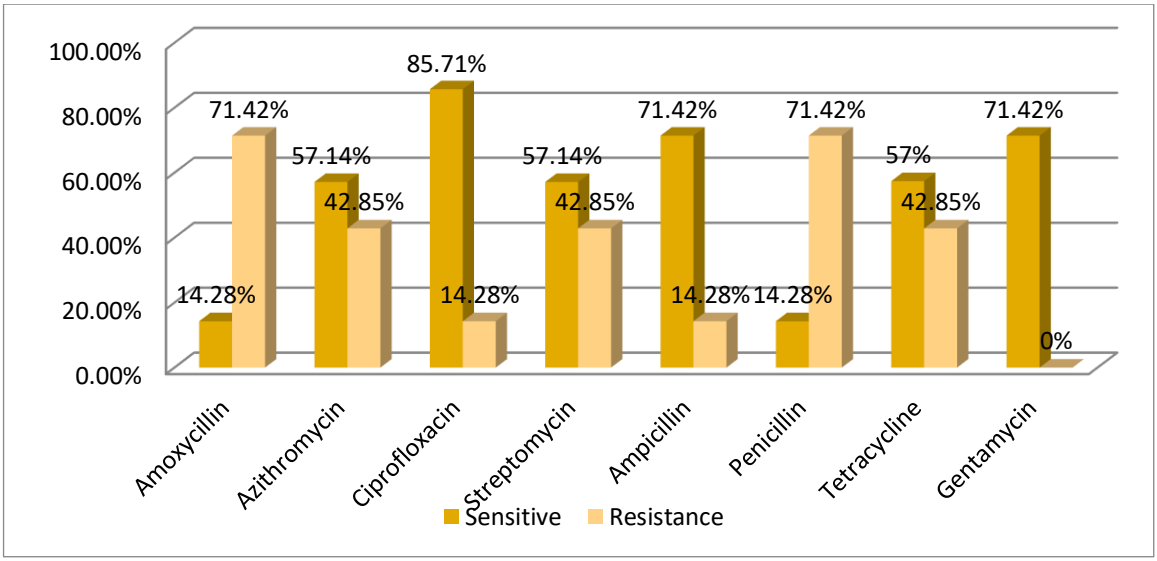


Figure 4: Antibiogram of *Salmonella* spp.

4.4.3 Antibiogram assay of *Staphylococcus* spp.

Staphylococcus isolates were tested against eight different antibiotics. Among them Ciprofloxacin showed the highest susceptibility pattern followed by the gentamycin.

Also, ampicillin, streptomycin, azithromycin and tetracycline are found sensitive inthis study. Highest resistant pattern showed by amoxycillin and penicillin.

Table No. 13: Antibiogram assay of *Staphylococcus* spp.

Name of antibiotic disc	Interpretation
Amoxicillin	R
Azithromycin	S
Ciprofloxacin	S
Streptomycin	S
Ampicillin	S
Penicillin	R
Tetracycline	S
Gentamycin	S

R=Resistant, S=Sensitive

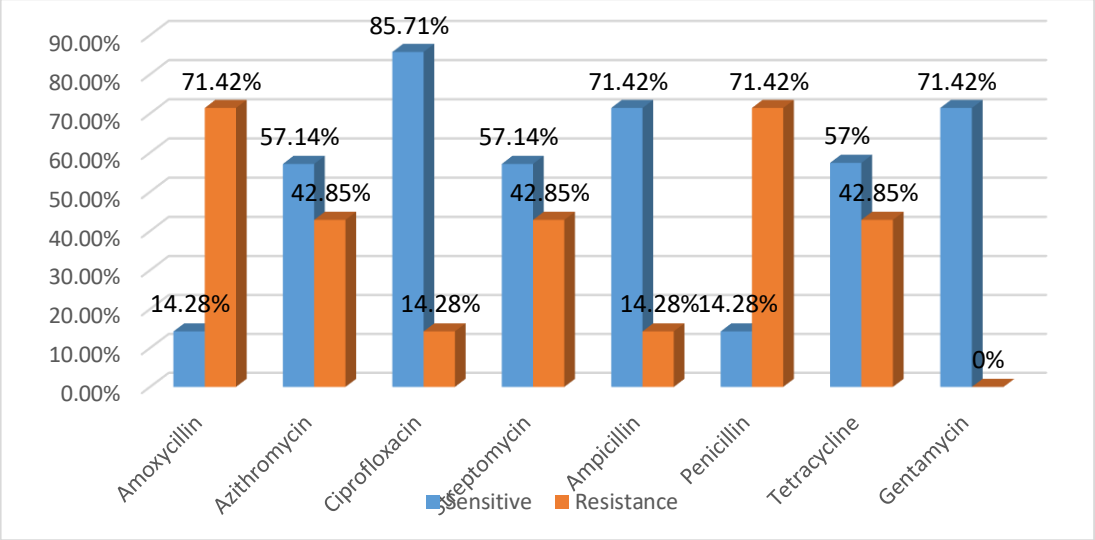


Figure 5: Antibiogram of *Staphylococcus* spp.

DISCUSSION

Present study revealed the presence of pathogenic bacteria namely, *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. in broiler meat collected from 5 selected markets of Dhaka city.

The isolates were identified by basic microbiological techniques including cultural and staining characteristics, motility, and biochemical test. Furthermore antibiogram study of isolated bacteria against commonly used antibiotics was performed. Different kinds of culture media including enrichment media and selective media were used to facilitate the growth of *E. coli*, *Salmonella spp.* and *Staphylococcus spp.* 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 Nazir *et al*., (2005); Hassan *et al., (*2014). The cultural properties of *E. coli* were the production of metallic sheen on EMB agar and rose pink coloured colony on the MacConkey agar which agreed with the findings of others (Kabir *et al*., 2017; Parvej *et al.,* 2018;). In Gram's staining, the morphology of the isolated *E. coli* was exhibited as Gram negative, short plump rod arranged in single, paired or in short chain which was reported previously (Tanzin *et al.* 2016; Mamun *et al*., 2016; Kabir *et al*., 2017; Parvej *et al.,* 2018;). *E. coli* isolates were able to ferment the five basic sugars by producing both acid and gas which was supported by (Beutin *et al.,* 1997; Sandhu and Clarke, 1996). The isolated *E. coli* were found MR and indole test positive but VP test negative. Those similar results were reported by many investigators (Mishra *et al.,* 2002; Ali *et al.,* 1998).

Specific enriched media like SS and XLD as described earlier by others were used for the isolation and identification of *Salmonella* spp. (Kabir *et al.,* 2017; Habrun and Mitak, 2003). Organisms grown on the selective media for *Salmonella* spp. were further subjected to detailed study on morphology, colony characteristics and biochemical properties. The colony characteristics of the organisms grown on selective media for *Salmonella* in this study were in accordance with the findings reported by other authors as characteristics for *Salmonella* spp. (Habrun and Mitak., 2003; Hossain, 2002). In addition, the isolated organism was Gram's negative, short plump rod arranged singly or in pair which also indicative of *Salmonella* (Musa *et*

al.,2017; Kamal *et al.*,2018). Furthermore, suspected *Salmonella* spp. were able to ferment dextrose, maltose and mannitol with the production of both acid and gas but did not ferment lactose and sucrose, and those characteristics of *Salmonella* spp. were satisfied the statement of Han *et al.,(*2011) and Musa *et al.,* (2017). The isolated *Salmonella* spp. were found MR test positive but indole and VP test negative that satisfied the statement of OIE, 2000; Douglas *et al.,* 1998. These all cultural, morphological and biochemical properties indicated that the isolated organism as species belonging to the genus *Salmonella.*

The colonies of *Staphylococcus* spp. on mannitol salt agar showed colonies that fermented mannitol and appeared golden yellow were characteristically similar to those reported previously (Shapna *et al.,* 2018; Das *et al.*,2019; Haque *et al.*,2018) Microscopically gram's stained smear of *Staphylococcs* spp. was gram positive cocci arrange in grape like cluster reported by Kabir *et al.,* (2017) also mentioned that the bacterium is non-sporulated, non-capsulated and non-flagellated. The isolated *Staphylococcus* spp. ferment of glucose, maltose, lactose, sucrose and mannitol fermentation with only acid. These findings are in close agreement with Haider *et al.,(*2018;); Shapna *et al.,* (2018). All are positive in Methyl red test. *Staphylococcus* spp. is positive in Voges-Proskauer test.

The overall prevalence of *E. coli* in broiler meat from different markets in Dhaka city was 74% where the highest prevalence (100%) at Bihari Camp and the lowest (60%) at SAU Mini Bazar & Krishi Market. It might be due to the very unhygienic practice in Bihari Camp Market than Mini Bazar & Krishi Market. The study findings were more or less similar to the findings of Al-Salauddin *et al.,* (2015) who reported the prevalence of *E coli* was 83.33% in broiler meat at various market of Mymensingh, Gazipur, and Sherpur districts. This slight difference might be due to variation of working methodology or environmental variation in different study areas.

The overall prevalence of *Salmonella* in meat from different markets in Dhaka city was 42% which was slightly lower than the previous report of Al-Salauddin*et al.,* (2015) who found 31.66% prevalence of *Salmonella* species in various markets of Mymensingh, Gazipur, and Sherpur districts. This slight difference might be due to

variation of working methodology or environmental variation in different study areas. As *Salmonella* is waterborne pathogen, high water contamination in Dhaka city than other city could be the reason of higher prevalence rate in Dhaka city. Besides, in Dhaka the butcher use same water repeatedly during meat handling & processing.

Staphylococcus found in meat from different market was 18% which is lower than Das *et al.,* (2016) who found 48.57% contamination in chicken meat and 43.33% in goat meat in Southern Assam. In Southern Assam, the prevalence was high probably due to soil contamination or environmental variation.

In this study, *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. all showed highest prevalence in Bihari Camp Market and lowest prevalence in SAU Mini Bazar because of very poor hygienic practices in Bihari Camp Market.

In Bangladesh, the use of broad spectrum antibiotics for any disease conditions is very common which is clear indication of the development of multi-drug resistant organisms. It is due to lack of proper knowledge of using of antibiotics. Antibiotic resistant bacteria are known to spread from meat to human via food chain. In this study, eight different antibiotics available in the market were used to study antimicrobial susceptibility profiles of the *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. In this study, isolated bacteria such as *E. coli*, and *Salmonella* spp. were found to grow multidrug resistant (resistant against 4-6 antibiotics) and *Staphylococcus* spp. were found to grow resistance against 2 antibiotics. From that study, it was revealed that the isolated *E. coli* were susceptible to ciprofloxacin, gentamicin, azithromycin and tetracycline which was not supported fully by Akond *et al.,(*2009) who reported resistant to tetracycline. They were resistant to penicillin, amoxycillin, streptomycin and ampicillin. The results were supported by Al-Ghamdi *et al.,(*2001).*Salmonella* spp. were susceptible to ciprofloxacin, gentamycin and azithromycin which was supported by Al-Ferdous *et al.,(*2013) where isolates were sensitive to ciprofloxacin. They were resistant to tetracycline, streptomycin, penicillin and amoxycillin which were not similar to the report of De *et al.,(*2012) who described azithromycin resistant to *Salmonella* spp. *Staphylococcus* spp. were found sensitive to gentamicin, ciprofloxacin, ampicillin, streptomycin, azithromycin which similar the report of Haider *et al.,(*2018) and Shapnan *et al.,(*2018) who

reported that ciprofloxacin, azithromycin resistant to *Staphylococcus* spp. and tetracycline, amoxycillin and penicillin resistant to *Staphylococcus* spp. which agree with Jahan *et al.*,2015; Das *et al.*,2019; Haque *et al.*,2018;).

Broiler meat is very popular in Bangladesh due to its cheap price and availability. But the presence of different pathogenic bacteria which are resistant to multiple antibiotics is threating for human health. To reduce this problem, proper hygienic management is needed to maintain by the broiler producers and proper hygienic processing by broiler meat sellers.

CHAPTER 5

SUMMARY AND CONCLUSION

This study was designed to isolate the bacteria from poultry meat at Krishi market, Bihari camp, Agargaon bazar, Taltola bazaar and SAU mini bazaar, Dhaka from January to May 2018. A total of 45 raw broiler meat samples were collected. Collected samples were immediately transported on ice to the Microbiology & parasitology laboratory of the Sher-e-Bangla Agricultural University for analysis. The samples were directly transferred in an icebox to the laboratory for further preparation and examination.

After processing of samples primary culture was done in nutrient broth and nutrient agar then pure culture was obtained from different selective media. From the pure culture staining and biochemical tests were done by maintaining standard procedures. The prevalence of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus* spp. was 74%, 42% and 18% respectively.

All pure isolates were subjected to Antibiogram study test by disc diffusion method against 8 different antibiotics. *E. coli* isolates were showed sensitive to ciprofloxacin (91.6%), gentamycin (87.5%), azithromycin (66.66%), tetracycline (58%) and resistant to penicillin (79.16%) then amoxycillin (75%), streptomycin (75%) and ampicillin (58.3%). Among all *Salmonella* spp. isolates ciprofloxacin (81.81%) showed the highest susceptibility pattern followed by the gentamycin (72.72%) and azithromycin (63.63%). Highest resistant pattern was showed by tetracycline (58%), streptomycin (72.72%), penicillin (72.72%) and amoxycillin (63.63%). in case of *Staphylococcus* spp. ciprofloxacin (85.71%) showed the highest susceptibility pattern followed by the gentamycin (71.42%). also, ampicillin (71.42%), streptomycin (57.14%), azithromycin (57.14%) and tetracycline (57.14%) found sensitive in this study. Highest resistant pattern showed by amoxycillin (71.42%) and penicillin (71.42%).

The prevalance of these bacteria was highest in *Bihari Camp Market* and lowest in *SAU Mini Bazar*. Ciprofloxacin, gentamycin, azithromycin were showed the highest susceptibility and highest resistant pattern was showed by penicillin,

amoxycillinOverall, the prevalence of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus* spp. in broiler meat and their drug resistance is very alarming. Therefore, broiler meat industry should be provided with an immediate attention by the government to maintain strict biosecurity and hygienic managements in farm and live bird markets all over the country.

From the present study, it may concluded that

- The prevalence of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus* spp. was 74%, 42% and 18% respectively.
- *E. coli, Salmonella* spp., *Staphylococcus* spp. successfully isolated and confirmed by different biochemical media, biochemical tests.
- *E. coli, Salmonella* spp., *Staphylococcus* spp. is more prevalent in Bihari Camp Market compare to other Market.
- Most of the isolates showed multi-drug resistance, but sensitive to ciprofloxacin.

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