ISOLATION AND IDENTIFICATION OF BACTERIAL PATHOGENS FROM RAW CHICKEN MEAT IN DINAJPUR DISTRICT

A THESIS

BY

SAID MOHAMED SALAD

REGISTRATION NO. 1805501

SEMESTER: JULY-DECEMBER, 2019

SESSION: 2018

MASTER OF SCIENCE (M.S.)
IN
VETERINARY PUBLIC HEALTH AND FOOD HYGIENE

DEPARTMENT OF MICROBIOLOGY

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Approved as to style and content by

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DECEMBER, 2019

Dedicated

To

My Beloved Mother

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"In the name of Allah, the most beneficent, most gracious and most merciful"

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The author

ABSTRACT

The present research work was under taken to isolate and identify the associated bacteria of chicken raw meat and to find out the effective antibiotics against the bacteria through antibiogram studies in Dinajpur district Bangladesh. July to November, 2019. A total of 48 samples were randomly collected from markets and transported to the Microbiology laboratory of Hajee Mohammad Danesh Science and Technology University, Dinajpur. After processing of samples primary culture was done in nutrient broth and nutrient agar then pure culture was obtained from different selective media. The level of bacterial load were significantly higher the first sampling in comparison with the second and third one respectively. The prevalence of *Escherichia coli, Salmonella spp. and Staphylococcus* spp. were 47.9%, 39.6% and 12.5% respectively. All pure isolates were subjected to Antibiogram assay test by disc diffusion method against 8 different antibiotics. *E. coli* isolates were sensitive moxifloxacin. Highest resistant to chloraphenicol, tetracycline and streptomycin. Among all *Salmonella spp.,* isolates, chloraphenicol showed the highest susceptibility pattern followed by the tetracycline, azithromycin, ampicillin and Erythromycin. Highest resistant pattern was showed by penicillin, Cefalexin and amoxicillin. In case of *Staphylococcus spp*., chloraphenicol showed the highest susceptibility pattern followed by the streptomycin and streptomycin found sensitive in this study. Highest resistant pattern showed by the tetracycline, azithromycin, ampicillin and tetracycline. This study revealed that broiler meat sold at some local markets in Dinajpur city were contaminated with multiple species of multidrug resistant bacteria which may risk for human health.

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LIST OF ABBREVIATIONS AND SYMBOLS

-	= Negative
%	= Percentage
/	= Per
+	= Positive
E. coli	= *Escherichia coli*
EMB	= Eosin Methylene Blue
et al.	= Associated
gm	= Grams
H_2O_2	= Hydrogen Peroxide
H_2S	= Hydrogen Sulfide
Hrs	= Hours
HSTU	= Hajee Mohammad Danesh Science and Technology
KOH	= Potassium Hydroxide
L	= Lactose
Ltd	= Limited
MC	= MacConkey
Mg	= Milligram
Ml	= Milliliter
ML	= Maltose
Mm	= Minutes
Mn	= Mannitol
MR	= Methyl Red
N	= Number
Na	= Nutrient Agar

NB = Nutrient Broth

NO = Number

PBS = Phosphate Buffered Saline

Prof. = Professor

Spp. = Species

TSI = Triple Sugar Iron

VP = Voges Proskauer

CHAPTER 1

INTRODUCTION

Bangladesh is an agriculture based country. As such poultry rearing is considered superior to the others in agricultural sector because of an almost assured in a relatively short period of time. Commercial poultry industry (mostly broilers and layers) plays an important role in the economy of Bangladesh. But the advancement of poultry industry is being hampered by various pathogenic bacterial infections causing nearly 30% mortality of chickens that has been estimated to cost about Tk. 8,000 crores annually in Bangladesh. The bacterial count in poultry housing systems is particularly high in comparison to those of pig and cattle. These pathogens get access to poultry flocks from various sources. Little is known about the bacterial presence in the poultry environment such as in poultry litter and in the poultry house air (Saleh *et al.,* 2003).

Intestinal bacteria play an important role on health through their effects on gut morphology, nutrition, pathogenesis of intestinal diseases and immune responses (Mead, 2000). Various pathogenic microbes, such as *Escherichia coli*, *Salmonella* spp., *Bacillus* spp., *Streptococcus* spp. and *Staphylococcus* spp., have been implicated to reduce the growth of poultry (Duke, 1986). Broiler is a major fast growing source of meat in the world today. The modern poultry industry can produce market-ready broiler chickens in less than six weeks.

This accomplishment has been achieved through genetic selection, improved feeding and keen health management practices including usage of antibiotics as therapeutic agents to 2009). Resistance against frequently used antibiotics has been observed in bacteria present in poultry since the introduction of these antimicrobial agents in poultry. The rise in antibiotic resistance has been reported in the past two decade in many countries including Bangladesh (Kapil, 2004). In Bangladesh, the economic aspect of poultry disease and their mortality and morbidity due to bacterial infection is a matter of great concern to the livestock owners. The antibiotic resistance pattern increases the incidence of disease in poultry and subsequently affects the economy of Bangladesh.

The main public health and economic problem is food borne disease which increase worldwide particularly in individuals eat meal outside their home, due to uncontrolled hygienic preparation of these type of the food, food borne disease was define according

to WHO, infectious or toxic nature of the disease occur through consumption of contaminated food (le Loir *et al.,* 2003). Food borne diseases (FBD) were affected one third population every year world word .In the developing countries and developed countries such as in USA in which billions of dollars were spent in the treatment of food borne pathogens that affected approximately 48millions patients each year (Scallan *et al.,* 2011). However, the main source of food borne disease is the animals (Busani *et al.,* 2006), these disease cause huge public health and economic problems (Gajadhar and Allen, 2004), high mortality (2.2 million individual dead) in human population was occur by food and water borne diarrheal disease every year (FAO/WHO, 2006).

Due to rapidly increasing in human population and changing in urbanization food habits, increasing in animal products consumption such as meats were recorded, FAO suspected consumption of meats and milk products would significantly increase associated with mass production and movement meat products, globally in meats production (FAO, 2006), these condition may lead to give a good chance for food contamination and spread food borne pathogens that animal origin which including *Sallmonella* spp, *Campylobacter*, *E. coli*, *Staphylococcus* spp, *Closytidium*, *Yersinia*, *Listeria*, *Acrobacter*, *Mycobacterium*, *Trichinella*, *Sarcocystis*, *Toxoplasma gondii*and *Cryptosporidium parvum* (Dhama *et al.,* 2013).

Beef and chicken meat contaminated with fecal organisms may consider essential food hygiene problem particularly Enterobacteriaceae including *Salmonella* spp, *E. coli*, *Proteus* as well as *Klebsiella* spp (Paterson 2006). Malpractices handling of poultry meat lead to food contamination with food borne microbes, in addition poultry may considered important food borne pathogen reservoir such as *Salmonella* spp and *Campylobacter enteritis* as a result of these organisms appear asymptomatic in live birds as well as large number of bird carcasses remain together during the operation and processing methods (Cavitte, 2003). Widely using antibiotic in the poultry as treatment prophylacticing or growth promoters in livestock lead to widely spread antibiotic resistant pathogens that cause problem in the humans particularly Salmonellosis (Schroeder *et al.,* 2004).

Threlfall *et al.,* (2003) found that 40 percentage of *Salmonella typhimurium* isolated from humans expressed single or multidrug resistant in certain European countries in 2000, also Chung *et al.,* (2004) isolated antibiotic resistant pathogen from poultry meats particularly *Salmonella* and *E. coli*. Nadeau *et al.,* (2002) recorded that bad handling and

consumption undercook meat of poultry were the main source of meats contamination with *Salmonella* and Campylobacter, these two organisms were associated with raw chicken (Hernandez *et al.*, 2005). Also these organsms were isolated from food and water of poultry (Padungtod and Kaneene, 2005). Majority of poultry meats were come to Iraq from differences sources particularly from India, Iran and China, meat of chicken are storage in freezers but there is not constant power of electric supply in the local markets which are using fuel powered generating sets, therefore these meats may exposure to growth contaminated pathogens which may originated from contaminated the chicken carcasses by their gut containing such as *Salmonella* and *E. coli* which were considered a main food borne pathogens (Adesiji *et al.* 2011).

By the considering above mentioned information this study was designed to isolate and identify the associated bacteria of chicken raw meat and to find out the effective antibiotics against the bacteria through antibiogram studies in Dinajpur district Bangladesh. Therefore, this research will play a great significance role in public health concerns in developing countries like Bangladesh.

Objectives

- ✓ To determine the bacterial load from raw chicken meat.
- ✓ To isolate and identify the bacterial pathogens from collected samples by cultural, morphological and biochemical tests.
- ✓ To determine antibiotic sensitivity of identified isolates.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Determination of bacterial load

Mawia *et al.* (2012) founded the mean values of log10 cfu/g of standard plate count (SAC), *E. coli* count (ECC), *S. aureus* count (SAC) and *Entero faecal* count (EFC) of 85 chevon samples to be 6.37± 0.06, 3.85± 0.85, 3.98± 0.12 and 4.15± 0.15 and for poultry meat to be 6.65± 0.06, 3.81±0.11, 4.09±0.13, 4.02± 0.12 from Jammu. The overall prevalence of *E. coli* in chevon and poultry meat samples was 47(28.14%) out of 167 samples which include 22(25.88%) from chevon samples and 25(30.49%) from poultry meat samples.

Singh *et al.* (2014) assessed samples of poultry, carabeef, chevon and pork from Agra and reported SPC of poultry meat to be satisfactory but the level of contamination of samples of beef, chevon and pork were higher. For carabeef it was found to be 7.03±0.07, pork to be 6.86±0.02 and chevon to be 6.96±0.78.

Sengupta *et al.* (2011) reported that total aerobic bacterial count in chicken meat samples taken from Kolkatta procured from semi-urban and urban markets ranged from 51-55 x 104 and 4-25 x 104 cfu/g of chicken meat respectively. Mean coliform count per gram of poultry meat from semi-urban and urban markets were 3.20 x 102cfu/g and 6.50 x 102cfu/g for chicken meat, respectively.

Bhandare *et al.* (2010) investigated chevon and mutton samples and found an average *Staphylococcus aureus* and *Staphylococcus epidermidis* counts as 3.15±0.18 and 3.46±0.17 log10cfu/cm2, respectively. *Bacillus cereus, Bacillus subtilis* and *Clostridium* spp. counts were 3.10±0.08, 3.41 ±0.19 and 0.76±0.06 log10 cfu/cm2 respectively. The *Escherichia coli* count was 3.54±0.06 and the *Klebsiella aerogenes* count was 3.22±0.22 log10 cfu/cm2. Count for *Proteus vulgaris* and *Proteus mirabilis* were 3.44±0.14 log10 cfu/cm2 and 3.71±0.1log10 cfu/cm^2, respectively highest prevalence was that of *S. epidermidis* followed by *K. aerogenes, B. subtilis* and *P. vulgaris* in from Mumbai.

Abdellah *et al.* (2007) reported levels of mesophiles, coliforms, *Escherichia coli* and *Staphylococcus aureus*on carcasses from 96 samples of chicken meat from popular

market and artisanal (manually) slaughterhouses to be significantly higher ($P < 0.05$) than in poulterers shops and supermarket in Morocco.

Al Dughaym (2009) analysed 100 samples of 10 poultry meat products in Saudi Arabia and reported the mean total bacterial counts to range from 2.7 × 104 cfu/g for nuggets to 3.3 × 107 cfu/g for burger and other products in the range of 105–106 cfu/g while *S. aureus* mean count ranged from < 102 cfu/g for all samples.

Bhandare *et al.* (2007) investigated the microbial load on sheep/goat carcasses in Deonar abattoir and traditional meat shops in Mumbai. The average total viable count after flaying, evisceration and washing in the abattoir was 5.51± 0.36, 6.06±0.53 and 5.13±0.58 cfu/cm2, respectively. Pooled average TVC in the shops after flaying, evisceration and washing was 5.83±0.42, 6.48±0.27 and 6.17± 0.14 log cfu/cm2, respectively.

Willayat *et al.* (2006) in their study reported that 76(60.8%) of 125 fresh chicken sample in Srinagar city were contaminated with food borne micro-organisms and had mean viable counts of 3.74 cfu/g**.**

Alvarez *et al.* (2002) reported mean counts (log10 cfu/g) that ranged from 5.56 to 7.28, 5.96 to 7.87, 3.49 to 5.42, 2.60 to 4.33 and 2.47 to 3.48 for mesophiles, psychrotrophs, coliforms, *E. coli* and *S. aureus* respectively from retail chicken parts and processed chicken products in Spain.

Khalifa and Abd El-Shaheed (2004) reported the aerobic plate count from raw chicken meat in Alexandria with an average of 3.0 x 104.*Enterobacteriaceae* were detected in all examined samples (100%) of raw chicken meat with mean values of 4.1 x 103 cfu/g. *Escherichia coli* occurrence was 22.6percent. *Staphylococcus aureus* was detected in 34.3percent of examined samples of raw chicken meat with an average counts of 2.8 x 102 cfu/g.

Pattanaik *et al.* (1997) reported that the total viable count (TVC) and coliform count of the market chicken samples were 7.93 and 7.22 log10 cfu/g, respectively in Bhubaneshwar city.

Lillard (1989) studied the incidence and recovery of *Salmonella* and other bacteria from commercially grown poultry carcasses at selected pre and post evisceration steps and reported that total aerobic count of 3.71 log10 cfu/g.

Bachhil from Izatnagar (1998) reported that on an average, 30percent each of fresh and frozen buffalo meats, 50 percent kabab and 10percent curry samples were positive for *Staphylococcus aureus* with mean population of 1.00 x 104, 4.4 x 103, 1.09 x 103 and 2.10x 102 per gram respectively. Out of 64 strains, 34.4 produced enterotoxin.

Abu-Ruwaida *et al.* (1994) determined the microbiological quality of broilers during processing in a modern commercial slaughterhouse in Kuwait and reported the mean TVC, *E. coli* count and *S. aureus* count in chicken carcass as 6.5-6.6log10 cfu/g, 3.6 log 10 cfu/g and 4.1log10 cfu/g, respectively.

Al-Mohizea *et al.* (1994) reported that the mean initial microbial counts (log10 count/cm2) in chicken carcass were 4.67, 4.14, 2.21, 2.78 and 2.96 for total aerobes, psychrotrophs, coliforms, *Staphylococcus aureus* and yeasts and moulds, respectively from Riyadh, Saudi Arabia.

Sofos (1994) determined the microbial load in poultry meat and reported that the total aerobic counts, *E. coli* count and *S. aureus*counts were 2-5 log10 cfu/g, 1-5 log10 cfu/g and 3log10 cfu/g respectively in London. Waldroup (1996) studied pathogens commonly associated with processed poultry meat and recorded that *S. aureus*counts in poultry meat varied more than 3 and less than 5 log10 cfu/g poultry meat and its by products from Nigeria.

Johnston and Tompkin (1992) studied the microbiological quality in fresh chicken carcasses in the United States and reported total aerobic count to be 2 to 4 log cfu/cm2.

Mead *et al.* (1993) reported total aerobic count of 3.08-5.50 log10 cfu/g and coliforms count 2.2-3.80 log10 cfu/g and *Staphylococcus aureus* counts ranged between 2.3-3log10 cfu/g in fresh chicken meat processed under standard hygienic procedure in Britain.

Krishnaswamy and Lahiry (1964) investigated mutton samples and reported the count to be 4.6 to 5.3 log10cfu/gm from market meat in India. Armitage (1995) on assessment of the microbiological quality of New Zealand beef and lamb reported that ±772 lamb carcasses had a mean Aerobic Viable Count (AVC) of 3.35cfu/cm2. Gill and Baker

(1998) assessed the hygienic performance of a sheep carcass dressing process in Canada and found unchilled sheep carcasses to have log10 AVC/cm2 at the shoulder, loin and leg to be 2.81, 2.80 and 2.56, respectively.

2.2 Isolation and identification of bacteria

Hyeon *et al.* (2012) isolated *Salmonella* from 118 of the 180 samples (65.5%). *Salmonella* were detected in 105 samples (88%) plated on XLD and 111 samples (94%) plated on SM-ID 2 when RVS broth was used for enrichment, and 43 Samples (36.4%) plated on XLD and 67 samples (56.8%) plated on SM-ID 2 when the MKTTn broth was used. The highest sensitivity was found in the RVS-XLD Combination (0.99), followed by RVS-SM-ID 2 (0.97).

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.

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).

Kwon *et al.* (2010) reported that the phenotypic analysis, *Salmonella gallinarum* strains (n=142) isolated during 2001 to 2007 showed the same pattern in the majority of the biochemical tests such as carbohydrate fermentation and amino acid decarboxylation. Interestingly, all of the strains could not ferment rhamnose, but SG 9R could, making rhamnose a potential biomarker to distinguish the vaccine strain.

Selvam *et al.* (**2010)** reported that Cloacal swabs of birds were subjected for isolation and identification of *Salmonella pullorum* and *Salmonella gallinarum*. The biovars *Salmonella gallinarum* and *pullorum* were differentiated based on TSI agar slant inoculation and different sugar fermentation tests. They found that none of the biovars fermented galactose and dulcitol and this indicated the isolates were not biovar *Gallinarum.* All the isolates fermented glucose and were confirmed as *Pullorum.*

Muktaruzzaman *et al.* (**2010)** conducted several types of biochemical media and reagents like bacteriological peptone, methyl red, phenol red, liquid paraffin wax, MR-VP media, potassium hydroxide, V-naphthol, alcohol and dulcitol were used in this study to identify *Salmonella* isolates. In Methyl red test, Positive reaction was indicated by the persistence of red color, indication of acidity and the negative one by the yellow color. In Voges-Proskauer (V-P) test the appearance of pink color indicated positive test. In case of Indole test a red color in the reagent layer indicated indole and negative case, there was no development of red colour. In the carbohydrate fermentation test acid production was indicated by the color change from red to yellow of the medium and the gas production was noted by the appearance of gas bubbles in the inverted Durham's tube.

Motility test was performed by the hanging drop slide method. The motile and non-motile organisms were identified by observing motility in contrasting with to and fro movement of bacteria.

Islam *et al.* (2006) studied on the seroprevalence, isolation and characterization of *Salmonellae* from layer chickens during the period from January to May 2006. The used

materials were blood sample, cloacal and liver swabs from live and dead birds respectively and visceral organs (liver, lungs, spleen and intestine). The detection methods used were serum plate agglutination (SPA) test; necropsy and histopathology; cultural, morphological and biochemical test. The overall seroprevalence was 43.4%. A total of 33 (21.02%) Salmonellae from live and dead birds were isolated. The isolation rate of Salmonellae was higher in seronegative (31.6%) group than seropositive (3.2%) group. Out of 33 *Salmonella* isolates, 25 were *S. pullorum*, 3 were *S. gallinarum*and the rest 5 were motile Salmonellae.

Mahendra *et al.* (2006) conducted a cross-sectional study of raw meat samples obtained from the local meat market of Kathmandu Metropolitan City, Nepal, during September 2002-May 2003, with special emphasis on isolation and identification of *Salmonella* spp. A total 123 raw meat samples (55 chicken meat, 37 buffalo meat and 31 goat meat) were collected and analysed relative to season. *Salmonella spp*. was found in 14 (11.4%) meat samples. Eight (14.5%) samples of chicken meat, 5 (13.5%) samples buffalo meat, and in one sample (3.3%) of goat meat were positive for *Salmonella*. The *Salmonella* spp. identified were *S. pullorum* (3.3%), *S. gallinarum* (0.8%), *S. typhi* (1.6%), *S. choleraesuis* (0.8%) and *Salmonella* of subgenus I or II group (4.9%). The seasonal prevalence of *Salmonella* was highest in the months of April/May.

Sujatha *et al.* (2003) reported that all isolates of *Salmonella* showed positive reaction to M.R., citrate, nitrate, and H< sub>2</ sub>S. Sugar fermentation tests revealed acid without gas from glucose, maltose, dulcitol, galactose, trehalose, xylose, and rhamnose. All the isolates were confirmed as *S. gallinarum* with antigenic structure 9, 12, -, by N.S.E.C.

Hossain (2002) stated that among five basic sugars the *Salmonella* ferment dextrose, maltose and mannitol with production of acid and gas but no fermentation was observed in lactose and sucrose.

Proux *et al.* (2002) reported that the biovar *Salmonella pullorum* and *Salmonella gallinarum* were differentiated by the use of sugars such as maltose, dulcitol and glucose.

2.3 Antibiogram study

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*.

Albarri *et al.* (2017) collected a total of 48 samples of vegetables, meat and chicken from retail stores and supermarkets. MNP with the medium Fluor cult Lauryl Sulfate Broth (FLSB) was used as the conventional method to isolate and detect *E. coli* from the samples, while Polymerase chain reaction with uid A-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.

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%).

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.

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), amoxicillin (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 *et al.* (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.

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%.

Datta *et al.* (2012) isolated *S. aureus*. From seventy nine samples, percentage resistance of the *S. aureus* samples to penicillin, ampicillin, streptomycin, tetracycline, amoxicillin and neomycin were found to be 85.71percent, 71.42 percent, 100 percent, 71.42 percent, 100 percent and 85.71 percent. The percentage of multidrug resistant (MDR, resistant against more than three antibiotics) *S. aureus* was 20 percent, respectively in Dhaka, Bangladesh. Karmi (2013) observed that 44 percent (11/25), 51 percent (13/25), 40 percent (10/25), 24 percent (6/25) and 44 percent (11/25) of isolates of *S. aureus* were positive for methicillin-resistance tests for freshly slaughtered whole chicken carcasses, chicken portions, chicken luncheon, chicken sausages and chicken burgers respectively. Higher contamination rate of MRSA (methicillin resistant *Staphylococcus aureus*) was found in raw poultry meat and the lower rate in poultry meat products subjected to heat treatment and preservatives in Egypt.

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.

Waters *et al.* (2011) characterized the prevalence, antibiotic susceptibility profiles, and genotypes of *S. aureus* from meat and poultry samples. Resistance (intermediate and complete) to tetracycline, ampicillin, penicillin, and erythromycin was highly prevalent but resistance to other antimicrobial was also observed, including dalfopristine, fluoroquinolones, oxacillin, daptomycin and vancomycin from the United States of America.

K**hatoon *et al.* (2010)** isolated 115 *S. aureus* from laboratories situated in different areas of Karachi out of which 85percent were resistant to ampicillin, 43percent against kanamycin, 23 percent against gentamycin, 5percent against chloramphenicol and 40percent against methicillin. Only 8percent were resistant to ciprofloxacin and vancomycin.

Sharma and Singh (2008) detected *E. coli* from meat samples taken from Himachal Pradesh. The prevalence of *E. coli* was highest in poultry meat (61.76 %), mutton (25.64 %) and chevon (22.09%).

Singh *et al.* (2009) collected a total of 86samples comprising of 35samples of chicken meat and 51 of carcasses swabs from local poultry farms and retail shops of Bareilly, Uttar Pradesh and were processed for detection and isolation of *C. jejuni* and *C. coli*. A total of 11isolates of *C. jejuni* and one of *C. coli* analyzed 86 samples processed. Of the samples of chicken meat, 4 isolates of *C. jejuni* and one of *C. coli*, and from carcasses samples, 6 isolates of *C. jejuni* were recovered and the overall prevalence for *Campylobacter spp*. in poultry meat and carcasses was found to be 12.79 percent.

Lee *et al.* (2009) reported that out of 3000 meat samples in Korea, 273 *E. coli* isolates were obtained from beef, poultry, and pork, resulting in an overall isolation rate of 9.1percent. Of these isolates, 201 were obtained from 1350 pork samples (14.9%), followed by 41 of 900 poultry samples (4.6%) and 31 of 750 beef samples (4.1%).

Çadircia *et al.* (2009) investigate the presence of *E. coli* O157 and O157:H7 strains from 200 ground beef and raw beef samples in Turkey. *E. coli* O157 was detected in five of the 200 (2.5%) samples tested, whereas *E. coli* O157:H7 was not detected in any sample.

Boston *et al.* (2009) investigated the prevalence of thermophillic *Campylobacter spp*. (TCS) in 198 beef and 120mutton carcass excision samples, and 232 chicken carcasses samples randomly collected from different retail stores and meat processing plants in Istanbul. TCS were isolated from 11.1 percent, 21.6 percent and 50.4percent of beef, mutton and chicken samples tested, respectively. A total of 292 *Campylobacter* isolates were obtained from the samples (56.5%) *C. jejuni*, (33.9%) *C. coli* and (9.6%) *C. jejuni* was the species most commonly isolated from chicken meat (56.5%) while *Campylobacter coli* was the most common in beef (63.3%) and mutton (63.9%) carcasses. There was no significant seasonal variation in the prevalence of TCS.

Hossnera *et al.* (2007) observed 100percent resistance for nalidixic acid and ampicillin whereas high sensitivity for ciprofloxacin, erythromycin and cloxacillin of *E. coli* isolates from broiler meat in Bangladesh.

Yadav *et al.* (2007) reported that among the 15 isolates of *E. coli* tested for resistance against various antibiotics all the isolates (100%) were found to be resistant to erythromycin and streptomycin, followed by sulphadiazine (95.84%) and cephaloridine (87.50%). Moderately high resistance was detected towards cephalexin (41.69%), penicillin G (37.60%), ceftiofur (33.36%) and norfloxacin (33.36%), enrofloxacin (27.40%) and carbenicillin (25.30%). Multiple drug resistance was demonstrated in ten isolates of *E. coli* in 100 mutton sample showing simultaneous resistance to 2 to 10 antibacterial agents in Mhow, India.

Dhanushree and Mallya (2008) observed that 77.5% of the *E. coli* isolates were resistance for ampicillin whereas 80percent, 90percent and 82percent isolates shows

sensivity for cephoaxime, ciprofloxacin and ceftriazone, respectively from the meat samples in Mangalore.

Rathod *et al.* (2004) reported that out of 60 chevon samples in Parbhani, the highest prevalence of 83.33 percent and 100 percent was recorded for Coliform and *Staphylococcus*, respectively.

Adwan *et al.* (2004) isolated Shiga toxigenic *Escherichia coli* (STEC) from raw beef samples in Palestine and STEC was identified in 44 (14.7%) of 300 raw beef samples and 12 (27.3%) of the STEC isolates were serotype O157.

Vazgecer *et al.* (2004) investigated the microbiology of 72 chicken donar kebabs in Ankara, Turkey. The mesophillic aerobic counts ranges at 1.0x 102- 6.4x 105/g. *B. cereus*, *Staphylococci* and coliforms counts were less than 102 g for the total of 48percent, 50percent and 61percent of the samples, respectively. *E. coli* was found in 31percent of the samples ranged between 2.0x 10 and 5.0x 102/g.

Saha *et al.* (2003) studied the occurrence of *E. coli* from broiler birds in Bengal and their antibiogram. The highest sensivity was recorded against cefotaxime (79.17%) followed by norfloxacin (77.08%), enrofloxacin (73.96%) and amikacin (67.71%). Lowest sensivity was recorded against ampicillin (2.08%), penicillin G (3.13%), cephalexin (13.54%), erythromycin (13.54%) and nalidixic acid (15.63%). Yadav and Sharda (2006) studied the drug resistance of *Escherichia coli* isolated from mutton and revealed highest sensitivity to chloramphenicol (95.92%) followed by colistin (89.80%), ceftriazone (75.51%), amikacin (69.39%), ciprofloxacin (67.35%), gentamicin(67.35%), tetracycline (59.18%), nalidixic acid (48.98%), cotrimoxazole (46.94%) and ampicillin (8.16%). Aksoy*et al.*, (2007) observed the verotoxin production in strains of *Escherichia coli* isolated from cattle and sheep, and their resistance to antibiotics. The antibiotic resistance rates of *E. coli* strains reported as follows: tetracycline (51.6%), streptomycin (24.2%), ampicillin (13.1%), amoxicillin/clavulanic acid (5.2%), gentamycin (4.6%), ciprofloxacin (4.6%), trimethoprim-sulfamethoxazole (4.3%), ceftaxime (0.7%). None of the strains were found resistant to cefepime or ceftazidime.

Al-Gallas *et al.* (2002) detected Shiga toxin-producing *E. coli* (STEC) strains. Among 250, *E. coli* strains isolated from 204 food samples (meat and dairy products) in Tunisia and found that Serotype O55:B5 to be the most prevalent type among *E. coli* isolates.

Lukasova and Jarchovska (1979) studied resistance to selected antibiotics in 325 strains of *Staphylococcus aureus,* isolated from foodstuffs. Out of these strains, 50.46percent were resistant to penicillin, 15.7% percent ampicillin, 4 percent to streptomycin, 18.2percent to oxytetracyclin, 2.5 percent to gentamicin, 1.6percent to kanamycin, 53.5percent to colistin and 9.2 percent to bacitracin. Eighty five strains (26.15%) were sensitive to all the antibiotics used; 107 strains (32.92%) were resistant to one antibiotic and 133 strains (40.93%) to two or more antibiotics from Czechoslovakia. Uzeh *et al.* (2006) studied the bacterial contamination of raw meat and Tshire Suya, a Nigerian meat product and found out that *S. aureus* shows high sensivity against ciprofloxacin, ofloxacin and erythromycin.

Pereira *et al.* (2007) studied the antibiotic susceptibility of *S. aureus* isolates from various foods in Portugal and observed that 70 percent and 73 percent of *S. aureus* strains were resistant to ampicillin and penicillin, respectively. No resistance to nitrofuantoin, vancomycin and ciprofloxacin was found. A small percentage of the isolates demonstrated resistance to rifampicin, gentamicin, gentamicin, erythromycin, chloramphenicol and tetracycline.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

The study was conducted at bashirat meat markets located distance of 13km north of Dinajpur Rangpur Division in Bangladesh. The research work was carried out in the microbiology laboratory of the department of Microbiology, Hajee Mohammad science and Technology University, Dinajpur district, during the period of July to November 2019. Dinajpur is located 413 km north-west of Dhaka in Bangladesh.

3.2 Study Design

A cross-sectional study was conducted from July to November 2019. To isolate and identify the bacterial pathogens collected from chicken raw meat. The study involves Dinajpur meat markets and chicken raw meat was collected for bacteriological analysis.

3.3 Sample Collection

A total of 48 samples each of chicken raw meat were collected from four different market places in Dinajpur. About 12 samples were collected from different retail outlets in each different market place. About 100 grams of meat samples were collected in clean, dry and sterile polythene bags with ice bag to transport to the laboratory for microbiological analysis within one hour or refrigerated at 4°C till further analysis was carried out and processed no later than 96 hours after purchase.

3.4 Processing of samples

The samples were aseptically cut into thin smaller pieces using sterile knife. The analytical portions were placed in separate sterile plastic bags to which 250 ml of buffered peptone water was added. The bags were shaken vigorously and the sample rinsate was collected.

3.5 Statistical analyses

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.

3.6 Bacteriological analysis

The samples were analyzed within 2-6 hours of collection. The different media such as nutrient Agar (NA), nutrient Broth (NB), SS Agar (Salmonella-Shigella Agar), EMB (eosin methylene blue), McConkey, and Mannitol Salt Agar were prepared separately. The last five media are called selective media. The above media were prepared separately by the following method:

3.6.1 Media for culture

3.6.2 Liquid Media

3.6.2.1 Preparation of nutrient broth

The Nutrient Broth media were prepared by suspending 6.5 gm nutrient broth in 500 ml distilled water. The media were heated to dissolve completely. The media were sterilized by autoclaving at 121°C for 20 minutes at 15lbs pressure. Then media were kept on the Petridis sterilizing by laminar air flow.

3.6.2.2 Preparation of peptone water

This medium was prepared by dissolving 10 g of peptone water and 5 g sodium chloride in 1litre of distilled water. The mixture was distributed in 5 ml volumes into clean bottles, and sterilized by autoclaving at 121°C (15lb/inch^2) for 15 minutes.

3.6.3 Solid media

3.6.3.1 Preparation of nutrient Agar (NA) media

The Nutrient Agar media were prepared by suspending 14gm nutrient Agar in 500ml distilled water in a bicker and boiled to dissolve completely. The media and some Petridis were sterilized by autoclaving at 121°C for 20 minutes at 15lbs. Then media were kept on the Petridis sterilizing by laminar air flow.

3.6.3.2 Preparation of plate Count Agar (PCA)

Add 17.5 g to 1 liter of distilled water. Dissolve by bringing to the boil with frequent stirring, mix and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes. After autoclaving, the medium was poured into each sterile petri dish and allowed to solidify. After solidification of the medium in the petri dishes, these were

incubated at 37°C for overnight to check their sterility and used for cultural characterization or stored at 4°C refrigerator for future use (Cater 1979).

3.6.3.3 Preparation of SS Agar media

The SS Agar media were prepared by suspending 31.5 gm SS Agar in 500ml distilled water. The media were heated to boiling with frequent agitation to dissolve completely but not autoclaved or overheated, because overheating may destroy the selectivity of the medium. The media were cooled to about 50°C. The media were mixed well and poured into sterile Petridis sterilizing by laminar air flow.

3.6.3.4 Preparation of McConkey media

The media were prepared by suspending 27.75 gm Mac Conkey Agar in 500 ml distilled water. The media were heated to boiling with gentle swirling to dissolve completely.

Media were sterilized by autoclaving at 121°C for 20 minutes at 15lbs pressure. Overheating was avoided. Then media were cooled to 45-50°C and poured into sterile Petridis. The surface of the medium was dried when inoculated.

3.6.3.5 Preparation of EMB Agar media

The media were prepared by suspending 18 gm EMB Agar in 500 ml distilled water. The media were heated to boiling to dissolve completely. The media were sterilized by autoclaving at 121°C for 20 minutes at 15lbs pressure. Overheating was avoided and was cooled to 50°C. Then the medium was shaken in order to oxidize the methylene blue (i.e., to restore its blue color) and to suspend the flocculent precipitate. The samples were first cultured into the nonselective media such as nutrient Agar and nutrient broth media for total bacterial count. Then these samples were subcultured into the selective media for identification of the bacteria by their colony morphology. Again the samples were direct cultured to the selective media for enumeration of the total identified bacteria.

3.6.3.6 Preparation of Mannitol Salt Agar

111 grams Mannitol Salt Agar base powder was added to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely (necessary calculation was done for required number of plates). The medium was then sterilized by autoclaving at 1.2 kg/cm2 pressure and 121°C for 15 minutes. After autoclaving the medium was put into water

bath at 45°C- 50°C to decrease the temperature. Then medium was poured in 10 ml quantities in sterile glass petri dishes (medium sized) and in 15 ml quantities in sterile glass Petri dishes (large sized) to form thick layer there. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the Petri dishes partially removed. The sterility of the medium was checked by incubating at 37°C for overnight. The sterile medium was used for cultural characterization or stored at 4°C in refrigerator for future use. Petri dishes, these were incubated at 37° C for overnight to check their sterility and used for cultural characterization or stored at 4°C in refrigerator for future use (Cater 1979).

3.6.3.7 Preparation of Mueller Hinton Agar

Mueller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method. 38 grams of Mueller Hinton agar powder was suspended in 1000 ml of distilled water. It was mixed well. It was heated agitating frequently and boiled for about one minute. It was dispensed and sterilized in autoclave at 116 - 121°C (15 lbs. sp) for 15 minutes. It was cooled to 45° or 50°C (Carter, 1979).

3.6.4 Reagents preparation

3.6.4.1 Methyl Red-Voges Proskauer broth

A quantity of 3.4 grams 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 test tubes were autoclaved. After autoclaving, the tubes containing medium were incubated at 37°C for overnight to check their sterility and used for biochemical characterization or stored at 4°C in refrigerator for future use (Cheesbrough, 1984).

3.6.4.2 Methyl Red solution

The indicator methyl red (MR) solution was prepared by dissolving 0.1 gm of Bacto methyl red (Difco) in 300 ml of 95% alcohol and diluting this to 500 ml with the addition of 200 ml of distilled water.

3.6.4.3 Voges-Proskauer solution

3.6.4.3.1 Alpha-naphthol solution

Alpha-naphthol solution was prepared by dissolving 5 grams of 1-naphthol in 100 ml of 95% ethyl alcohol.

3.6.4.3.2 Potassium hydroxide solution

Potassium hydroxide (KOH) solution was prepared by adding 40 grams of potassium hydroxide crystals in 100 ml of cold distilled water.

3.6.4.4 Indole reagent

3.6.4.4.1 Kovac's reagent

This solution was prepared by mixing 25 ml of concentrated Hydrochloric acid in 75 ml of amyl alcohol and to this mixture 5 grams of paradimethyl-aminohenzyldehide crystals were added. This was then kept in a flask equipped with rubber cork for future use (Merchant and Packer, 1967).

3.6.4.4.2 Phosphate buffered saline solution

For preparation of Phosphate buffered saline (PBS) solution, 8 gram of sodium chloride (NaCl), 2.89 gram of disodium hydrogen phosphate (Na_2HPO_4, $12H_2O$), 0.2 gram of potassium chloride (KCl) and 0.2 gram of potassium hydrogen phosphate (KH2PO4) were suspended in 1000 ml of distilled water. The solution was heated to dissolve completely. The solution was then sterilized by autoclave at 121 °C 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, 1984).

3.7 Methods

3.7.1 Laboratory preparation

All items of glass wares including test tubes, pipettes, cylinder, flasks, conical flask, glass plate, slides, vials and agglutination test tubes in a household dishwashing detergent solution for overnight contaminated glass wares were disinfected in 2% sodium

hypo chloride prior to cleaning, the glass wares were then cleaned by brushing washed thoroughly and finally sterilized either by dry heat at 160 ^{0}C for 2 hours or by autoclaving for 15 minutes at 121^{0}C under 15 lbs pressure by square inch. Autoclaved items were dried in a hot air oven over 50 ^{0}C. Disposable plastic was sterilized by autoclaving all the glass ware was kept in oven at 50 ^{0}C for future use.

3.7.2 Experimental layout

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.

The second step included the study of response of the isolated bacteria against commercially available antibiotic discs.

Poultry raw meat samples were collected from the four different areas of Dinajpur. 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 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) and Agar no. 110. Then Gram's staining, biochemical tests was performed.

Finally the isolated organisms were subjected to Antibiogram study test to observe the resistant characteristics of organism on some specific antibiotic disk.

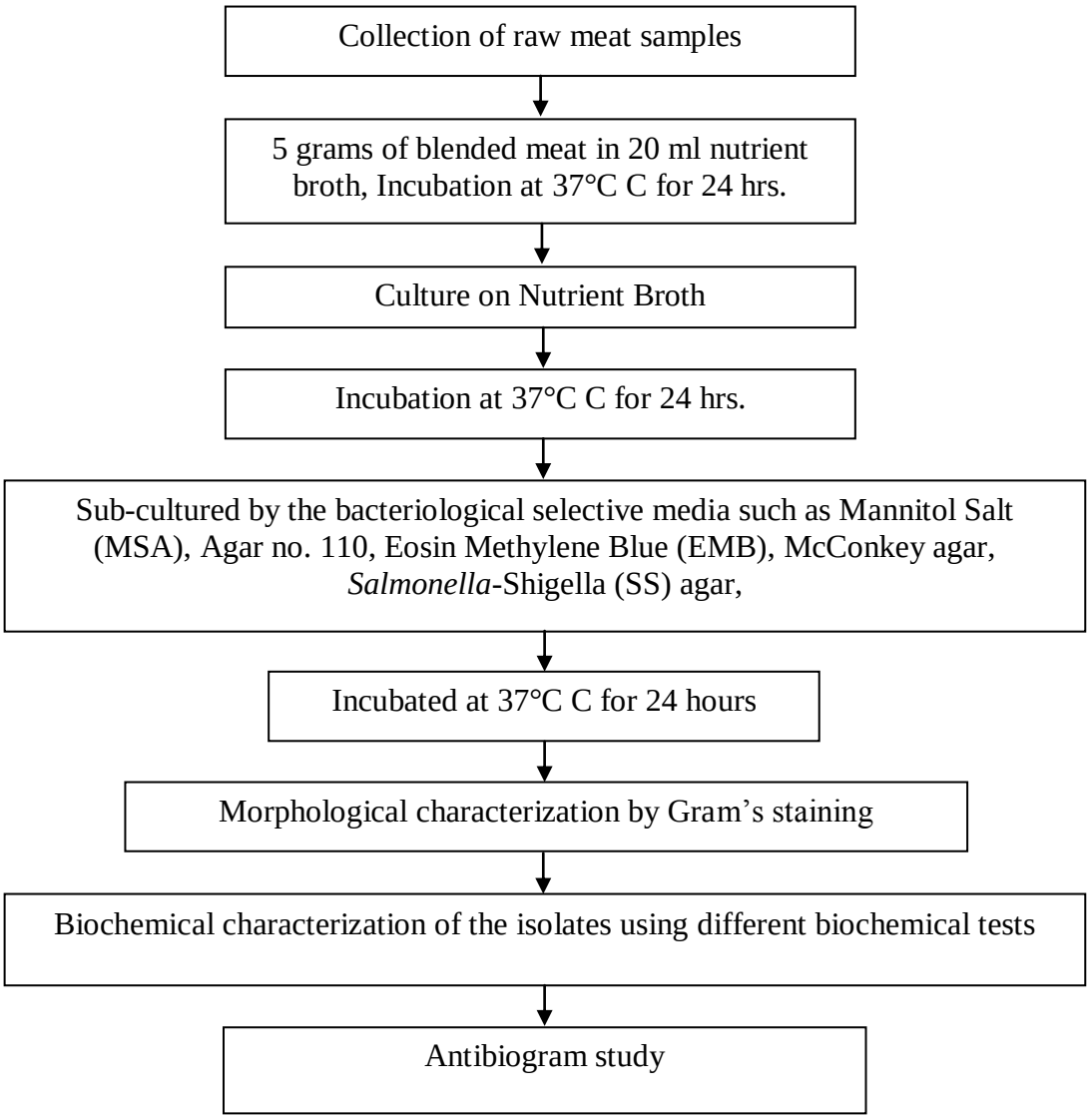

Fig. 3.1: Experiment layout

3.8 Materials required for anti-biogram study

3.8.1 Muller Hinton Agar (MHA)

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

3.8.2 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.

3.8.3 Antibiotic discs

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

Table 3.1: Antimicrobial agent with their disc concentration

Antimicrobial agent with their disc concentration are presented below

Antibacterial agents	Symbol	Disk concentration
Gentamycin	GEN	10µg/disc
Moxifloxacin	MO	30µg/disc
Streptomycin	S	10µg/disc
Azithromycin	AZM	30µg/disc
Chloramphenicol	C	30µg/disc
Ampicillin	AMP	5µg/disc
Erythromycin	E	30µg/disc
Streptomycin	S	10µg/disc
Tetracycline	TE	10µg/disc

3.8.4 Culture in ordinary media

Samples were inoculated separately into ordinary media like nutrient agar, nutrient broth and were incubated at 37°C for overnight. The colonies on primary cultures were repeatedly sub-cultured by streak plate method (Cheesbrough, 1984) until the pure culture with homogenous colonies were obtained.

3.8.5 Isolation of bacteria in pure culture

For isolation of bacteria in pure culture, the mixed culture was inoculated into nutrient agar media by streak plate technique to obtain isolated colonies as per:

Step-1: An inoculum was picked up with a sterile loop and spread on an area of themedium in the petridish.

Step-2: The loop was sterilized by being heated as red hot in a flame.

Step-3: The inoculum was spread over the reminder of the plate by drawing the cooledparallel line.

This method was repeated as many times as necessary to obtain a culture containing only one type of colony and usually at least two more times to ensure purity.

3.8.6 Morphological Characteristics of organism by gram's staining method

The gram staining was followed to study the morphological and staining characterization of bacteria and provide information about the presumptive bacterial identification as per recommendation of (Cowan and Steel 1979).

Gram stain

A pure colony was spread and fixed on the slide by drying using a Bunsen burner flame. The slide was allowed to cool, and then flooded with crystal violet solution for 30 sec, followed with Grams iodine solution for 1 min, followed by draining excess iodine by decolorizing using acetone for at least 10 sec and then washed with water. Counter staining was done using Basic fuchsin and allowed to stand for 30 seconds. This was followed by washing the slide and dried in the air. The slide was observed under light microscopy at X40. Short rods that stained red / pink were considered gram negative.

Gram stain

Procedure

- A loopful of tap water was transferred onto the microscopic slide by flamed loop.
- A loop was flamed again and a colony of bacteria was picked up from blood agar media and placed on slide and spread thinly.
- The smear was air dried and fixed by passing on the flame of Bunsen burner 2-3 times
- The slide was placed on the staining rack and the crystal violate stain was poured on the smear and allowed to act for a minute.

- then washed by tap water and iodine was added and kept for a minutes
- It was washed by tap water again and decolorized by 95% ethanol for 30 second
- The slide was counter stained by safaranin for about 1 minute and washed with tap water, dried and examined under microscope using with oil emersion.
- The slide was observed under light microscopy at X40. Short rods that stained red / pink were considered gram negative.

3.8.7 Culture into differential media

Mac-Conkey agar

Samples were sub-culture on Mac-conkey agar media and incubated at 37°C for overnight. After that both lactose fermenter bacteria (rose pink color colony) and lactose non-fermenter bacteria (pale color colony) were selected.

3.9 Culture on selective media

3.9.1 Eosin Methylene Blue (EMB) agar

Samples of positive lactose fermenter were taken and sub-culture on EMB agar media and incubated at 37°C for overnight. Some EMB agar plate showed slightly circular colonies with dark center metallic sheen. Also, in some EMB agar, the growth was indicated by smooth, characteristics mucoid and pink colored colonies which are a consequence of the organism's abundant polysaccharide capsule.

3.9.2 *Salmonella* -Shigella agar

Sample of non-lactose fermenter were taken and sub-culture on SS agar media and incubated at 37°C for overnight, which after inoculation, raised, black centered, smooth round colony was present.

3.9.3 Mannitol salt agar (MSA)

Materials from nutrient agar were inoculated into Mannitol salt agar containing plates and incubated at 37°C for overnight, which after inoculation, raised, yellow, smooth colony was present.

3.9.4 Agar No. 110

Materials from nutrient agar were inoculated into Agar No.110 containing plates and incubated at 37°C for overnight, which after inoculation, raised, grayish, smooth colony was present.

3.9.5 Identification of isolates

The purified isolates were identified according to criteria described by Barrow and Feltham (1993). This included staining reaction, cell morphology, cultural characteristics and biochemical characteristics.

3.9.6 Microscopic examination

Smears were made from each type of colony on primary culture and from purified colonies. Then fixed by heating and stained by Gram staining method as described by Barrow and Feltham (1993). The stained smears were examined microscopically under oil immersion lens for cell morphology, cell arrangement and staining reaction.

3.10 Biochemical preparation

3.10.1 Indole production

Two to five pure colonies were inoculated using a sterile wire loop in 2 ml of peptone water in bottles and incubated overnight at 37°C. 0.5 ml of Kovac's reagent was added and examined after 1minute. Presence of rose red colour on upper layer was considered positive (+), while absence of rose red or pale colour was considered negative (-).

3.10.2 Methyl Red test

The test was conducted by inoculating a colony of the test organism in 0.5ml sterile buffered glucose broth. It was incubated at 37°C for 48 hours. Then 2-3 drops of methyl red solution was added. The result shown the red color in the upper part of the test tubes in positive cases and in negative cases there was no development of yellow color (Cheesbruogh, 1984).

3.10.3 Voges-Proskauer (VP)

In each bottle, 2.5 ml of Methyl red-VogesProskauer broth was added and inoculated with pure colonies of test organisms. The bijous bottles were then incubated at 37°C for

48 h, followed by addition of 0.6 ml or 6 drops of VP reagent A (α-naphthanol 34 solution), then 0.2ml (2 drops) of VP reagent B (40% KOH). The bottles were shaken and allowed to stand for 15 minutes. Pink red colour (reddish pink) of the broth culture in the bottles was considered positive (+), while colourless (pale) were considered negative (-)

3.10.4 Triple sugar Iron (TSI) agar slant

65 grams TSI agar base powder was mixed in 1000 ml of cold distilled water in a flask and mixed thoroughly, then heated to boiling for dissolving the medium completely (necessary calculation was done for required number of test tubes).The medium was then sterilized by autoclaving for 15 minutes at 121°C maintaining a pressure of 1.2 kg/.Then 20/10 ml of medium was poured into each sterilized test tubes and allowed to cool and to solidify (kept in horizontal position). After solidification test tube were used for biochemical characterization and incubated at 37°C for 24 hours.

3.10.5 Microscopy and Colony morphology Identification

Characterization and identification of the colony isolates was achieved by initial morphological examination of the colonies in the plate (macroscopically) for colonial appearance, size, colour and consistency were recorded. Gram's staining from the colonies provided a preliminary identification of the pathogenic bacteria.

3.10.6 Characterization of bacteria

Individually isolated colonies of the same morphology were selected from appropriate agar plates cloned and checked for purity of growth prior to characterization of the respective genera and species. Biochemical characterization of the bacteria was done by performing specific tests such as TSI, Indole, Methyl red and Voges Proskauer.

Characterization of the respective genera and species were done on the basis of morphological cultural biochemical and serological reaction the classification and specification of organisms was based on the scheme presented in Bergey's manual of systemic bacteriology (Holt, 1985).

3.11 Antibiotic Sensitivity testing for the isolates

Antibiotic sensitivity testing by the Kirby Bauer's disc diffusion method was performed for the isolates using commercially available antibiotic discs on Muller – Hinton agar (MHA). Standard suspensions of the isolates were adjusted to 0.5 McFarland Standard. Immediately after standardization a sterile cotton swab was immersed into bacterial suspension and a lawn culture was performed on the surface of MHA plate. Commercially available antibiotic discs were arranged on the surface of inoculated plates. The plates were incubated at 37°C for 16-18 hours.

Procedure

- The covers of each of the agar plates were labeled with name of the test organism to be inoculated.
- Using a septic technique, inoculated all agar plates with their respective test organism as follows:
    - A sterile cotton swab Dipped as into a well-mixed saline test culture and excess inoculums by pressing the saturated swab against the inner wall of culture tube.
    - Using the cotton swab the entire agar surface was streaked horizontally, vertically and around the outer of the plate to ensure a heavy growth over the entire surface
- All culture plates were allowed to dry for about 5 minutes.
- Using the sense disk dispenser, the antibiotics discs were applied by placing the dispenser over the agar surface and pressing the plunger, depositing the disc simultaneously on to the agar surface. If dispensers are not available, distribute the individual discs at equal distances with forceps dipped in alcohol and flamed.
- Gently pressed each disc down with the wooden end of a cotton swab or sterile forceps to ensure that the discs adhere to the surface of the agar.
- All plate cultures were incubated in an inverted position for 24 to 48 hours at 37^0C.

3.12 Antimicrobial susceptibility testing

The standard kirbay-bauer disk diffusion method was used to determine antimicrobial susceptibility profile of the isolates (Bauer, 1999) according to the recommendations of national committee for clinical laboratory standards (CLSI-2015) bacterial inoculums was prepared by suspending the freshly grown bacteria in 4-5 ml sterile nutrient broth and the turbidity was adjusted to that of a 0.5 McFarland standard. Antimicrobial susceptibility testing was performed using Mueller-hinton medium, antibiotic disks were applied using same sterile forceps. Agar plates were incubated at 37^0C for 18 hours. After overnight incubation at 37^0C, the diameter in millimeters of the zones of inhibition around each of the antimicrobial disks were recorded and categorized as a resistant or sensitive in accordance with company recommendations. All isolates were tested for sensitivities to 7 of routine and practical antibiotics.

3.13 Total bacterial count

The total bacterial count was made by adding 1 ml of sample into sterile test tube having 9 ml normal saline solution (NSS). After thoroughly mixing, the sample was serially diluted up to 1: $10^{-4}$and 1: 10^{-5}then duplicate samples (1 ml) were pour plated using 15-20 ml standard plate count agar solution and mixed thoroughly. The plated sample was allowed to solidify and then incubated at 37ºC for 48 hours. Finally, counts were made using a colony counter after incubation of the cultures at 37 ºC for 48 hours. All colonies including those of pin point size were counted on selected plates using colony counter. Results from plates, which contained 30 to 300 colonies per plate were recorded. Plates with more than 300 colonies could not be counted and were designating as TNTC (too numerous to count) while plates with fewer than 30 colonies were designate as TFTC (too few to count).

The plate counts were expressed as colony forming unit of the suspension (CFU/ml) (Marth, 1978) and the average for each sample were recorded as CFU/ml. Samples were graded as very good if the total bacterial count did not exceed $2x10^{-5}$ CFU/ml, good if it was between $2x10^{-5}$ and 1x106 CFU/ml and fair if the count was between $1x10^{-6}$ and $5x10^{-6}$ CFU/ml. Samples having bacterial count above $5x10^{-6}$ CFU/ml were graded as poor quality (Sherikar *et al.,* 2004).

Cfu/ml= (no. of colonies × dilution factor) / volume of culture plate.

CHAPTER 4

RESULTS

The present study was conducted to isolate and identify bacteria from poultry meat in four selected meat markets in Dinajpur, Bangladesh. A total of 48 samples of poultry meat were processed for isolation and identification of bacteria through culture test, staining, biochemical test and antibiotic sensitivity test.

4.1 Bacterial load

Table 4.1: Total Viable Count

Categories of chicken meat markets	Dilution	Number of colony	Total viable count
Chicken meat markets 1	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	220	2.2 x10^{5}CFU/ml
	10^{-4}	195	1.95x10^{6}CFU/ml
	10^{-5}	130	1.3 x10^{7}FU/ml
Chicken meat markets2	10^{-1}	Over 300	TNTC
	10^{-2}	Over 300	TNTC
	10^{-3}	210	2.1 x10^{5}CFU/ml
	10^{-4}	185	1.85x10^{6}CFU/ml
	10^{-5}	140	1.4 x10^{7}FU/ml
Chicken meat markets 3	10^{-1}	Over 300	TNTC
	10^{-2}	95	9.5 x10^{4}CFU/ml
	10^{-3}	85	8.5 x10^{5}CFU/ml
	10^{-4}	65	6.5 x10^{6}CFU/ml
	10^{-5}	50	5.0 x10^{7}CFU/ml
Chicken meat markets 4	10^{-1}	Over 300	TNTC
	10-^{2}	Over 300	TNTC
	10-^{3}	210	2.1 x10^{5}CFU/ml
	10-^{4}	135	1.35x10^{6}CFU/ml
	10^{-5}	125	1.25 x10^{7}CFU/ml

Table 4.2: Morphological and staining properties of isolated bacteria by Gram's staining

Sl. No.	Bacterial isolates	Shape	Arrangement	Gram's Staining character
1.	***Escherichia coli* spp**	Rod in shape	Single, pair or in short chain	Gram (-)
2.	***Salmonella* spp**	Rod in shape	Single or pair	Gram (-)
3.	***Staphylococcus* spp**	Cocci in shape	Arranged in cluster	Gram (+)

4.2 Results of cultural examinations

The cultural properties of bacteria isolated from four different chicken meat markets were studied for the isolation, identification of various bacteriological media. The staining property of primary culture of each of the different samples indicated the presence of more than one type of bacteria in the same smear. The pure cultures of the organism from each mixed culture were obtained by repeated streak plate method using different simple and selective solid media for study. The individual cultural properties of bacterial isolates are presented in table 2. The cultural characteristics of *E. coli*, *Salmonella spp* and *Staphylococcus spp* exhibited on the media are presented in following figure.

Table 4.3: Cultural characteristics of the bacterial isolates

Name of media used for culture	*E. coli*	*Salmonella* spp	*Staphylococcus spp.*
MacConkey Agar	Rose pink lactose fermenter colony	Colorless pale translucent	No growth
EMB Agar	Metallic sheen (greenish black)	No growth	No growth
***SS* Agar**	No growth	Small non-lactose fermented with black center colony	No growth
Mannitol Salt Agar	No growth	No growth	Medium yellowish colony
Agar no. 110	No growth	No growth	Medium whitish colony

Legends:

AGAR NO. 110, EMB = Eosin Methylene Blue, SS=*Salmonella Shigella,*

4.3 Results of Biochemical tests

Biochemical tests of the bacteria was done by performing specific tests such as TSI, Indole, Methylred, and Voges Proskauer

Table 4.4: Identification of *Salmonella spp.*, *E. coli* and *Staphylococcus spp.*by biochemical test

Biochemical test	Change of the media	Results obtained
	Salmonella spp.	
Indole test	No colour change	negative
MR test	Red colour	positive
Triple sugar iron (TSI) test	S-Red, B-yellow	S-Al, B-A, gas (+), H_2S (+)
VP test	No colour change	negative
	E. coli	
Indole test	Pink rose color ring at the top of the media	positive
MR test	Red colour	positive
Triple sugar iron (TSI) test	Yellow color with gas	S-A, B-A, gas (+), H_2S (-)
VP test	No colour change	negative
	Staphylococcus spp.	
Indole test	No colour change	negative
MR test	Red colour	positive
Triple sugar iron (TSI) test	S-Red, B-yellow	S-Al, B-A, gas (-), H_2S (-)
VP test	Red colour	positive

Table 4.5.Frequency percentage of isolated bacteria from chicken meat markets

Selected Chicken meat markets	**No. of samples**	**No. of isolated *E. coli spp.***	**No. of isolated *Salmonella spp.***	**No. of isolated *Staphylococcus spp.***
market 1	**12**	3(25%)	6(50%)	3(25%)
market 2	**12**	5(41.6%)	7(58.3%)	0(0%)
market 3	**12**	8(66.6%)	2(16.6%)	2(16.6%)
market 4	**12**	7(58.3%)	4(33.3%)	16(8.3%)
Total	**48**	23(47.9%)	19(39.6%)	6(12.5%)

4.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test results with resistance to two antibiotics, or more, were considered as multiple resistances. Among all the strains, 40.98% were sensitive to all the antimicrobial agents used, while 59.02% were resistant to at least one or more antimicrobial agents tested. In the study, various antimicrobial agents were tested against *Escherichia coli, Staphylococcus* and *Salmonella* which included Ampicillin, Chloraphenicol, Tetracycline and streptomycin. The antimicrobial sensitivity response (zone diameters in mm) for each antimicrobial agent is indicated in the (Table 6).

Table 4.6: Result of antibiotic sensitivity test of *E. coli*, *Staphylococcus* and *Salmonella*

Anti-bacterial agents	Disk concentration	Number of inhibition(mm)	Interpretation
	E. coli		
Moxifloxacin	10 µg/disc	30	Sensitive
Tetracycline	µg/disc	0	Resistant
Moxifloxacin	30µg/disc	30	Sensitive
Streptomycin	15µg/disc	13	Intermediate
Chloraphenicol	10µg/disc	0	Resistant
	Staphylococcus		
Chloraphenicol	10 µg/disc	28	Sensitive
Tetracycline	µg/disc	0	Resistant
Azithromycin	30µg/disc	0	Resistant
streptomycin	15µg/disc	15	Intermediate
Ampicillin	10µg/disc	0	Resistant
	Salmonella		
Chloraphenicol	5µg/disc	25	Sensitive
Tetracycline	µg/disc	0	Resistant
Ampicillin	10µg/disc	0	Resistant
Azithromycin	30µg/disc	0	Resistant
Erythromycin	15µg/disc	0	Resistant

Fig. 4.1: Yellowish colony of *Staphylococcus* spp on Mannitol salt agar

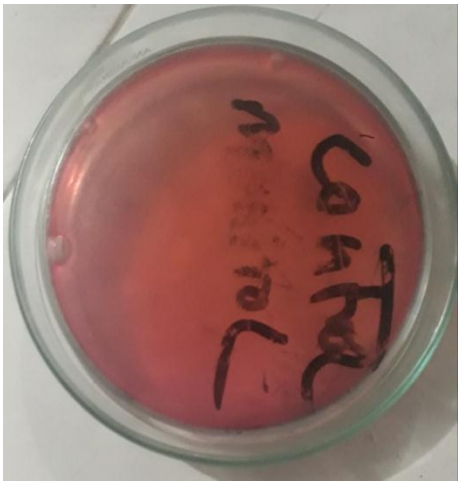

Fig. 4.2: Mannitol salt agar (Control)

Fig. 4.3: Green metallic sheen colony of *E. coli* on Eosin Methylene Blue agar

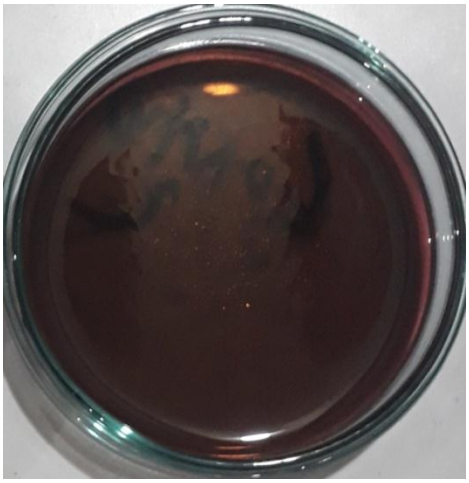

Fig. 4.4: Eosin Methylene Blue agar (Control)

Fig. 4.5: Black colony of *Salmonella* SS Agar

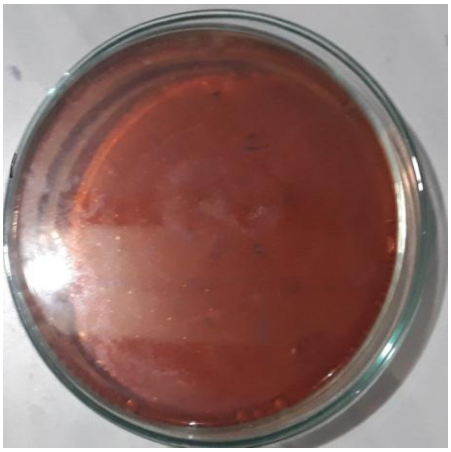

Fig. 4.6: SS Agar (Control)

Fig. 4.7: Whitish colony of *Staphyllococcus spp* on *Staphyllococcus* agar 110

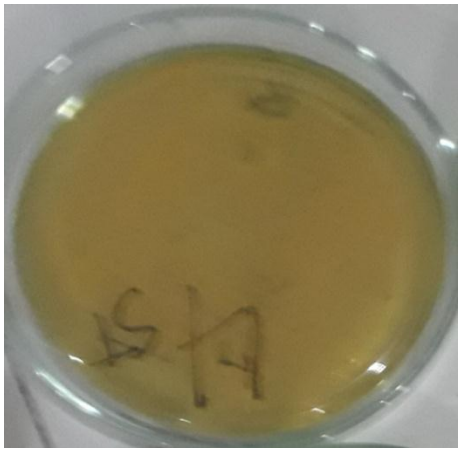

Fig. 4.8: *Staphyllococcus* agar 110 (Control)

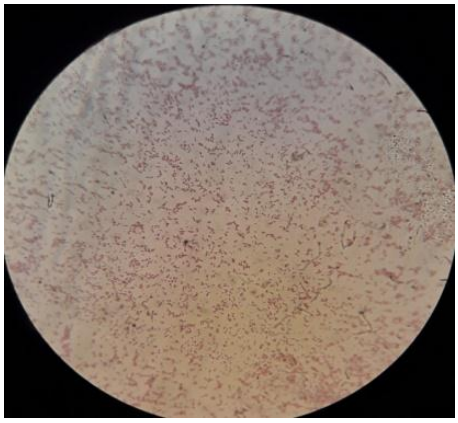

Fig. 4.9: Gram negative large pink color *E. coli* under 100 × microscopes

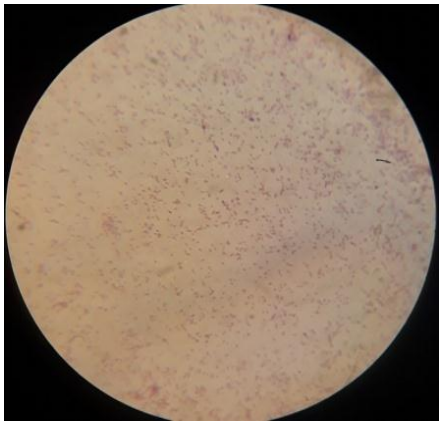

Fig. 4.10: Gram negative short pink color *Salmonella spp* under 100 × microscopes

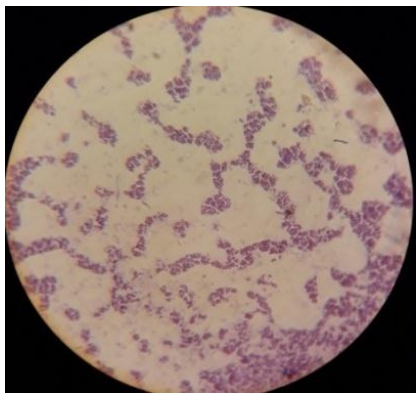

Fig. 4.11: Gram negative long pink color *Staphyllococcus spp* under 100 × microscopes

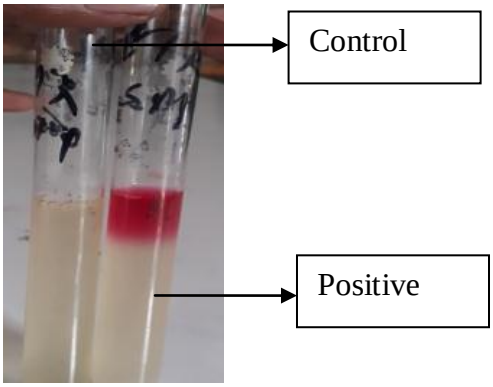

Fig. 4.12: *Salmonella* spp showing positive result (left) on MR test with (right)

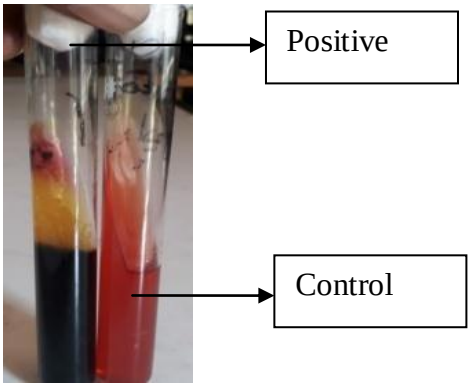

Fig. 4.13: *Salmonella* spp showing positive Result (left) on TSI test with right

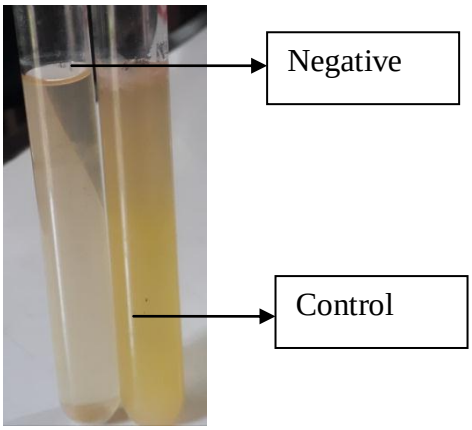

Fig. 4.14: *Salmonella* spp showing negative result (left) on indole test with (right)

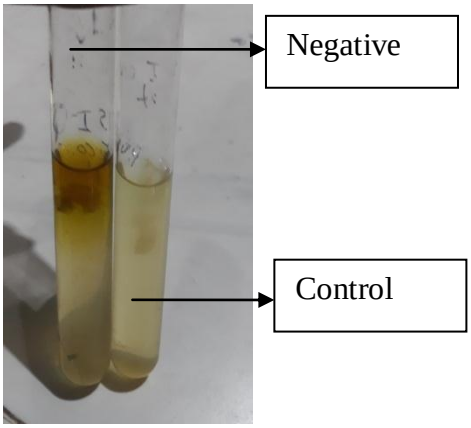

Fig. 4.15: *Salmonella* spp showing negative Result (left) on VP test with right

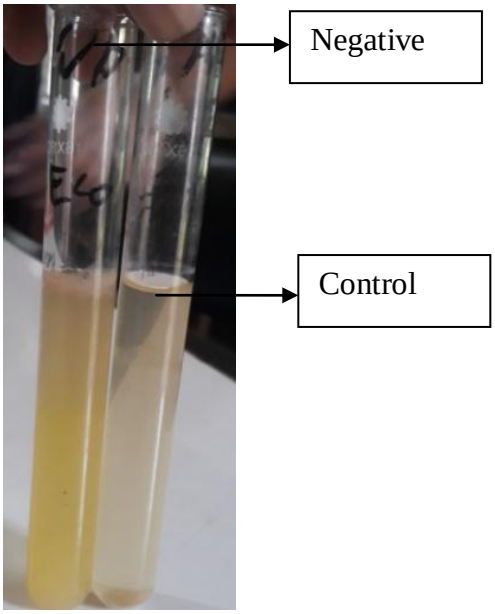

Fig. 4.16: *E. coli* spp showing negative result (left) on VP test with control (Right).

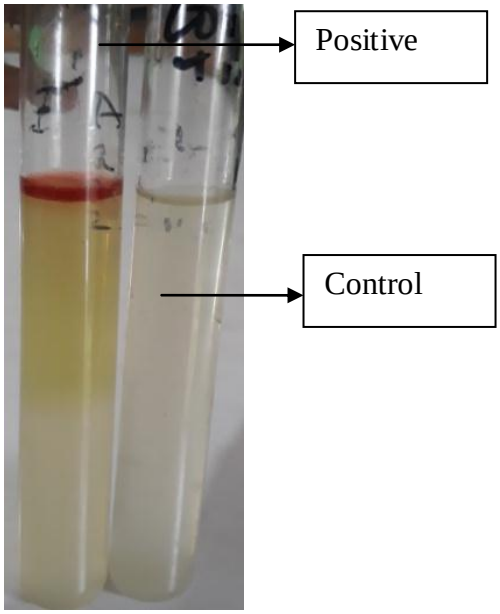

Fig. 4.17: *E. coli* spp showing positive result (left) on Indole test with control (Right).

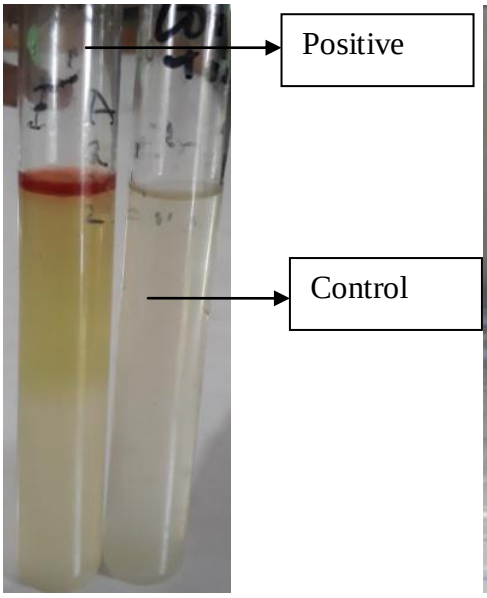

Fig. 4.18: *E. coli* spp showing positive result (left) on MR test with control (right).

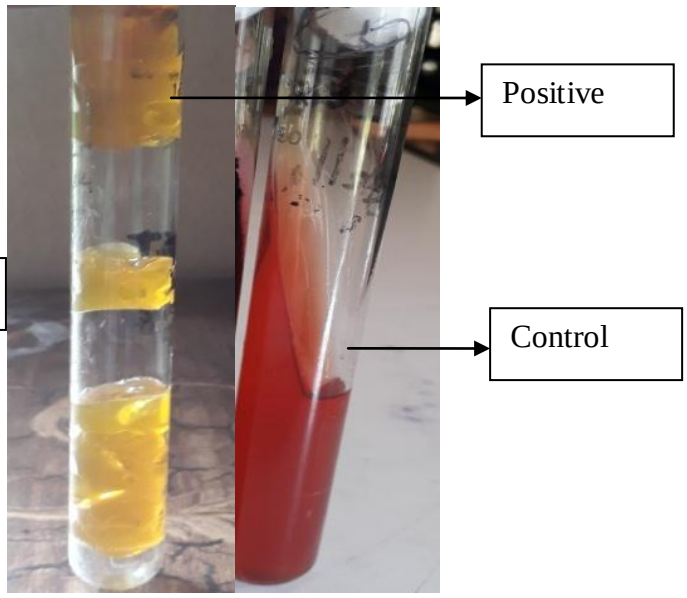

Fig. 4.19: *E. coli* spp showing positive result (left) on TSI test with control (right)

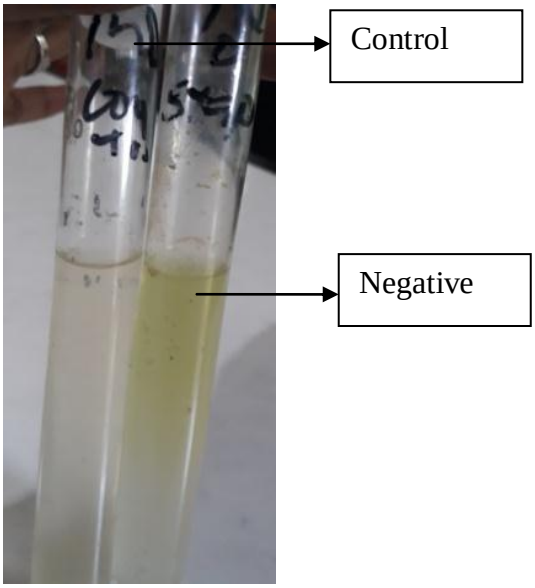

Fig. 4.20: *Staphylococcus* spp showing negative result (right) on indole test with (left)

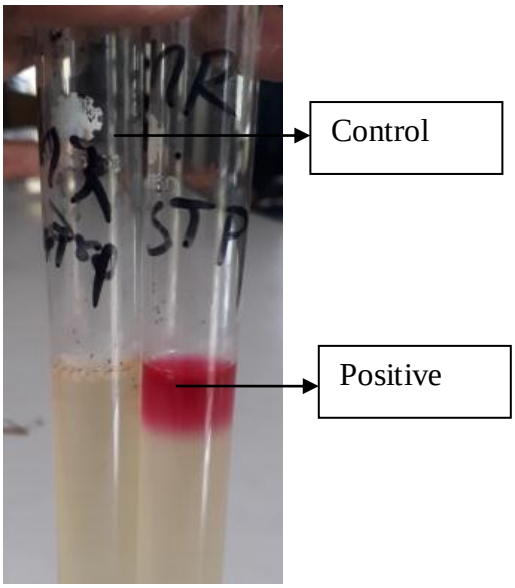

Fig. 4.21: *Staphylococcus* spp showing positive result (right) on MR test with (Left)

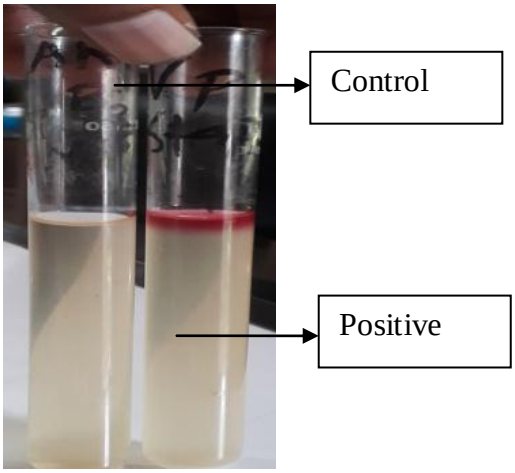

Fig. 4.22: *Staphylococcus* spp showing positive result (right) On VP test with (left)

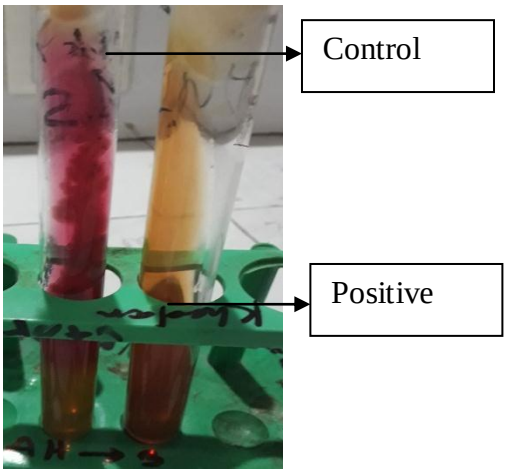

Fig. 4.23: *Staphylococcus* spp spp showing positive result (right) on TSI test with (Left)

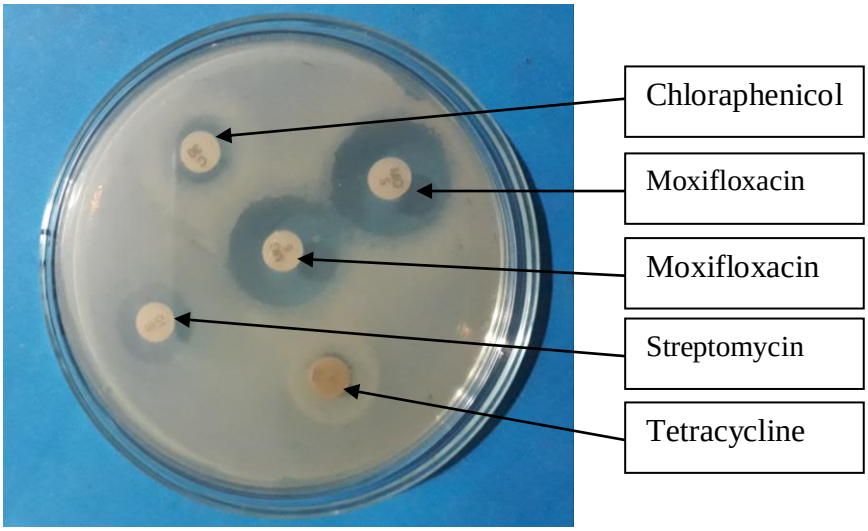

Fig. 4.24: Antibiotic sensitivity test of *E. coli*

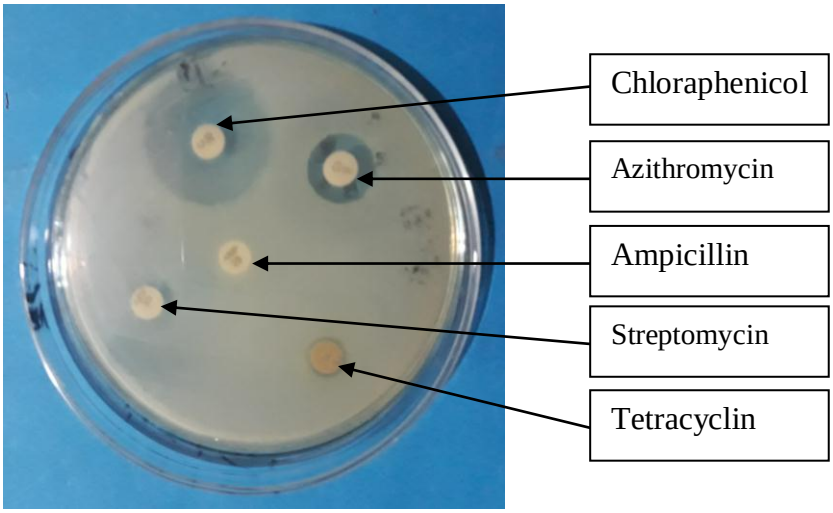

Fig. 4.25: Antibiotic sensitivity test of *Staphylococcus*

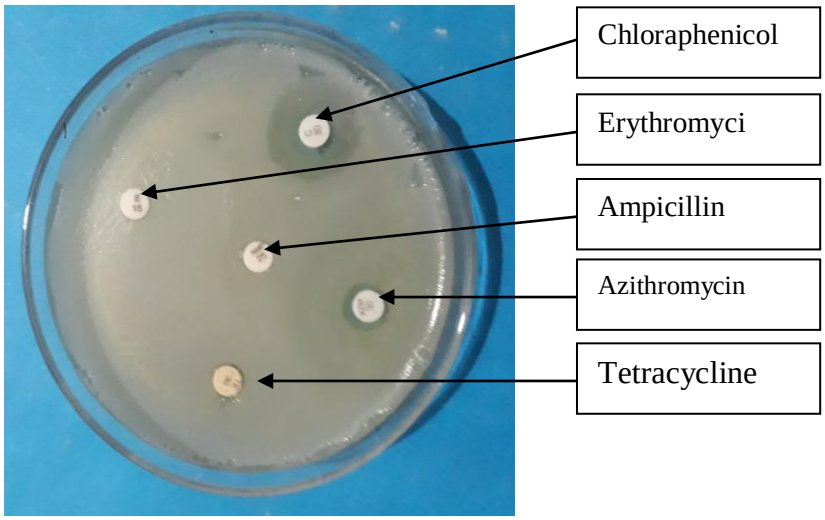

Fig. 4.26: Antibiotic sensitivity test of *Salmonella*

CHAPTER 5

DISCUSSION

Present study revealed the presence of pathogenic bacteria namely, *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. in chicken raw meat collected from four selected markets of Dinajpur district during the period from July to December 2019. In this study there were 48 samples selected four different meat markets of Dinajpur and brought to the microbiology laboratory of the Department of microbiology, Hajee Mohammad Science and Technology University, Dinajpur for bacteriological examination, Isolation and identification of microorganisms were confirmed by their colony of different cultural media, staining characteristics and biochemical tests. The bacteriological media used in this study were selected according to (Buxton and Frazer, 1977).

The level of bacterial load were significantly higher the first sampling in comparison with the second and third one respectively. The findings are similarly with that of (Nicholas and leaver 1967) who reported that high level bacterial contamination on the first sampling. Table (4.1).

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).

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 (table 4.2), which was reported previously (Tanzin *et al.,* 2016; Mamun *et al.,* 2016; Kabir *et al.,* 2017; Parvej *et al.,* 2018).

The cultural properties of *E. coli* were the production of metallic sheen on EMB agar and rose pink coloured colony on the MacConkey agar (table 4.3), which agreed with the findings of others (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 (table 4.4). Those similar results were reported by many investigators (Mishra *et al.*, 2002; Ali *et al.*, 1998).

The colonies of *Staphylococcus* spp. on mannitol salt agar showed colonies that fermented mannitol and appeared golden yellow were characteristically (table 4.3), 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 (table 4.2), reported by Kabir *et al.,* (2017) also mentioned that the bacterium is non-sporulated, non-capsulated and non-flagellated. These findings are in close agreement with Haider *et al.,(*2018;); Shapna *et al.,* (2018). The *Staphylococcus* spp are positive in Methyl red test and Voges-Proskauer test but negative in indole (table 4.4).

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* (table 4.2), reported by the (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 (table 4.4), 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 current study showed highly prevalence of bacterial pathogens isolated in the raw chicken meat of *Escherichia coli, Salmonella* and *Staphylococcus* spp, were 47.9%, 39.6%, and 12.5%, respectively. The predominant bacterial pathogen isolated from chicken meat was *Escherichia coli* (47.9%).

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 Dinajpur district was 39.6% 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 Dinajpur district than other city could be the reason of higher prevalence rate in Dinajpur district.

Staphylococcus found in meat from different market was 12.5% 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 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 *a*nd *Staphylococcus* spp.

In this study, isolated bacteria such as *E. coli,* and *Salmonella* spp. were found to grow multidrug resistant (resistant against 2 antibiotics) and *Staphylococcus* spp. were found to grow resistance against 3 antibiotics. From that study, it was revealed that the isolated *E. coli* were susceptible to moxifloxacin which was supported fully by Akond *et al.,* (2009) who reported resistant to tetracycline. They were resistant to chloraphenicol,

tetracycline and streptomycin (figure 4.24). The results were supported by Al-Ghamdi *et al.,* (2001). *Salmonella* spp. were susceptible to ciprofloxacin, gentamicin and azithromycin which was not supported by Al-Ferdous *et al.,* (2013) where isolates were sensitive to chloraphenicol (figure 4.26). They were resistant to tetracycline, azithromycin, ampicillin and Erythromycin 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 chloraphenicol which slightly similar the report of Haider *et al.,(*2018) and Shapnan *et al.,* (2018) who reported that ciprofloxacin, azithromycin resistant to *Staphylococcus* spp. And tetracycline resistant to *Staphylococcus* spp (figure 4.25). 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 6

SUMMARY AND CONCLUSSION

Present study revealed the presence of pathogenic bacteria namely, *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. in chicken raw meat collected from four selected markets of Dinajpur district during the period from July to December 2019. In this study there were 48 samples selected four different meat markets of Dinajpur and brought to the microbiology laboratory of the Department of microbiology, Hajee Mohammad Science and Technology University, Dinajpur for bacteriological examination, Isolation and identification of microorganisms were confirmed by their colony of different cultural media, staining characteristics and biochemical tests. The bacteriological media used in this study were selected according to (Buxton and Frazer, 1977).

The current study showed highly prevalence of bacterial pathogens isolated in the raw chicken meat of *Escherichia coli, Salmonella* and *Staphylococcus* spp, were 47.9%, 39.6%, and 12.5%, respectively. The predominant bacterial pathogen isolated from chicken meat was *Escherichia coli* (47.9%).

In this study, isolated bacteria such as *E. coli*, and *Salmonella* spp. were found to grow multidrug resistant (resistant against 2 antibiotics) and *Staphylococcus* spp. were found to grow resistance against 3 antibiotics. From the present study, *E. coli* were susceptible to moxifloxacin.

Recommendation

- The presences of *Escherichia coli, Salmonella* and *Staphylococcus* spp, in poultry raw meat poses a risk to consumers of this most popular food this is should be needed the remedial measures.
- Most of the isolates showed multi-drug resistance, but sensitive to moxifloxacin, moxifloxacin should be used to treat these bacteria isolates.

REFERENCES

Abdellah, C., Fouzia, R.F., Abdelkader, C., Rachida, S. B. and Mouloud, Z. 2007. Microbiological Quality of Poultry Meat on the Meknès Market (Morocco). *International Journal of Food Safety,* 9: 67-71.

Abu-Ruwaida, A.S., Sawaya, W.N., Dashti, B.H., Murard, M. and Al-Othman, H.A. 1994. Microbiological quality of broilers during processing in a modern commercial slaughterhouse in Kuwait. *Journal of Food Protection*, 57: 887-890.

Adesiji, Y.O., Alli, O.T., Adekanle, M.A. and Jolayemi, J.B. 2011. Prevalence of *Arcobacter, Escherichia coli, Staphylococcus aureus* and *Salmonella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. Sierra Leone. *J Biomed Res.,* 3(1): 8-12.

Adugna, F., Pal, M. and Girmay, G. 2018. Prevalence and Antibiogram assessment of *Staphylococcus* aureus in beef at municipal abattoir and butcher shops in Addis Ababa, Ethiopia. *Bio. Med. Res. Int*. 5: 607-625.

Akond I.O.2009. The incidence and antibiotics susceptibility of *Escherichia coli* O157: H7 from beef in Ibadan Municipal, Nigeria. *Afr. J. Biotechnol*. 9(8).

Albarri, O.M., Var, I., Meral, M., Heshmati, B. and Köksal, F. 2017. Prevalence of *Escherichia coli* isolated from meat, chicken and vegetable samples in Turkey. *J Biotech Sci. Res*. 4(3).

Al-Ferdous, D., and Hohmann, E.L. 2013. Nontyphoidal salmonellosis. *Clin Infect Dis*. 32(2): 263-269.

Al-Gallas, N., Ben Aissa, R., Attia Annabi, T.H., Bahri, O. and Boudabous, A. 2002. Isolation and characterization of shiga toxin- producing *Escherichia coli* from meat and dairy products. *Food Microbiology*, 19(4): 389-398.

Al-Ghamdi, M.R. and Moss, M.O. 2001 Food Microbiology. 3rd Edition. The Royal Society of Chemistry, Cambridge. p. 463.

Al-Salauddin, A.S., Hossain, M.F., Dutta, A., Mahmud, S., Islam, M.S., Saha, S. and Lutful, K.S. 2015. Isolation, identification, and antibiogram studies of *Salmonella* species and *Escherichia coli* from boiler meat in some selected areas of Bangladesh. *Int J Basic Clin Pharm*. 5(4): 999-1003.

Alvarez, M. A., Capita, R., Alonso-Calleja, C., Moreno, B., Maria, D. and Garcia-Fernandez, C. 2002. Microbiological quality of retail chicken by products in Spain. *Meat Science*, 62(1): 45-50.

Aly, M.E., Essam, T.M. and Amin, M.A. 2012. Antibiotic resistance profile of *E. coli* strains isolated from clinical specimens and food samples in Egypt. *Int. J Microbiol Res*. 3(3): 176-82.

Barrow P, Feltham, G 1993. Infection of the reproductive tract and eggs with *Salmonella enterica* serovar Pullorum in the chicken is associated with suppression of cellular immunity at sexual maturity. *Infect. Immun*., 73 (5), 2986–2990.

Bauer AW, Kirby WM, Sherris JC, Turck M. 1995. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 45(4): 493-496.

Beutin, L., Aleksic, S., Zimmermann, S. and Gleier, K. 1994. Virulence factors and phenotypical traits of verotoxigenic strains of *Escherichia coli* isolated from human patients in Germany. *Med. Microbiol. Immunol*. 183(1): 13–21.

Bhandare, S. G., Sheikar, A. T., Patukar, A. M., Waskar, V. S. and Zende, R. J. 2007. A comparison of microbial contamination on sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. *Food Control*, 18: 854-858.

Bhandare, S., Paturkar, A. M., Waskar, V. S. and Zende, R. J. 2010. Prevalence of microorganisms of hygienic interest in an organized abattoir in Mumbai, India. *Journal of Infection in Developing Countries*, 4(7): 454-458.

Busani, G., Scavia, A., Luzzi, I. and Caprioli, A. 2006. "Laboratory surveillance for prevention and control of foodborne zoonoses," Annalidell' Istituto Superiore di Sanità, 42: 401–404.

Buxton, A. and Fraser, G. 1977. *Escherichia coli*. In Animal Microbiology. 1: 78-80.

Carter, G.R. 1979. Diagnostic procedures in veterinary bacteriology and mycology 3rd ed Charles C. Thomas publisher, Springfield, lllinosis, USA pp. 157-161

Cavitte, J.C. 2003. Present and future control of food-borne pathogens in poultry; revision of the European Community legislation on zoonoses. In: Proceedings of the XVI European Symposium on the Quality of Poultry Meat; Saint-Brieuc, France. 1: 4658.

Cheesebrough, M. 1985. Medical laboratory manual for tropical countries. *Microbiology*. 2: 400-480.

Chung, Y.H., Kwon, Y.I., Kim, S.Y., Kim, S.H., Lee, B.K. and Chang, Y.H. 2004. Antimicrobial susceptibilities and epidemiological analysis of *Salmonella* enteritidis isolates in Korea by phage typing and pulsed-field gel electrophoresis. J. Food Prot. 67: 264–270.

Cowan and Steel 1979. Manual for the Identification of Bacteria. Cambridge University Press, Cambridge.

Das, M., Sabuj, A.A.M., Haque, Z.F., Barua, N., Pondit, A., Mahmud, M.M., Khan, M.F.R. and Saha, S. 2019. Characterization of *Staphylococcus* aureus isolated from human dental infection. *Afr J Microbiol Res*. 13(14): 273-278.

Das, P. and Mazumder, P.B. 2016. Prevalence of *Staphylococcus* in raw meat samples in Southern Assam, India. *IOSR J Agric Vet Sci*. 9(1): 23-29.

Datta, S., Akter, A., Shah, I.G., Fatema, K., Islam, T.H., Bandyopadhyay, A., Khan, Z. U.M. and Biswas, D. 2012. Microbiological Quality Assessment of Raw Meat and Meat Products, and Antibiotic Susceptibility of Isolated *Staphylococcus aureus. Agricultural and Food Analytical Bacteriology,* 2: 187-194.

De, M. P. 2012. Introduction. Microbiological food safety. *Microb Infect*. 4(4): 395-397.

Dhama, K., Chakraborty, S., Barathidasan, R., Tiwari, R., Rajagunalan, S. and Singh, S. 2013. *Escherichia coli,* an economically important avian pathogen, its disease manifestations, diagnosis and control, andpublic health significance: A review. *Res. Opin Anim. Vet. Sci*., 3: 179-194.

Duke, G.E. 1986. Alimentary canal: secretion and digestion, special digestive function and absorbtion. *Avian physiol.* pp. 295.

FAO/WHO 2006. Food safety risk analysis. A guide for national food safety authorities. Food and Nutrition Paper 87, FAO, Rome, Italy, WHO, Geneva, Switzerland.

Ferede, B. 2014. Isolation, identification, antimicrobial susceptibility test and public awareness of *Salmonella* on raw goat meat at Dire Dawa Municipal Abattoir, eastern Ethiopia (Doctoral dissertation, Addis Ababa University).

Gajadhar, A. A. and Allen, R. J. 2004. "Factors contributing to the public health and economic importance of waterborne zoonotic parasites," Veterinary Parasitology, vol. 126, pp. 314.

Gwida, M. and El-Gohary, A. 2015. Prevalence and characterization of antibiotic resistance food borne pathogens isolated from locally produced chicken raw meat and their handlers. *J Dairy Vet Ani Res*. 3(1): 00062.

habrun, E.C. and Mitak, R.P. 2003. Antimicrobial susceptibility pattern of shiga toxin-producing *Escherichia coli* strains. *J. Microbiol. Antimicrob*. 2(8): 113-117.

Haider, M.A., Ievy S., Sobur, M.A., Mahmud, M.M., Hossain, M.T., Rahman, A.M.M.T., Nazir, K.H.M.N.H. and Rahman, M.T. 2018. Isolation and characterization of antibiotic resistant bacteria from pre and post microwave oven treated burger sold in Mymensingh. *Asian Australas. J. Food Saf. Secur.* 2(1): 6-11.

Haque, Z.F., Sabuj, A.A.M., Mahmud, M.M., Pondit, A., Islam, M.A. and Saha, S. 2018. Characterization of *Staphylococcus* aureus from Milk and Dairy Products Sold in Some Local Markets of Mymensingh District of Bangladesh. *J Nutr*. 8(6): 1000743.

Hassan G. Development of a dairy cattle drinking waterquality index (DCWQI) based on fuzzy inference systems. Ecological Indicators.2014; 20: 228-237.

Hernandez, T., Sierra, A., Rodriguez-Alvarez, C., Torres, A., Arevalo, M.P., Calvo, M. and Arias, A. 2005. *Salmonella enterica* serotypes isolated from imported frozen chicken meat in Canary Islands. *J. Food Prot.* 68: 2702–2706.

Hiroi, M., Takahashi, N., Harada, T., Kawamori, F., Iida, N., Kanda, T. and Masuda, T. 2012. Serotype, Shiga toxin (Stx) type, and antimicrobial resistance of Stx-producing *Escherichia coli* isolated from humans in Shizuoka Prefecture, Japan (2003–2007). *Jpn. J. Infect Dis*. 65(3): 198-202.

Holt, P.E. 1985. Urinary incontinence in the bitch due to sphincter mechanism incompetence: surgical treatment. *Journal of Small Animal Practice*. Vol.26(5): pp. 237-246.

Hossain, K.M. 2002. Characterisation of bacteria isolated from diarrheic calves. M.S Thesis, Department of Microbiology and Hygiene. Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

Hossnera, A. A., Khan, M. S. R., Islam, M. J., Nazir, K. H. M. N. H. and Rahman, M. T. 2007. Detection of colicinogenic *Escherichia coli* isolates and interrelatedness with their enteropathogenicity and anitibiotic resistant pattern. *Journal of Bangladesh Social Agriculture Science and Technology*, 4(1 & 2): 173-176.

Hyeon, J.Y., Chon, J.W., Hwang, I.G., Kwak, H.S., Kim, M.S., Kim, S.K., Choi, I.S., Song, C.S., Park, C. and Seo, K.H. 2012. Prevalence, antibiotic resistance, and molecular characterization of *Salmonella* serovars in retail meat products. *J. Food Protec*. 74: 161-166.

Islam, M.M., Haider, M.G., Chowdhury, E.H., Kamruzzaman, M. and Hossain, M.M. 2006. Seroprevalence and pathological study of *Salmonella* infections in layer chickens and isolation and identification of causal agents. *Bang. J. Vet. Med.* 4(2): 79-85.

Jahan, M., Rahman, M., Parvej, M. S., Chowdhury, S. M. Z. H., Haque, M. E.,Talukder, M. A. K. and Ahmed, S. (2015). Isolation and characterization of *Staphylococcus aureus* from raw cow milk in Bangladesh. *J Adv Vet Anim Res*. 2(1): 49-55.

Jarallah, E.M., Sahib, S. and Yassen, K. 2014. Isolation and Identification of some pathogenic Bacterial Species Contaminated from Meats in Butchers Shops and Kebab Restaurants in AL-Kut city. *Euphrates J. Agri. Sci*. 4(6): 30-37.

Johnston, R. W. and Tompkin, R. B. 1992. Meat and Poultry products. In C. Vanderzant and D. F. Spitstoesser (ed.). Compendium of methods for the microbiological examination of foods APHA. Washington. 821-835.

Kabir, M.H., Ershaduzzaman, M., Giasuddin, M., Nazir, K.H.M.N.H., Mahmud, M.M., Islam, M.R. and Ali, M.Y. 2017. Prevalence and molecular detection of the causal agents of sub-clinical mastitis in dairy cows in Sirajganj and Pabna districts, Bangladesh. *J. Adv. Vet. Anim. Res*. 4(4): 378-384.

Kamal, T., Nazir, K.H.M.N.H., Parvej, M.S., Rahman, M.T., Rahman, M., Khan,M.F.R., Ansari, W.K., Ahamed, M.M., Ahmed, S., Hossen, M.L., Panna, S.N., Rahman, M.B. 2018. Remedy of contamination of multidrug resistant *Salmonella* and *Escherichia coli* from betel leaves (Piper betle) keeping them fresh for long time. *J Adv Vet Anim Res*. **5**:73-80.

Kapil, A. 2004. The challenge of antimicrobial resistance: need to contemplate. *Indian J. Med. Res.*, 121: 83-91.

Khatoon, A., Kamal, M., Hussain, S.F., Wasi, A., Rauf, O. and Sahid, S.M. 2010. Antimicrobil susceptibility pattern and identification of plasmid borne Methicillin Resistance *Staphylococcus aureus. American Eurasian Journal of Agriculture and Environmental Science*, 7(2): 139-145.

Krishnaswamy, M. A. and Lahiry, N. L. 1964. Microbiological examination of market meat. *Indian Journal of Public Health.* 8(3): 105-106.

le Loir, Y., Baron, F. and Gautier, M. 2003. *Staphylococcus aureus* and food poisoning. *Genetics and Molecular Research*, 2(1): 63–76.

Lukasova, J and Jarchovska, H. 1979. Resistance of *Staphylococcus-aureus* strains, isolated from food, to antibiotics. *Veterinary Medicine (Praha),* 24(6): 363-368.

Mahanti, A., Samanta, I., Bandyopadhyay, S., Joardar, S.N., Dutta, T.K. and Sar, T.K. 2014. Isolation, molecular characterization and antibiotic resistance of enterotoxigenic *E. coli* (ETEC) and necrotoxigenic *E. coli* (NTEC) from healthy water buffalo. *Veterinary Arhiv*. 84: 241-250.

Mahendra, M., Vandana, J., Joshi, D.D. and Poornima, M. 2006. Prevalence of *Salmonella* species in various raw meat samples of a local market in Kathmandu. *Annals of the New York Academy of Sciences*. 1081: 249-256.

Mahmoud, A.H., Abdellrazeq, G.S., Akeila, M.A. and Khadr, A.M. 2013. Association of antimicrobial resistance with plasmid and protein profiles in enterohemorrhagic *Escherichia coli* isolated from calves, lambs and fish. *Alex. J. Vet. Sci.* 38: 137-145.

Mamun MA, Rahman MM and Turin TC., 2016.Microbiological quality of selected street food items vended by schoolbased street food vendors in Dhaka, Bangladesh.*International Journal of Food Microbiology*, 166(3): 413—418.

Marth, E. H. 1978. Standard Methods for the Examinations of Dairy Products. *American Public Health Association, Washington*, DC.P. 416.

Mawia, K. Kotwal, S. K., Lone, J. A., Kumar, A., Kumar, Y., Sharma, P., Rashid, M., Najimana and Singh, M. 2012. Bacteriological assessment of chevon and poultry meat from local markets of Jammu. *Journal of Veterinary Public Health*, 10(1): 27-30.

Mead, G.C. 2000. Prospects for competitive exclusion treatment to control *Salmonella* and other food home pathogen in poultry. Vet. J., 159: 111-123.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee. 1999. Food- related illness and death in the United States. *Emerging infectious diseases*, 5: 607-625.

Merchant LA and Packer RA, 1967.Veterinary Bacteriology and Virology. Seventhedi. *The Iowa University Press, Ames, Iowa USA.*286-306.

Mohammed, O., Shimelis, D., Admasu, P. and Feyera, T. 2014. Prevalence and antimicrobial susceptibility pattern of *E. coli* isolates from raw meat samples obtained from abattoirs in Dire Dawa City, Eastern Ethiopia. *Int. J. Microbiol. Res.* 5(1): 35-39.

Moses, A.E., Egwu, G.O. and Ameh, J.A. 2012. Antimicrobial resistant pattern of *E. coli* O157 isolated from humans, cattle and surface water samples in Northeast Nigeria. *J Anim Vet Adv*. 2(5): 209-215.

Muktaruzzaman, M., Haider, M.G., Ahmed, A.K.M., Alam, K.J., Rahman, M.M., Khatun, M.B., Rahman, M.H. and Hossain, M.M. 2010. Validation and Refinement of *Salmonella pullorum* (SP) Colored Antigen for Diagnosis of *Salmonella* Infections in the Field. *Int. J. Poul. Sci.* 9(8): 801-808.

Musa, Z., Onyilokwu, S.A., Jauro, S., Yakubu, C. and Musa, J.A. 2017. Occurrence of *Salmonella* in ruminants and camel meat in Maiduguri, Nigeria and their antibiotic resistant pattern. *J Adv Vet Anim Res.***4**(30: 227-233.

Nadeau, E., Messier, S. and Quessy, S. 2002. Prevalence and comparison of genetic profile cases of Campylobacteriosis in humans. J. Food Prot. 65: 73–78.

Nazir M.M. 2005. Bacterial flora : a review. Journal Microbiology Ecology Vol. 19 (1): pp. 21-41.

Nicholas d., leaver, T., 2002.Microbiological guidelines for ready to eat food.Road and Evirinmental Hygein department, Hong Kong, 115-116.

Noori, T.E. and Alwan, M.J. 2016. Isolation and Identification of zoonotic bacteria from poultry meat. *Int. J. Adv. Res. Biol. Sci*. 3(8): 57-66.

Parvej, M.S., Mamun, M., Hassan, J., Mahmud, M.M., Rahman, M., Rahman, M.T., Rahman, M.B. and Nazir, K.H.M.N.H. 2018. Prevalence and characteristics of Shiga-toxin producing *Escherichia coli* (STEC) isolated from beef slaughter house. *J Adv Vet Anim Res.* 5(2): 218-225.

Paterson, D. L. 2006. Resistance in Gram-negative bacteria: Enterobacteriaceae. *The American Journal of Medicine*, 119(6, supplement 1): S20–S28.

Pattanaik, P., Kar, B. C. and Roy, P. K. 1997. Microbiological studies on chicken carcasses processed in local slaughter units. *Indian Journal of Poultry Science*, 32: 89-92.

Proux, K., Humbert, F., Jouy, E., Houdayer, C., Lalande, F., Oger, A. and Salvat, G. 2002. Improvements required for the detection of *Salmonella* Pullorum and Gallinarum. *The Canadian. J. Vet. Res.*, 66: 151-157.

Radwan, I.A., Salam, H.S.H., Alwanis, S.A.A. and Yahia Al-Sayed, M.A. 2014. Frequency of some virulence associated genes among multidrug-resistant *Escherichia coli* isolated from septicemic broiler chicken. *Int. J. Adv. Res*. 2(12): 867-874.

Rahman, M., Rahman, A.K.M.A., Islam, M.A. and Alam, M.M. 2017. Antimicrobial Resistance of *Escherichia coli* Isolated from Milk, Beef and Chicken Meat in Bangladesh. *Bangladesh j. vet. med*. 15(2): 141-146.

Rajput, S.K., Gururaj, K. Singh, D.V. and Gitam, S. 2013. Antibiogram study profile of the test isolates of *E. coli* isolated from clinical cases of diarrhea in the kids of goat breeds. *Ind. Res. J. Genet Biotech.* 5(3): 160-164.

Rathod, K.S., Ambadkar, R.K., Zanjad, P.N., Deshmukh, V.V. and Raziuddin. 2004. Microbial Quality of chevon sold in Parbhani city. *Journal of Veterinary. Public Health*, 2 (1&2): 19-22.

Saad, M.S., Edris, A.M., Shaltout, F.A. and Edris-Shimaa, N. 2011. Isolation and identification of *Salmonella*e and *E. coli* from meat and poultry cuts by using multiplex PCR. *Benha Vet Med J*. 22(2): 152-160.

Saha, A., Hui, A.K., Das, R., Roy, J.P., Ray, N. and Mahata, T.K. 2003. Occurrence of *Escherichia coli* from broiler birds in Bengal and their antibiogram. *Indian Journal of Animal Health*, 42(2): 136-141.

Saleh, M., Seedorf, J. and Hartung, J. 2003. Total count of bacteria in the air of three different laying hen housing systems. Dtsch Tierarztl Wochenschr, 110: 94-97.

Samaha, I. A., Ibrahim, H. A. A. and Hamada, M. O. 2012. Isolation of some enteropathogens from retailed poultry meat in Alexandria Province. *Alex J. Vet Sci*. 37(1): 17-22.

Sasaki, Y., Usui, M., Murakami, M., Haruna, M., Kojima, A., Asai, T. and Yamanda, Y. 2012. Antimicrobial resistance in shiga toxin-producing *Escherichia coli* O157 and O26 isolates from beef cattle. *Jpn J Infect Dis*.65: 117-121.

Scallan, R. M. Hoekstra, F. J. Angulo et al.,"Foodborne illness acquired in the United States— major pathogens," Emerging Infectious Diseases,vol. 17, no. 1, pp. 7–15, 2011.

Schroeder, C.M., White, D.G. and Meng, J. 2004. Retail meat and poultry as a reservoir of antimicrobial-resistant *Escherichia coli*. Food Microbiol. 21: 249–255.

Selvam, A., Gunaseelan, Kumar, S.K. and Sekar, M. 2010. Assessment of carrier status of *Salmonella* Pullorum and Gallinarum infection in health flocks. *Tamilnadu J. Vet. Ani. Sci.* 6(2): 99-101.

Sengupta, R., Das R., Ganguly, S. and Mukhopadhayay, S. K. 2011. Survey on microbial quality of chicken meat in Kolkatta. *International Journal of Research in Pure and Applied Microbiology*, 1: 32-33.

Shapna, T.A., Mahmud, M.M, Uddin, M.S., Islam, M.M., Khanam, S., Ripon, J.H., Hossain, M.T. and Nazir, K.H.M.N.H. (2018). Impact of heat treatment on organoleptic and microbial quality of hotdog. *Food Safety and Health*. 1(1): 15-21.

Sharma, M. and Singh D.P. 2008. Recovery of Bacterial Contaminants from Various Foods in the State of Himachal Pradesh, India FAVA -OIE Joint Symposium on Emerging Diseases.

Sherikar, A. T., Bechhil, V. N. and Thaplyal, D. C. 2004. Taxt book of elements of Veterinary Public Health. Indian Council of Agricultural Research, New Delhi. pp: 75-120.

Sofos, J. N. 1994. Microbial growth and its control in meat, poultry and fish. In A. M. Pearson, and Dutson T. R. (eds.). Quality attributes and their measurement in meat, poultry and fish products. Blackie Academic and Professional, London. 358.

Sujatha, K., Dhanalakshmi, K. and Rao, A.S. 2003. Isolation and characterisation of *Salmonella* gallinarum from chicken. *Ind. Vet. J*. 80(5): 473-474.

Tanzin S. 2011. Street Vended Food in Developing World: Hazard Analyses. Indian Journal of Microbiology. 51(1): 100-106.

Threlfall, E.J., Fisher, I.S.T., Berghold, C., Germer-Smidt, P., Tschäpe, H., Cormican, M., Luzzi, I., Schneider, F., Wannet, W., Machado, J. and Edwards, G. 2003. Antimicrobial drug resistance in isolates of *Salmonella* enterica from cases of salmonellosis in humans in Europe in 2000: results of international multicentre surveillance. *Eurosurveillance,* 8:41-5.

Willayat, M. M., Sheikh, G. N., Ahmed, R. and Gunjan, D. 2006. Isolation of *Salmonella* serotypes from fresh and frozen chicken. *Indian Veterinary Journal,* 83: 1253-1255.

Yadav, M. M., Roy, A., Sharda, R. and Arya, G. 2007. Detection of toxin genes and antibiogram pattern in *Escherichia coli* isolates from sheep meat on Indian market. *Journal of Veterinary Science,* 77(6): 485-494.