ISOLATION, IDENTIFICATION AND ANTIBIOTIC SENSITIVITY PROFILING OF *ESCHERICHIA COLI* AND *SALMONELLA SPP.*: AN ISSUE OF PUBLIC HEALTH

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ISOLATION, IDENTIFICATION AND ANTIBIOTIC SENSITIVITY PROFILING OF *ESCHERICHIA COLI* AND *SALMONELLA SPP.*: AN ISSUE OF PUBLIC HEALTH

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CERTIFICATE

This is to certify that the thesis entitled "**Isolation, Identification and Antibiotic Sensitivity Profiling of *Escherichia coli* and *Salmonella spp.*: An Issue of Public Health**" submitted to the department of Medicine and Public Health, faculty of Animal Science & Veterinary Medicine, Sher-e- Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, in partial fulfillment of the requirements for the degree of **Master of Science (MS) in Medicine**, embodies the result of a piece of bona fide research work carried out by **Sharmin Khatun**, registration no. : **14- 05860**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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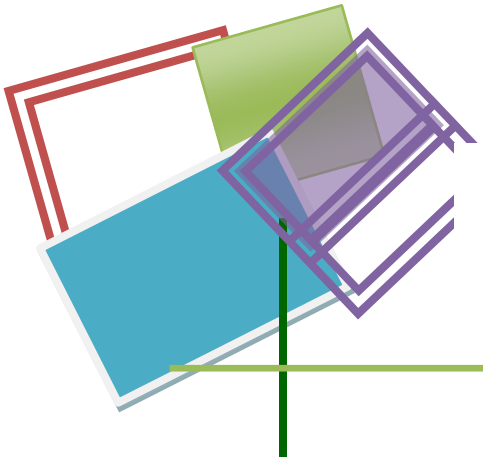

Dedicated
To My
Beloved Parents

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The Author
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LIST OF ABBREVEATIONS AND SYMBOLES

ABBREVIATION	FULL WORD
AMP	Ampicillin
AMX	Amoxicillin
Approx.	Approximately
BG	Brilliant Green
CFU	Colony Forming Unit
CIP	Ciprofloxacin
DNA	Deoxyribonucleic acid
EMB	Eosin Methylene Blue
et al.	and others
ESBL	Extended-Spectrum Beta-Lactamase
E.coli	*Escherichia coli*
etc.	Etcetra
Fig.	Figure
GM	Gentamicin
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen Sulphide
hrs.	Hours
IN	Intermediate
Lbs.	Pound
Ltd.	Limited
MC	MacConkey
Mg	Milligram
MH	Muller Hinton
Ml	Millilitre
Mm	Milimeter
mon.	Month
Min.	Minute
MR	Methyl Red

NCCLS	National Committee for Clinical Laboratory Standard
NM	Non motile
No.	Number
PCR	Polymerase chain Reaction
PBS	Phosphate buffered solution
R	Resistant
Rpm	Revolutions Per Minute
S	Sensitive
SAU	Sher-e-Bangla Agricultural University
Sp	Species
SS	Salmonella Shigella
SLT	Shiga-like toxin
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
TET	Tetracycline
TSI	Tripple Sugar Iron
UTI	Urinary tract infection
UV	Ultraviolet
VP	Voges-Proskauer
yrs.	Years
ºC	Degree Celsius
-	Negative
%	Percentage
~	Tilde
+	Positive
®	Registard trade mark
µg	Microgram
µl	Microlitre

ABSTRACT

The study's goal was to look at the isolation and identification of *Escherichia coli* and *Salmonella spp*. bacteria and their antibiotic resistance patterns. A total of 40 samples of duck liver and intestinal contents was collected and selected from different village on Savar upazila and tested for *E. coli* and *Salmonella spp*. bacteria with the isolates subjected to antibiotic sensitivity tests. Cultural characteristics, biochemical testing, and gram's staining were used to isolate and identify bacterial genera/species. The prevalence rates are different for different bacteria. Highest prevalence rate found in *E. coli* (52.5%) followed by *Salmonella spp.* (37.5%). Antibiotic sensitivity test by disc diffusion method or Kirby-Bauer test was performed against five most used different antibiotics. Ciprofloxacin was the most sensitive to *E. coli* isolates (95.24 %), followed by gentamycin (66.67%), while amoxicillin (90.47%) followed by tetracycline & amoxicillin both (80.95%) was the most resistant. *Salmonella spp.* isolates were found to be the most susceptible to Ciprofloxacin (100%), followed by Gentamycin (86.67%). Highest resistant pattern of *Salmonella spp.* was showed against amoxicillin (100%) followed by ampicillin (80%) and tetracycline (60%) with intermediate resistant 40%. According to the findings of this study, duck contains multidrug resistant *E. coli* and *Salmonella spp*. pathogens on both duck liver and intestinal contents. *Salmonella spp.* and multidrug-resistant *E. coli* are dangerous bacteria that can spread to people by contact with them or through the food chain, raising major public health problems.

Keywords: Isolation, Antibiotic sensitivity, *E. coli, Salmonella spp.*

CHAPTER I

INTRODUCTION

Duck is a large billed, short-necked, and relatively small waterfowl species belong to the family Anatidae. Duck is the species of poultry that provides the second-most poultry meat and eggs, just after chicken. Duck population has been estimated at 61.746 million occupying second position in poultry in Bangladesh (DLS, 2021).

Escherichia coli is characterized as a gram-negative, rod-shaped bacterium belonging to the family Enterobacteriaceae (Tenaillon *et al.,* 2010). Many common bacterial illnesses, such as cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveller's diarrhoea, as well as other clinical infections such as neonatal meningitis and pneumonia, are caused by this bacterium. Some *E. coli* bacterium strains (such as the O157:H7 strain) can cause severe anemia or renal failure, which can lead to death (Lowenfels, A. 2013). Enteropathogenic *E. coli* (EPEC) is a common cause of diarrhea in people. EPEC infection of cultured intestinal epithelial cells induces attaching and effacing (A/E) lesions, alters intestinal ion transport, increases paracellular permeability, and stimulates inflammation (Savkovic *et al.,* 2005). Shiga toxin-producing *E. coli* (STEC) causes the hemolytic uraemic syndrome, which is produced by the release of Shiga toxins (Stxs) during intestinal infection and subsequent absorption into the circulation. *E. coli* can get into the meat during processing. If the infected meat is not cooked to 160°F (71°C), the bacteria can survive and infect when eating the meat. This is the most common way people become infected with *E. coli*. Food that has meet raw meat can become infected as well. The bacteria can also be transmitted from one person to another if an infected individual does not thoroughly wash their hands after a bowel movement. *E. coli* can be transmitted from infected hands to other individuals or things.

Salmonella spp. may be involved in a variety of pathogenic processes in humans and domestic animals (Freeman, 1985). The organisms are motile (exclude *S. pullorum* and *S. gallinarum),* gram-negative, rod-shaped, non-spore forming, non-capsulated, facultative anaerobic bacteria belong to family Enterobacteriaceae (Agbaje *et al*., 2011). Salmonella is consistently one of the most common causes of food poisoning in the world. Over 36,000 serotypes have been identified and consider as potentially pathogenic. *Salmonella* is primarily found in the intestines of humans and animals. Contaminated meats, primarily from avian and cattle sources, are the most likely source of human salmonellosis and thus the most serious meat-borne public health threat (Buncic *et al*. 2014, Kabir, 2010).

Diarrhea, vomiting, fever, and abdominal cramps are all symptoms of *Salmonella spp.* *Salmonella* infections can cause people to become so unwell that they need to go to the hospital. Severe diarrhoea may necessitate medical treatment, such as intravenous fluid rehydration. Symptoms of septicaemia or bacteraemia, in which the infection enters the bloodstream, include a high temperature, lethargy, thoracic and abdominal discomfort, chills, and anorexia. Long-term complications or sequelae have been reported in some patients, including arthritis, osteoarthritis, appendicitis, endocarditis, pericarditis, meningitis, peritonitis, and urinary tract infections (Bell, C. & KYRIAKIDES, A. 2002). Typhoid fever, often known as enteric fever, is caused by a small number of serotypes (Bell, C. & KYRIAKIDES, A. 2002).

Antimicrobial resistance (AMR) occurs when microbes evolve mechanisms that protect them from the effects of antimicrobials (WHO, 2004). Antimicrobial resistance is one of the most common reasons for antimicrobial therapy failure. This survival mechanism given by bacteria might occur naturally or be acquired. Antibiotic resistance and its transmission to human pathogens are important because antibiotic-resistant bacteria can colonize the human intestine and contribute resistance genes to human endogenous microflora via R-factor, conjugative plasmids, and chromosomal elements, as reviewed by (Kabir, 2010). As a result, disease-causing bacteria that have developed resistance to antibiotic medication therapy are becoming more important to public health. Many governments have outlawed the use of prophylactic antibiotics to curb the development of antibiotic-resistant zoonotic pathogens (Chessbrough, 2006).

As a result, studying the susceptibility and resistance patterns of isolated organisms to various antibiotics is critical for the better treatment of animal diseases. Duck is one of the world's most important poultry species, especially for the Indian subcontinent. In Bangladesh, duck contributes to a major part of its economy. The economic aspect of duck disease, as well as their mortality and morbidity due to bacterial infection, is of great concern to livestock owners and the government, but due to a lack of awareness and research, the presence of bacteria in duck and the consequences of this on human health remain unknown.

Considering the above discussion, the current study was designed with the following objectives in mind:

I. To isolate and identify the pathogenic *E. coli* and *Salmonella spp.* from duck

II. To demonstrate the prevalence of *E. coli* and *Salmonella spp.* on duck

III. To determine the antimicrobial resistance pattern of isolated bacteria

CHAPTER 2

REVIEW OF LITERATURE

Isolation, identification, characterization, prevalence and antibiogram determination of the bacteria isolated from duck liver and intestinal content from different village of Savar upazila, Dhaka was performed using the information gained from the following related review of literature.

2.1. Isolation, Identification of pathogenic *E. coli* and *Salmonella spp.* bacteria

2.1.1 Isolation, Identification of pathogenic *E. coli* bacteria

Eid *et al.* (2019), collected total of 500 samples randomly from different duck farms at Ismailia Governorate, Egypt. The collected samples were subjected to the bacteriological examination. The most common pathogens isolated from apparently healthy and diseased ducks were *Escherichia coli* (3.6% and 22.8%), *Salmonella spp.* (2.8%). The antibiotic sensitivity test revealed that most of the isolated strains were sensitive to enrofloxacin, norfloxacin, and ciprofloxacin.

Majumder *et al.* (2017) examine a total of 60 cloacal swab samples were collected from two duck farms of Bangladesh Agricultural University and Shamvuganj. Out of 60 samples, 26 (43.33%) were confirmed to be *E. coli* positive. Among the *E. coli* positive samples, 12 (46.15%) samples were found positive for Stx-1 and 11 for Stx-2. Among 26, 11 (42.31%) samples possess both Stx-1 and Stx-2 genes, whereas only one isolate had Stx-1 gene. The prevalence of both Stx-1 and Stx-2 in Bangladesh Agricultural University Poultry Farm was 41.66%, and the prevalence of Stx-1 and Stx-2 in Shamvuganj was 50% and 42.86%, respectively.

Kim *et al.* (2016), conducted a study to investigate the prevalence and antimicrobial resistance of *Escherichia (E.) coli* isolated from ducks in Korea. A total of 400 cecal content samples were collected from 40 duck farms in Korea. Isolated *E. coli* strains were 364 of the 400 cecal samples. Resistance to the tested antimicrobial agents *E. coli* isolates to the tested antimicrobial agents was high and 90.9% (331/364) of *E. coli* isolates showed multi-antimicrobial resistance. Antimicrobial resistance in duck zoonotic pathogens should be of concern to the Korean duck industry, as these pathogens exhibit a high rate of antimicrobial resistance and pose a potential hazard to public health.

Darwish *et al*. (2015) carried out a study to investigate the possible transmission of diarrheagenic *E. coli* from consumption of duck meat and giblets. The examined duck meat and giblets contained *E. coli* that could be isolated. *E. coli* serogroups O86, O127, O114, O26, and O78 could all be found. The highest concentration of *E. coli* was found in the liver, followed by the gizzard, heart, spleen, and muscle. Separated *E. coli* serogroups carried various virulent components that caused hemorrhage and diarrhea. Further, isolated *E. coli* serogroups shown markedly reduced sensitivity or even resistance to the most widely used antibiotics in Egypt.

Khoo *et al*. (2010), conducted a study to establish the incidence of positive infections in different type of chickens with the hope to identify the pathogenic strains potentially infecting consumers. APEC isolates commonly belong to certain serogroups such as 01, 02 and 078. A total of 3768 samples from 471 cases were screened, of which 178 isolates were APEC. A total of 141 (79%) of the isolates were *E. coli* 01:K1, 16 (9%) were *E. coli* 02:K1 and another 21 (12%) were *E. coli* 078:K80. Of the 141 *E. coli* 01:K1 isolates, 125 (89%) were from chickens and 16 (11%) from ducks.

2.1.2 Isolation, Identification of pathogenic *Salmonella spp.* bacteria

Rahman *et al.* (2017), A total of 48 duck samples comprising of liver (n=16) spleen (n=16) and intestinal content (n=16) were collected from 16 ducks (8 sick and 8 dead). The samples were collected from the selected Duck farms at three *Upazillas* (sub-districts) in Dinajpur district, Bangladesh. *Salmonella spp*. could be isolated from 39.58% (n=19/48) duck. The isolated *Salmonella* organisms were found to be highly sensitive to Azithromycin, Ciprofloxacin and Levofloxacin.

Dey *et al.* (2016), A total of 12 small to medium sized duck farms and 28 individual households were visited for data collection. Based on history and clinical signs and as per the information provided by the farmers the prevalence rate of Duck Salmonellosis was recorded as 38.1% and the P value was calculated as 0.003 ($p<0.01$) which was noted as highly significant. Based on tentative occurrences of Duck Salmonellosis as per information taken from the structured questionnaire a total of 120 fecal samples were collected from apparently healthy and diseased ducks based on age, sex, season, location, and health status. Based on their cultural and biochemical characteristics it was found that among 120 fecal samples 32

(26.67%) were found to be positive for *Salmonella* and the P value was recorded as 0.0019 ($p<0.01$) which was also considered as highly significant.

Kim et al. (2016), made a study in Korea, in order to determine the prevalence and antibiotic resistance of *Salmonella spp.* isolated from ducks in Korea. From 40 duck farms in Korea, 400 cecal content samples in total were gathered. Of the 400 cecal samples, 83 and 364 contained isolated isolates of *Salmonella spp*. *Salmonella Typhimurium* was the most common serotype among the 83 *Salmonella* isolates (51 isolates; 61.45%). With the exception of erythromycin, *Salmonella* isolates showed little resistance to the investigated antimicrobial drugs. The Korean duck industry should be concerned about the high prevalence of antibiotic resistance seen in zoonotic infections that infect ducks since they could endanger public health.

Eid *et al.* (2019), gathered a total of 500 samples at random from several duck farms in Egypt's Ismailia Governorate. The samples that were taken were examined bacteriologically. *Salmonella spp.* (2.8%) was the most prevalent pathogen isolated from both healthy and ill ducks. The majority of the isolated strains were positive for enrofloxacin, norfloxacin, and ciprofloxacin during the antibiotic sensitivity test.

2.2 Prevalence of pathogenic *E. coli and Salmonella spp.* bacteria on duck

2.2.1 Prevalence of pathogenic *E. coli* bacteria on duck

Eid *et al.* (2019), Ismailia Governorate in Egypt's duck farms provided a total of 500 samples, which were chosen at random. The bacteriological analysis of the collected samples was performed. *Escherichia coli* (3.6% and 22.8%) was the most typical pathogen recovered from both healthy and ill ducks.

Majumder *et al.* (2017) examine a total of 60 cloacal swab samples were collected from two duck farms of Bangladesh Agricultural University and Shamvuganj. Out of 60 samples, 26 (43.33%) were confirmed to be *E. coli* positive.

Kim *et al.* (2016), did a study to find out how common and resistant to antibiotics *Escherichia (E.) coli* isolates from ducks were in Korea. From 40 duck farms in Korea, 400 cecal content samples in total were gathered. Of the 400 cecal samples, 364 had isolated *E.coli*

Khoo et al. (2010), conducted a study to establish the incidence of positive infections in different type of chickens with the hope to identify the pathogenic strains potentially infecting consumers. A total of 141 (79%) of the isolates were *E. coli* 01:K1, 16 (9%) were *E. coli* 02:K1 and another 21 (12%) were *E. coli* 078:K80. Of the 141 *E. coli* 01:K1 isolates, 125 (89%) were from chickens and 16 (11%) from ducks.

2.2.2 Prevalence of pathogenic *Salmonella spp.* bacteria on duck

Eid *et al.* (2019), Ismailia Governorate, Egypt, provided a total of 500 samples, which were chosen at random from various duck farms. Bacteriological testing was done on the samples that were obtained. *Salmonella spp.* (2.8%) was the pathogen that was most frequently isolated from both healthy and sick ducks.

Rahman *et al.* (2017), A total of 48 duck samples comprising of liver (n=16) spleen (n=16) and intestinal content (n=16) were collected from 16 ducks (8 sick and 8 dead). The samples were collected from the selected Duck farms at three *Upazillas* (sub-districts) in Dinajpur district, Bangladesh. *Salmonella spp*. could be isolated from 39.58% (n=19/48) duck.

Dey *et al.* (2016), In order to gather data, 28 different homes and 12 small to medium sized duck farms were all visited. Duck salmonellosis prevalence was determined to be 38.1% based on history, clinical symptoms, and the information provided by the farmers. The P value was assessed to be 0.003 ($p<0.01$), which was deemed to be very significant. A total of 120 fecal samples were acquired from ducks that appeared to be healthy and those that appeared to be sick, based on age, sex, season, location, and health condition, and tentative cases of Duck Salmonellosis as determined by data obtained from the structured questionnaire. There were 120 fecal samples, and 32 (26.67%) of them were found to be positive for *Salmonella* based on their cultural and biochemical characteristics, it was found that among 120 fecal samples 32 (26.67%) were found to be positive for *Salmonella* and the P value was recorded as 0.0019 ($p<0.01$) which was also considered as highly significant.

***Kim et al.* (2016),** investigated the prevalence and antibiotic resistance of *Salmonella spp.* isolated from ducks in Korea through this investigation. 40 duck farms in Korea provided a total of 400 cecal content samples. Of the 400 cecal samples, 83 and 364 had isolated *Salmonella spp.* strains. *Salmonella Typhimurium (*51 isolates; 61.45%) was the serotype that was most frequently found among the 83 *Salmonella* isolates.

Cha *et al.* (2013) In between 2011 and 2012, 7119 samples from 72 duck farms spread across five provinces were analyzed. *Salmonella* serotypes were found in 65.2 % (47/72) of the duck farms' flocks, or 43.4 % (69/159) overall. Eighty-five strains were found in 69 flocks of ducks. Except for one isolate, every other isolate was resistant to at least one antibiotic, and 27% of the isolates were resistant to five to sixteen different antimicrobials.

2.3. Antimicrobial sensitivity pattern of *E. coli* and *Salmonella spp.* isolates

2.3.1 Antimicrobial sensitivity pattern of *E. coli* isolates

Eid *et al.* (2019), obtained a total of 500 samples at random from several duck farms in Egypt's Ismailia Governorate. The collected samples were examined bacteriologically. *Escherichia coli* (3.6% and 22.8%) was the most typical pathogen found in both healthy and ill ducks. The majority of the isolated strains tested positive for enrofloxacin, norfloxacin, and ciprofloxacin in the antibiotic sensitivity test.

Kim *et al.* (2016), examined the prevalence and antibiotic resistance of *Escherichia (E.) coli* isolated from ducks in Korea. From 40 duck farms in Korea, a total of 400 cecal content samples were gathered. A total of 364 of the 400 cecal samples contained isolated *E. coli* bacteria. *E. coli* isolates had a high level of resistance to the tested antimicrobial drugs, and 90.9% (331/364) of them displayed multi-antibiotic resistance. The Korean duck business should be concerned about antibiotic resistance in zoonotic infections that infect ducks since these pathogens have a high antimicrobial resistance rate and could endanger public health.

Darwish *et al.* (2015) carried out a study to investigate the possible transmission of diarrheagenic *E. coli* from consumption of duck meat and giblets. The examined duck meat and giblets contained *E. coli* that could be isolated. *E. coli* serogroups O86, O127, O114, O26, and O78 could all be found. The highest concentration of *E. coli* was found in the liver, followed by the gizzard, heart, spleen, and muscle. Separated *E. coli* serogroups carried various virulent components that caused hemorrhage and diarrhea. Further, isolated *E. coli* serogroups shown markedly reduced sensitivity or even resistance to the most widely used antibiotics in Egypt.

Adzitey *et al.* (2013) examine Fifty-five (n=55) isolates of *Escherichia coli* isolated from ducks in Penang, Malaysia were examined for their susceptibility to eleven different

antibiotics and assayed for the presence of plasmid DNAs. All the 55 *Escherichia coli* isolates were resistant (100%) to vancomycin. Higher resistance (≥ 60) occurred for tetracycline 51 (92.7%), ampicillin 40 (72.7%), streptomycin 37 (67.3%), and sulfamethoxazole-trimethophrim 37 (67.3%). No and low resistance was observed for nitrofurantoin (0%) and gentamicin (1.8%), respectively. The isolates also showed some intermediate resistances to all antibiotics examined except for vancomycin.

2.3.2 Antimicrobial sensitivity pattern of *Salmonella spp.* isolates

Chen *et al.* (2020) collected 365 sample in between May 2017 and April 2019, the fresh duck meat from retail markets in six different Guangdong Province cities. Duck meat had a high percentage of *Salmonella* contamination (151/365, or 41.4%). Twenty-six distinct Salmonella serotypes were found; the most common serotypes were *S. Corvallis* (n = 25, 16.6%), *S. Kentucky* (n = 22, 14.6%), and *S. Agona* (n = 20, 13.3%). Each isolate had at least one antibiotic resistance, and 133 (88.1%) of the isolates had multidrug resistance (MDR). Most *Salmonella* isolates (86.1%) contained seven types of virulence-related genes.

Eid *et al.* (2019), gathered a total of 500 samples at random from several duck farms in Egypt's Ismailia Governorate. The samples that were taken were examined bacteriologically. *Salmonella spp.* (2.8%) was the most prevalent pathogen isolated from both healthy and ill ducks. The majority of the isolated strains were positive for enrofloxacin, norfloxacin, and ciprofloxacin during the antibiotic sensitivity test.

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Kim *et al.* (2016), carried out a study to look into the prevalence and antibiotic susceptibility of *Salmonella spp.* isolated from ducks in Korea. From 40 duck farms in Korea, a total of 400 cecal content samples were gathered. Between 83 and 364 of the 400 cecal samples included isolated isolates of Salmonella spp. *Salmonella Typhimurium* (51 isolates; 61.45%) was the most frequent serotype among the 83 *Salmonella* isolates. Except for erythromycin, *Salmonella* isolate resistance to the investigated antimicrobial drugs was minimal. Since duck zonotic viruses have a high rate of antibiotic resistance and could endanger public health, the

Korean duck business should be concerned about this issue.

Cha *et al.* (2013) In between 2011 and 2012, 7119 samples from 72 duck farms spread across five provinces were analyzed. *Salmonella* serotypes were found in 65.2 % (47/72) of the duck farms' flocks, or 43.4 % (69/159) overall. Eighty-five strains were found in 69 flocks of ducks. Except for one isolate, every other isolate was resistant to at least one antibiotic, and 27% of the isolates were resistant to five to sixteen different antimicrobials.

CHAPTER 3

MATERIALS AND METHODS

This study was conducted at the laboratory of the Department of Medicine & Public Health, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka 1207, during the period of January to June, 2021.

3.1 Materials

3.1.1 Study area

Sample ware collected from the selected duck farm at Savar Upazilla (*Sub district*), Dhaka. Collected sample then transported carefully with ice box and examined in the Department of Medicine & Public Health laboratory, Sher-e-Bangla Agricultural University.

3.1.2 Sample size

There were total 40 duck samples comprises of intestinal content (n = 20) and liver (n = 20) from 20 ducks (10 live and 10 dead). The samples were collected from different selected farm on Savar Upazilla. Selected whole bird samples were transported with ice box to Dept. of Medicine and Public Health Laboratory, Dhaka. Postmortem procedure was performed to collect the intestinal contents and liver.

Table 1. Number of different samples were collected from different area of Savar Upazilla

Sl. no	Village name	No of sample
1	Tetuljhora	**4**
2	Gonokpara	**4**
3	Shimulia Bazar	**4**
4	Noihati	**4**
5	Bagbari	**4**
Total		**20**

3.1.3 Bacteriological Media

3.1.3.1 Solid Culture Media

Nutrient Agar (NA), Eosin-Methylene-Blue (EMB), MacConkey agar (MC), Salmonella-shigella (SS), and Brilliant Green agar (BGA) were the media utilized for bacteriological examination.

3.1.3.2 Liquid Media (Broth)

The liquid media ware used for this study were Nutrient broth (NB) and Peptone Broth.

3.1.4 Chemicals and Reagents

3.1.4.1 Phosphate buffer saline (PBS)

Eight gram of sodium chloride, 2.89 gm disodium hydrogen phosphate, 0.2 gm potassium chloride, and 0.2 gm potassium hydrogen phosphate, were suspended in 1000 ml distilled water to make Phosphate buffered saline (PBS) solution. The solution was heated until it was entirely dissolved, and the p^H was adjusted using a p^H meter and maintain at 7.0-7.2 (Cheesbrough, 2006). After that, the solution was autoclaved and stored at 4°C for future use.

3.1.4.2 Other chemicals use on test

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

3.1.5 Glass wares and other appliances

The following types of glassware and appliances utilized in the experiment:

Petridishes, conical flasks (100, 500, and 1000 ml), cotton, slides, and coverslips, Eppendorf tube, test tube (with or without Durham's fermentation tubes and stopper), test tube stand, pipette, micropipette, incubator, refrigerator, sterilizing instruments, thermometer, ice carrier,

hand gloves, spirit lamp, match lighter, laminar air flow, hot air oven, centrifuge tubes, autoclave machine, electronic machine, glass spreader, inoculation loop, compound microscope, syringe and needle, tray, forceps, scalpel, scissors etc.

3.1.6 Materials required for anti-biogram study

3.1.6.1 Muller Hinton Agar (MHA)

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

3.1.6.2 McFarland standards

The turbidity of bacterial suspensions is adjusted using McFarland standards to keep the quantity of bacteria within the specified range.

3.1.6.3 Antibiotic discs

The antibiotics susceptibility pattern was determined using commercially available antibiotic discs (OXOID Limited, Canada).

Table 2: Disc concentration of antimicrobial agents

Antimicrobial agents	Disc Concentration (µg)
Ampicillin (AMP)	10
Amoxycillin (AMX)	30
Ciprofloxacin (CIP)	5
Gentamycin (GM)	10
Tetracycline (TE)	30

3.2 Methods

3.2.1 Brief description of the experimental design

The entire experimental design is carried out in two stages. On the first step bacteria were isolated from duck Liver & Intestinal content and then identified the *E. coli* and *Salmonella spp.* based on cultural and morphological characteristics. Motility test with hanging drop preparation and carbohydrate fermentation tests were also done to confirm the isolated

organism as *E. coli.* and *Salmonella spp*. The second step included the study of response of the isolated bacteria ware tested against commercially available antibiotic discs.

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

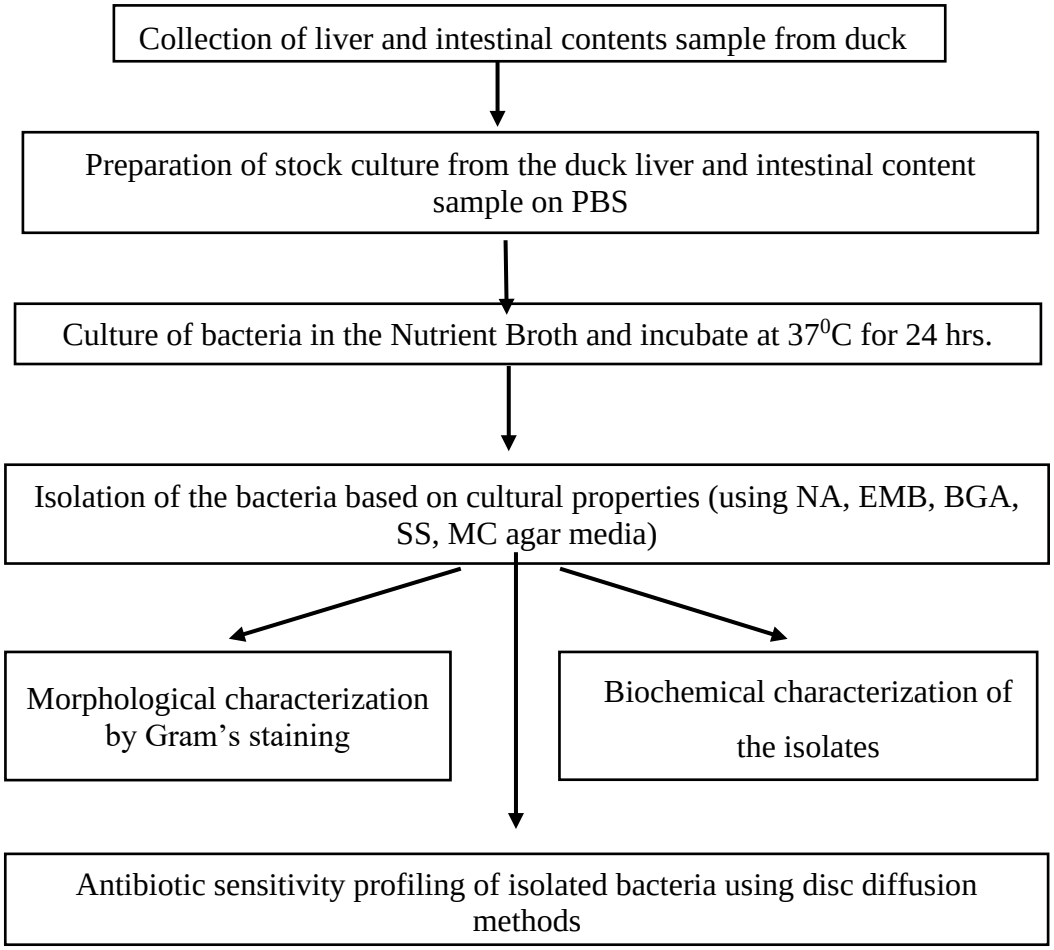

Figure 1: Schematic illustration of the experimental design

3.2.2 Preparation of different cultural media

3.2.2.1 Nutrient broth

The Nutrient Broth was prepared by 25 grams suspended in 1000 mL distilled water. If required, heat the medium to dissolve it completely. Autoclaved at 15lbs pressure (121°C) for 30 minutes to sterilize. The broth was placed in test tubes and incubated at 37°C overnight to ensure sterility before being stored at 4°C until needed.

3.2.2.2 Nutrient agar

Nutrient agar was made by dissolving 28 grams of dehydrated nutrient agar (Hi Media, India) in 1000 ml of distilled water and autoclaving at 121^0C for 15 minutes at the pressure of 15 lbs./inch^2. The agar was then distributed onto Petridis (90 mm and 100 mm) and incubated at 37°C overnight to ensure sterility before being stored at 4°C until required.

3.2.2.3 MacConkey's ager

To dissolve the medium fully, 49.53 grams of MacConkey agar (HiMedia, India) were suspended in 1000 ml of cold distilled water and autoclave at 15lbs pressure at 121^{o}C for 30 minutes. After that, it was put into sterilized Petridis's and let to set. Following the solidification of the media in the plates, the plates were incubated overnight at 37°C to ensure sterility.

3.2.2.4 Eosine Methylene Blue (EMB) agar

In 1000 ml of distilled water, 36 grams of powdered EMB agar base (HiMedia, India) were suspended. The suspension was brought to a boil for a few minutes to completely dissolve the powder in the water. To make the medium sterile, it was autoclaved for 30 minutes. The medium was autoclaved and then placed in a water bath at 45°C to cool down to 40°C. To produce EMB agar plates, 10-20 ml of media was poured from the water bath into small and medium sterile petri dishes. After the medium had solidified in the plates, they were incubated at 37°C overnight to ensure sterility.

3.2.2.5 Salmonella-Shigella agar (SS agar)

Sixty grams of dehydrated SS agar medium base were suspended in 1000 ml distilled water and heated to boiling to completely dissolve the medium, as directed by the manufacturer (HiMedia, India). The medium should not be autoclaved. The suspended medium should be cooled down by using water bath at 50°C. Following the solidification of the medium in the Petri dishes, then the Petri dishes were incubated at 37°C for an overnight check on sterility before being stored at 4°C for future use.

3.2.2.6 Brilliant Green agar

Fifty-eight grams of dehydrated medium were suspended in 1000 ml distilled water and heated to boiling to completely dissolve the medium, as directed by the manufacturer (HiMedia, India). Autoclaving was used to sterilize the media. After autoclaving, the medium was placed in a 45°C water bath to cool it down. Following the solidification of the medium in the petri dishes, the petri dishes were incubated at 37°C for an overnight check on sterility before being stored at 4°C for future use.

3.2.2.7 Mueller Hinton agar

Thirty-eight grams Mueller Hinton agar suspended in 1000 mL distilled water and heated to boiling to completely dissolve the medium. After sterilization, autoclave for 15 minutes at 15 lbs pressure at the temperature of 121°C. The temperature was lowered to 45-50°C. Then pour the petri dishes with those medium. After solidification of the medium in the petri dishes, incubate at 37ºC for overnight to check their sterility and then stored at 4ºC in a refrigerator for future use.

3.2.3 Preparation of different reagents

3.2.3.1 Methyl Red and Voges-Proskauer (MR-VP) broth

A total of 3.4 gm of MR-VP medium was diluted in 250 ml of distilled water and dispensed in 2 ml portions in each test tube before autoclaving at 121°C for 15 minutes at a pressure of 15 lbs/inch^2. After autoclaving, the medium-filled tubes were incubated overnight at 37°C to ensure sterility before being kept in the refrigerator for future use.

3.2.3.2 Kovac's reagent for Indole test

Kovac's reagent for Indole test was prepared by dissolving 0.1 gram of Bacto methyl-red in 300 mL of 95 % alcohol, then diluting to 500 mL with distilled water.

3.2.3.3 Methyl-Red solution

The medium is made up of 1% peptone water with fermentable sugars added to it. Peptone water was made by combining 1-gram Bacto peptone (Difco, USA) and 0.5-gram sodium chloride in 100 mL distilled water, boiling for 5 minutes, adjusting the pH to 7.6 with phenol

red (0.02%), cooling, and filtering through filter paper. The solutions were then poured into cotton-plugged test tubes containing inverted Durham's fermentation tubes in 5 ml increments. The sugars used for fermentation, dextrose (MERCK, India), maltose (s.d. fiNE-CHEM Ltd.), lactose (BDH, England), sucrose (MERCK, India), and mannitol (PETERSTOL TENBEG), were made separately as 10% solutions in distilled water (10 grams sugar was dissolved in 100 ml of distilled water). To dissolve the sugar, a little heat was required. After that, they were autoclaved for 15 minutes to sanitize them. For three days, the sugar solutions were sterilized in Arnold's steam sterilizer at 100^{o}C for 30 minutes. In each culture tube containing sterile peptone water, 0.5 mL of sterile sugar solution was added aseptically. To ensure sterility, the sugar solutions were incubated at 37°C for 24 hours. Biochemical tests were conducted using these solutions.

3.2.3.4 Preparation of 50% Buffered Glycerol Saline

In 700mL distilled water, 8.3 grams of Buffered Glycerol Saline Base were suspended. After that, 300mL glycerol was added. Heat was used to completely dissolve the medium, which was then well mixed and delivered in screw-capped tubes or other appropriate containers. Autoclaving at 15 lbs/inch^2 of pressure at the temperature of 121°C for 15 minutes was used to sterilize the items.

3.2.4 Isolation of bacteria

3.2.4.1 Serial dilution of bacterial culture (10-fold dilution)

To reduce the bacterial count for the total viable count (TVC) and total coliform count (TCC), the stock sample was serially diluted. It was performed by filling 10 (1-10) Eppendorf tubes with 900 µl of PBS. From the stock tube (2ml), 100 µl of the stock sample was transferred to the Eppendorf tube adjacent to the stock tube. Then, from the first Eppendorf tube to the next, 100 µl of diluted material is passed. To the last tube, make successive dilutions in the same manner, and discard 100 µl of diluted material from the last tube. To determine the total viable count and total coliform count, transfer 25 µl of liquid sample from the 7th tube to nutrient Agar plate and MacConkey agar plate.

3.2.4.2 Primary culture of microorganisms

Primary growth of all kinds of bacteria present in the collected samples was performed in nutrient broth. The samples were inoculated in nutrient broth and incubated at 37°C overnight to allow the organisms to proliferate.

3.2.4.3 Isolation in culture media

After primary culture of the organism, a tiny amount of inoculums from Nutrient broth were streaked through inoculating loop on MacConkey agar, Brilliant green agar, and Salmonella-Shigella agar to observe the colony morphology. The organisms had distinct colony shape, showing that *E. coli* was chosen for culturing on selective mediums such as EMB agar and *Salomonella* on *Salmonella-Shigella* agar (SS agar). The morphological characteristics (shape, size, surface texture, edge and elevation, color, opacity, and so on) of potential colonies on various agar medium that grew during 18 to 24 hours of incubation were meticulously documented.

3.2.4.4 Morphological identification of bacteria using Gram's staining methods

Merchant and Packer (1967) recommended using Gram's staining to identify the size, shape, and arrangement of bacteria. The steps were as follows: An inoculation loop was used to pick up a small colony, which was then spread on a glass slide and gently heated to fix it. The smear was then stained with crystal violet solution for 2 minutes before being cleansed under running tap water. After that, Gram's iodine was applied as a mordant for one minute before being rinsed with running water again. Then, acetone alcohol was added as a decolourizer. After rinsing with water, safranine was used as a counter stain and left to set for 2 minutes. The slide was then cleaned with water, blotted, and air dried before being inspected under a microscope using immersion oil and a high-power objective (100X).

3.2.4.5 Motility test

The motility test was carried out according to Cowan and Steel's (1985) method to distinguish motile bacteria from non-motile bacteria. A pure culture of the test organism was allowed to develop in nutrient broth before the test. To make hanging drop preparation, one drop of cultured broth was placed on the cover slip and inverted over the concave depression of the hanging drop slide. To avoid air movement and evaporation of the fluid, Vaseline was

applied to the concave depression of the hanging drop slide for better adhesion of the cover slip. The hanging drop slide was then inspected using a compound microscope with a 100X objective and immersion oil. Observing motility in contrast to bacterial to and from movement allowed the motile and non-motile organisms to be distinguished.

3.2.5 Biochemical tests to identify isolated *E. coli* and *Salmonella spp*.

E. coli and *Salmonella spp*. isolates were confirmed using a variety of biochemical tests.

3.2.5.1 Indole test

The test organisms were cultivated for 48 hours at 37°C in test tubes containing 3 ml of peptone water containing tryptophan. Then 1 ml diethyl ether was added, shaken thoroughly, and set aside until the ether rose to the top. Then, 0.5 ml of Kovac's reagent was carefully poured down the side of the test tube, forming a ring between the medium and the ether layer, and the colour of the ring was noted. Indole production was suggested by the development of a brilliant red coloured ring. There is no development of red colour in the negative scenario (Cheesbrough, 2006).

3.2.5.2 Methyl Red test

A single colony from a pure culture of the test organism was inoculated in 5 mL of sterile MR-VP broth for the test. After a 5-day incubation period at 37°C, 5 drops of methyl red solution were added, and colour development was seen. The development of red colour was positive, indicating an acid pH of 4.5-6 due to glucose fermentation. The appearance of a yellow colour indicated a negative outcome (Cheesbrough, 2006).

3.2.5.3 Voges-Proskauer test

The test *E. coli* organisms were cultured for 48 hours at 37°C in 3 mL of sterile MR-VP broth. Then, per ml of a broth culture of the test organism, 0.6 ml of 5% alpha-naphthol and 0.2 ml of 40% potassium hydroxide containing 0.3% creatine were added. Then give it a good shake and let it sit for 5-10 minutes to see how the colour develops. The appearance of a bright copper-red colour signified a positive case. There was no development of copper colour in the negative cases (Cheesbrough, 2006).

3.2.5.4 Citrate utilization test

By softly touching the tip of a needle to a colony that is 18 to 24 hours old, inoculate Simmons citrate agar on the slant. Incubate aerobically for 18 to 24 hours at 35°C to 37°C. Due to their slow development rate on citrate medium, certain organisms may require up to 7 days of incubation. Observe the formation of blue colour along the slant, which indicates alkalinization (Cheesbrough, 2006).

3.2.5.5 Sugar fermentation test

In the sugar fermentation test, a loop of NB culture of the organisms was inoculated into each tube containing five basic sugars (e.g., dextrose, sucrose, lactose, maltose, and mannitol) individually and incubated for 24 hours at 37^0C. The formation of gas bubbles in the inverted Durham tube indicated acid generation, which was shown by a colour change from reddish to yellow in the liquid.

3.2.6 Maintenance of stock culture

The stock culture was made by mixing 1 ml of sterilized glycerol with 1 ml of pure culture in nutrient broth and storing it at -20^0C.

3.3 Antibiotic sensitivity test

By disc diffusion or the Kirby-Bauer method, three isolates from five genera were tested for antimicrobial drug susceptibility against ten regularly used antibiotics. Selection of 3 to 5 isolated colonies from the SS, MC, EMB, and BGA agar plate. Using a sterile cotton bar, the isolated colony were uniformly scattered onto Muller-Hinton agar plates. The antibiotic disc was then placed on Muller-Hinton agar and incubated for 24 hours at 37°C. Then the plates are examined and used a meter ruler to measure the diameter of inhibitory zones in millimetres from the edge of the disc to the edge of the zone.

3.3.1 Antimicrobial discs

Table 2. shows a list of commercially available antibacterial discs (OXOID, Canada) and their concentrations. The discs are put to the plates as soon as possible after inoculation, but not

later than 15 minutes. The discs were individually placed on the agar with sterile forceps and gently pressed down. On a 100-mm plate, no more than 5 discs were placed.

3.3.2 Recording and interpreting results

After placing the discs on the plate, the plates were inverted and incubated at 37^{o}C for 16-18 hours. The diameter of the full inhibitory zones (including the diameter of the disc) was measured and recorded in millimeters after incubation. Without opening the lid, measurements were taken with a ruler on the underside of the plate. The zones of growth inhibition were compared to the Clinical and Laboratory Standards Institute's zone-size interpretive (CLSI, 2007). According to CLSI's (2007) zone diameter interpretation standards, antimicrobial testing findings were classified as sensitive, intermediate, or resistant on **Table 3.**

Table 3: Drugs with their disc concentration for the Enterobacteriaceae family

Name of Antibiotic	**Disc Conc. (µg /disc)**	**Zone Diameter Interpretive Standard (mm)**		
		Resistant	**Intermediate**	**Susceptible**
Gentamycin (GEN)	10	≤13	14--17	≥18
Tetracycline (TE)	30	≤14	15--18	≥19
Amoxicillin (AMX)	10	≤13	14--17	≥18
Ampicillin (AMP)	10	≤13	14--16	≥17
Ciprofloxacin (CIP)	5	≤15	16--20	≥21

3.4 Statistical Analysis

Data were entered into the Microsoft Office Excel 2021 spreadsheet. To explain the outcome of the prevalence rate and self-evaluation model, descriptive statistics was utilized. The prevalence was given as a percentage.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 *E. coli* bacteria identification based on cultural and biochemical properties

4.1.1.1 Cultural properties on nutrient broth

The growth of *E. coli* marked rapid turbidity in Nutrient broth. (Shown in **Fig. 2**)

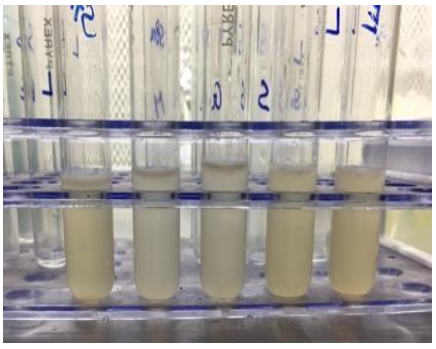

Figure 2: Turbidity of *E. coli* on Nutrient broth

4.1.1.2 Cultural properties on nutrient agar media

The experimental samples (rinsed with PBS) were streaked on nutrient agar to unveil the growth of *E. coli* after 24 hrs. of incubation at 37^0C aerobically and were indicated by the growth of smooth, circular, white to the grayish-white colony. (Shown in **Fig. 3**)

Figure 3: Colony of *E. coli* showing smooth, circular, white colony on Nutrient agar

4.1.1.3 Cultural properties on EMB agar media

Separate EMB agar plates were streaked with the organism and incubated aerobically at 37^0C for 24 hours. Smooth, round, black-colored colonies with a metallic sheen showed growth. Due to the generation of acid, EMB agar is used to distinguish coliform enteric bacteria from other enteric bacteria. In acidic conditions, the dyes produce a dark purple complex which is usually associated with a green metallic sheen which is an indication of the growth of *E. coli* colonies of other non-lactose fermenters that appear as translucent or pink. (Shown in **Fig. 4**)

Figure 4: EMB agar inoculated with *E. coli* demonstrating growth with green-metallic sheen colonies

4.1.1.4 Cultural properties on Mac-Conkey (MC) agar media

MC agar plates were streaked separately with the organism and revealed the growth of bacteria after 24 hrs. of incubation at 37^0C aerobically and were indicated by the growth of bright pink to the red colored colony due to fermentation of lactose by *E. coli.* (Shown in **Fig. 5**)

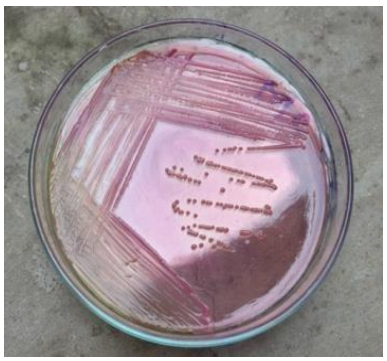

Figure 5: Colony of *E. coli* showing bright pink colour on Mac-Conkey (MC) agar

4.1.1.5 Identification of *E. coli* by Gram's staining

The microscopic examination of Gram's stained smears from NA, MC, EMB, and BG agar reveals that the isolated bacteria were Gram-negative, pink-colored, small rod-shaped organisms arranged in single, pairs or short chains. (Shown in **Fig. 6**)

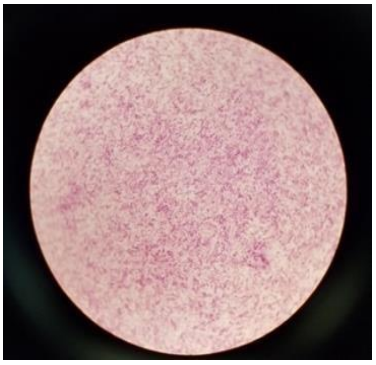

Figure 6: Gram negative, pink coloured, long rod shape *E. coli* under light microscope. (100x)

4.1.1.6 Results of motility test

All of the isolates were found to be motile using the hanging drop slide.

Table 4: Morphological and cultural properties of *E. coli* isolated from duck

Feature	Appearance
Nutrient agar	Smooth, circular, white to greyish white colonies were found.
Eosin Methylene Blue agar	Smooth, circular, black colour colonies with metallic sheen were produced.
Mac-Conkey agar	Rose pink lactose fermented colonies were formed.
Staining property	Gram negative, pink coloured, small rod-shaped organisms arranged in single, pairs or short chain was observed.
Motility	The organisms were motile.

4.1.1.7 Identification of *E. coli* by Biochemical test

All the isolates of *E. coli* showed a positive reaction on MR and Indole test by indicating red color development after adding the respective reagents. On the other side, *E. coli* showed no color change on Voges-Proskauer **Fig. 7**. And all the isolates of *E. coli* were fermented the five basic sugars (dextrose, maltose, lactose, sucrose, and mannitol) and produced both acid and gas. The color change of the sugar media from reddish to yellow showed acid production, while the accumulation of gas bubbles in the inverted Durham tube indicated gas production. (Shown in **Table 5**)

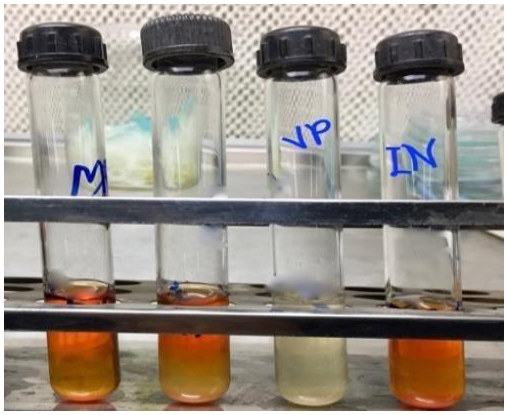

Figure 7: Indole positive, VP negative, and MR positive of *E. coli*

Table 5: Biochemical reaction patterns of *E. coli*

Fermentation properties with five basic sugars					**Indole test**	**VP Test**	**MR Test**	**Citrate test**
DX	**ML**	**L**	**S**	**MN**				
AG	AG	AG	A↓G↓	A G	+	−	+	−

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

4.1.2 *Salmonella spp.* bacteria identification based on cultural and biochemical properties

4.1.2.1 Cultural properties on nutrient broth

All *Salmonella spp.* isolates produced turbidity in nutrient broth. (Shown in **Fig. 8**)

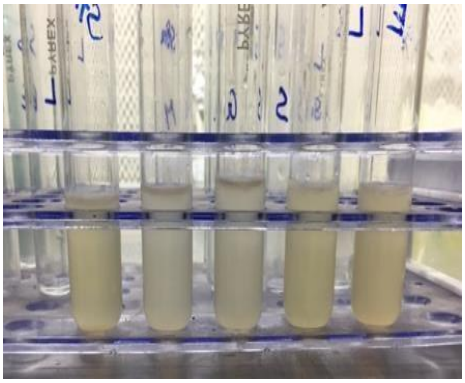

Figure 8: Turbidity of *Salmonella spp.* on Nutrient broth

4.1.2.2 Cultural properties on Mac-Conkey (MC) agar media

After overnight incubation, red to pink-white colonies surrounded by brilliant red zones were presumptively identified as *Salmonella spp.* (Shown in **Fig. 9**)

Figure 9: Pink-white colony surrounded by brilliant red zone on Mac-Conkey agar

4.1.2.3 Cultural properties on *Salmonella-Shigella* (SS) agar media

Because of the lactose nonfermanter, the isolated *Salmonella spp.* generated opaque, translucent, colorless, smooth, and spherical colonies with black centers on SS agar. **(Fig. 10)**

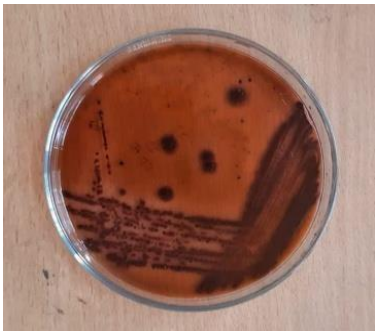

Figure 10: Colony of *Salmonella spp.* producing opaque, translucent, smooth, round colonies on Salmonella- shigella agar

4.1.2.4 Cultural properties on Brilliant Green agar (BGA) media

After overnight incubation, the *Salmonella spp.* organisms developed good growth; red-colored, pinkish-white colonies on BG agar, which were tentatively identified as *Salmonella spp*. (Shown in **Fig. 11**)

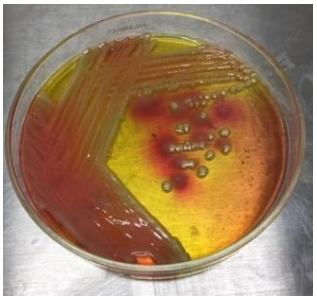

Figure 11: Pinkish-white *Salmonella spp*. colony on Brilliant green agar

4.1.2.5 Identification of *Salmonella spp.* by Gram's staining

The microscopic examination of Gram's stained smears from SS agar, BGA revealed Gram-negative, pink color, small rod-shaped organisms arranged in single or paired.

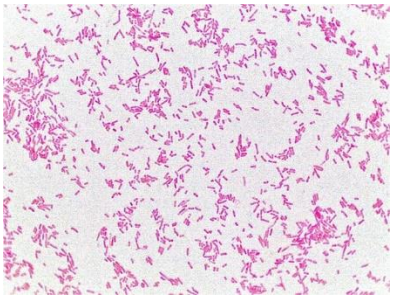

Figure 12: Gram negative, pink colour, small rod-shaped organisms arranged in single or paired under the light microscope (100x)

Table 6: Morphological and cultural properties of *Salmonella spp.* isolated from duck

Feature	Appearance
SS agar	Opaque, translucent, colourless, smooth, round colonies were found.
BG agar	Red-colored, pinkish-white colonies ware showed.
Staining property	Gram- negative, pink colour, small rod-shaped organisms arranged in single or paired was observed.

4.1.2.7 Identification of *Salmonella spp.* by Biochemical test

Salmonella spp ferments all the sugar and produced both acid and gas except sucrose and lactose shown in **Table 7**. *Salmonella spp.* show a positive reaction in MR and Citrate reduction test otherwise it produces a negative reaction on the VP and Indole test shown in **Fig. 13**.

Figure 13: Indole negative, VP negative, and MR positive of *Salmonella spp.*

Table 7: Biochemical reaction patterns of *Salmonella spp*.

Fermentation properties with five basic sugars					**Indole Test**	**MR Test**	**VP Test**	**Citrate Test**
DX	**ML**	**L**	**S**	**MN**				
AG	AG	NF	NF	A↓G	−	+	−	+

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

4.1.3 Prevalence of *E. coli* and *Salmonella spp.* on duck

Prevalence of *E. coli* and *Salmonella spp.* on duck intestinal contents and liver are given on different tables. Those bacteria are isolated on different cultural and biochemical tests.

4.1.3.1 Prevalence of *E. coli* bacteria on duck

Prevalence of *E. coli* and on duck intestinal contents and liver are given on **Table 8.** Those bacteria are isolated on different cultural and biochemical tests.

Table 8: Prevalence of *E. coli* bacteria on duck

Bird Type	Sample Type	Total Sample	*E. coli*	Prevalence of *E. coli*
Live	Liver	10	2	20%
Live	Intestinal Contents	10	7	70%
Dead	Liver	10	3	30%
Dead	Intestinal Contents	10	9	90%

Among the 40 samples there are 21 samples found positive of *E. coli*. The overall prevalence of *E. coli* on duck sample is 52.5%.

4.1.3.2 Prevalence of *Salmonella spp.* bacteria on duck

Based on different cultural, biochemical, and staining procedure *Salmonella spp.* are identified and the prevalence rate are given on **Table 9.**

Table 9: Prevalence of *Salmonella spp.* bacteria on duck

Bird Type	Sample Type	Total Sample	*Salmonella spp.*	Prevalence of *Salmonella spp.*
Live	Liver	10	1	10%
Live	Intestinal Contents	10	4	40%

Dead	Liver	10	3	30%
Dead	Intestinal Contents	10	7	70%

Among the 40 samples there are 15 samples found positive of *Salmonella spp*.. The overall prevalence of *Salmonella spp.* on duck sample is 37.5%.

4.1.4 Antibiotic sensitivity profiling of *E. coli* and *Salmonella spp.*

4.1.4.1 Antibiotic sensitivity profiling of *E. coli*

A total of 21 isolates of *E. coli* isolated from duck samples were further used to determine the antibiotic sensitivity pattern. Out of 21, *E. coli* positive isolates 19 samples are resistant to amoxicillin, 17 resistants to ampicillin & tetracycline. On the other hand, 14 samples are sensitive to gentamycin and 20 are ciprofloxacin. The antibiotic sensitivity profile of *E. coli* isolates presented in **Table 10**. All isolated bacteria showed significantly resistant to amoxicillin (90.47%) followed by tetracycline & amoxicillin both (80.95%). Sixty six percent sensitive to gentamycin and 95.24% to ciprofloxacin were found in this study (**Fig. 15**).

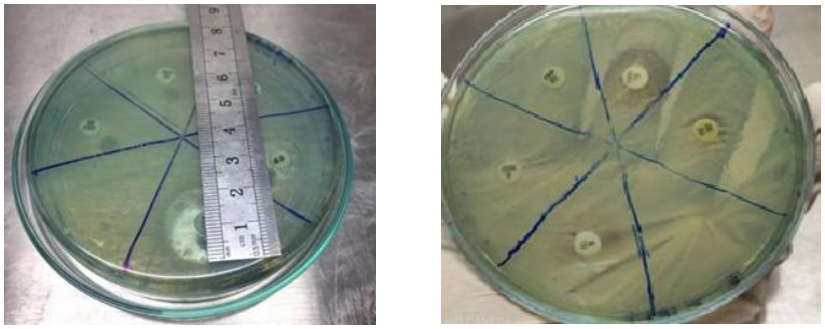

Figure 14: Antibiotic sensitivity test of *E. coli*

Table 10: Antibiotic sensitivity pattern of *E. coli* on different antibiotics

	AMX	**AMP**	**CIP**	**GEN**	**TE**
S	0	0	20	14	0
IN	2	4	1	6	4
R	19	17	0	1	17

Legend: AMX= Amoxicillin, AMP= Ampicillin, CIP= Ciprofloxacin, GEN= Gentamycin, TE= Tetracycline, S=Sensitive, IN= Intermediate, R= Resistant

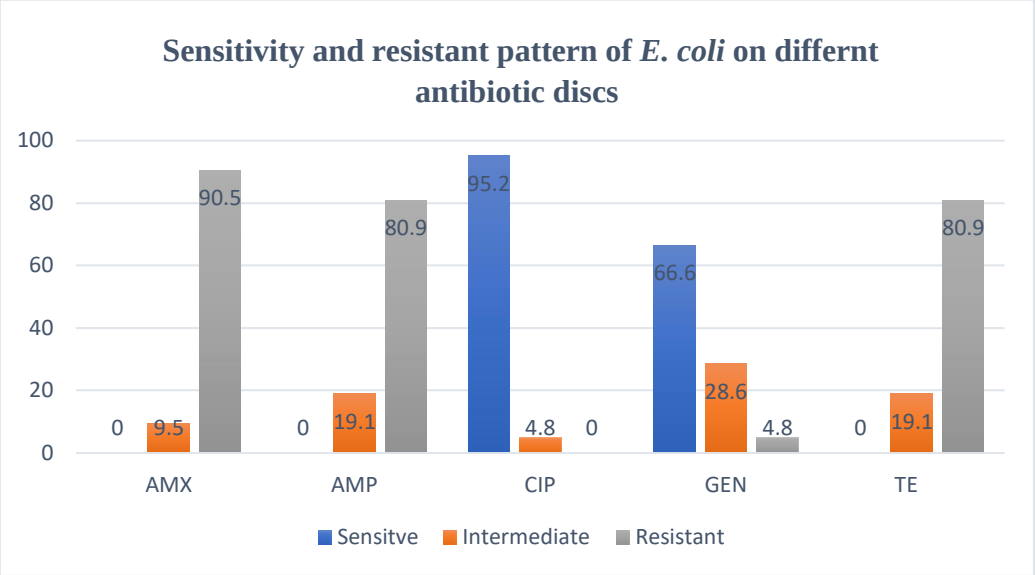

Figure 15: Antibiotic sensitivity pattern of *E. coli* on different antibiotic discs

This figure represents that 95.2% isolates were sensitive to ciprofloxacin and 66.6% sensitive to gentamycin with 28.6% intermediate. Amoxicillin was shown 90.5% resistant followed by both ampicillin & and tetracycline (80.9%) with 19.1% intermediate in reaction.

4.1.4.2 Antibiotic sensitivity profiling of *Salmonella spp.*

A total of 15 isolates of *Salmonella spp.* isolated from duck samples were further used to determine the antibiotic sensitivity pattern. Out of 15 *Salmonella spp.* positive isolates all the 15-sample ware resistant to amoxicillin, 12 ampicillin, 9 tetracycline with 6 intermediate resistant. On the other hand, 15 samples were sensitive to ciprofloxacin and 13 was gentamycin. The antibiotic sensitivity profile of *Salmonella spp.* isolates presented in **Table 11**. The antibiotic sensitivity profile of *Salmonella spp.* isolates presented in **Fig. 16.** All isolated bacteria showed significantly resistant to amoxicillin (100%) followed by ampicillin (80%), tetracycline (60%). Hundred percent sensitive to ciprofloxacin and 86.67% to gentamycin are found in this study (**Fig. 16)**.

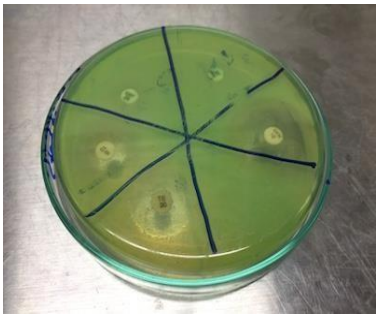

Figure 16: Antibiotic sensitivity test of *Salmonella spp.*

Table 11: Antibiotic sensitivity pattern of *Salmonella spp.* on different antibiotics

	AMX	AMP	CIP	GEN	TE
S	0	0	15	13	0
IN	0	3	0	2	6
R	15	12	0	0	9

Legend: AMX= Amoxicillin, AMP= Ampicillin, CIP= Ciprofloxacin, GEN= Gentamycin, TE= Tetracycline, S=Sensitive, IN= Intermediate, R= Resistant

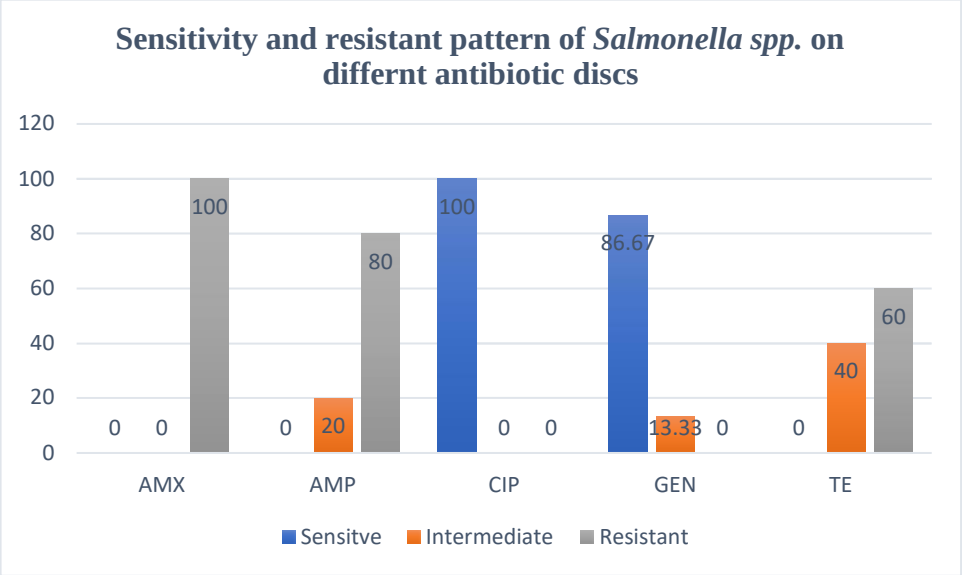

Figure 17: Antibiotic sensitivity pattern of *Salmonella spp.*

This figure represents that 100% isolates were sensitive to ciprofloxacin and 86.67% sensitive to gentamycin with 13.33% intermediate resistant. Amoxicillin was shown 100% resistant followed by ampicillin (80%) and tetracycline (60%) with 40% intermediate in reaction.

4.2 Discussion

The current study was conducted to determine the bacteriological state of sick and dead duck from different village of Savar upazila, as well as the isolation, identification, and characterization of the bacterial flora present in duck liver and intestine, and the public health significance. Cultural examination, morphological studies, staining properties, and biochemical analyses were carried out in the laboratory to characterize the isolated bacteria.

The antibiogram study was carried out on bacterial isolates to investigate their sensitivity and resistance pattern against the most used antibiotics on the market.
E. coli and *Salmonella spp*. were detected in duck liver and intestinal contents on the current investigation, which was supported by Dey *et al.* (2016), Rahman *et al.* (2017), Eid *et al.*, (2019) and Kim *et al*. (2016). In this work, the organism was cultured using several selective and enriched culture media simultaneously. The media utilized in this study were selected considering the experience of the past researcher worked in various fields relevant to the present study by Eid *et al.*, (2019), Kim *et al*., (2016) and Dey *et al.*, (2016).

In this study, colony characteristics of *E. coli* observed in EMB, MC and NA were like the findings of Cheesbrough (2006), Freeman (1985) and Buxton and Fraser (1977). The morphology of the isolated bacteria was Gram negative short rod, grouped in single or paired and motile, as supported by various writers including Britannica (2015), Cowan (1985) and Merchant and Packer (1967).

The *E. coli* isolates showed a complete fermentation of 5 basic sugars by producing both acid and gas which were supported by Merchant and Packer (1967), Freeman (1985) and Cheesbrough (2006). The isolates also revealed positive reaction in MR test and Indole test but negative reaction in VP test Cheesbrough (2006) and Buxton and Fraser (1977).

Salmonella spp. colony features obtained in NA, SS and BG agar were comparable to those observed by Cheesbrough (2006), Freeman (1985) and Buxton and Fraser (1977). In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative small rod arranged in single or paired and motile which was supported by several authors Cheesbrough (2006) Freeman (1985), Merchant and Packer (1967) and Cowan (1985).

Isolates of *Salmonella spp*. was showed a complete fermentation of 5 basic sugars and production of both acid and gas except sucrose and lactose which was supported by Cheesbrough (2006) and Freeman (1985). *Salmonella spp.* shows a positive reaction in MR and Citrate reduction test otherwise it produces a negative reaction on the VP and Indole test, which was supported by Buxton and Fraser (1977), Merchant and Packer (1967).

The total overall prevalence of *E. coli* in duck is 52.5%. Dead duck intestinal content having the greatest prevalence (90%) and live duck liver having the lowest (20%) of *E. coli* prevalence. The study findings were like the findings of Kim *et al.,* (2016), who reported the prevalence of *E. coli* was 91% in duck. On another study of Eid *et al.* (2019) on Egypt found 22.8% prevalence rate on duck sample. This discrepancy could be attributed to differences in season or environmental variation in different study areas.

The overall prevalence of *Salmonella* in duck is (37.5 %), dead bird intestinal content having the greatest prevalence (70%) and live bird liver having the lowest (10%). On another study Rahman *et al.* (2017) found slightly higher prevalence rate (39.1%) of *Salmonella spp.* on Dinajpur district. On previous study, Kim *et al.,* (2016) shows the prevalence of *Salmonella spp.* was 20.75% on Korea, which was lower compared to Savar. This slight difference might be due to variation of season or environmental variation in different study areas or sample handling and processing techniques.

In the present study, it was found that the *E. coli* isolated from duck samples are sensitive to ciprofloxacin (95.2%) followed by gentamycin (66.6%). The results strengthen the earlier observations of Eid *et al.,* (2019), who found around sensitive to ciprofloxacin and Gentamycin. Resistance of *E. coli* was observed against amoxicillin (90.5%), ampicillin (80.9%) and tetracycline (80.9%). The result was supported by Eid *et al.,* (2019), who found resistant to ampicillin and amoxicillin.

In the current investigation, it was found that the *Salmonella spp.* isolated from duck sample are sensitive to ciprofloxacin (100%) followed by gentamycin (86.67%). The results strengthen the earlier observations of Rahman *et al.* (2017) and Eid *et al.* (2019), they both found both the ciprofloxacin and gentamycin are sensitive to *Salmonella spp*. Resistance of *Salmonella spp.* was observed against amoxicillin (100%), ampicillin (80%) and tetracycline (60%) with 40% intermediate resistant. The results strengthen the earlier observation of Eid *et al.,* (2019) found 71.5% ampicillin and amoxicillin resistant on duck sample.

The presence of such characteristics in *E. coli* and *Salmonella spp.* isolates indicates that the organisms may have acquired resistance due to the indiscriminate use of antibiotics. The presence of isolated should be considered detrimental to health, and risk factors should be avoided. However, in the current investigation, ciprofloxacin and gentamycin was found to

be the most efficient antibiotic for treating *E. coli* and *Salmonella spp.* infection. Still, ciprofloxacin and gentamycin are best for the treatment of both bacterial infection, but they may get resistance in near future.

CHAPTER 5

SUMMARY AND CONCLUSION

The current study was conducted from January to June 2021 to determine the bacteriological state, prevalence rate, and antibiotic sensitivity pattern of zoonotic *E. coli* and *Salmonella spp*. bacteria of dead and live duck from different villages of Savar upazila Dhaka, Bangladesh. For this purpose, a total of 40 samples were collected from 5 different villages to suggest public health importance based on present hygienic condition, prevalence rate and antibiotic sensitivity pattern of bacteria isolated from duck samples.

In the present study, a total of 40 samples were collected from 5 different villages named Tetuljhora, Gonokpara, Shimulia Bazar, Noihati and Bagbari at Savar upazila area in Bangladesh. Using standard bacteriological technique among 40 samples, 21 samples were found positive for *Escherichia coli* and 15 samples of *Salmonella spp*. The prevalence of *Escherichia coli* and *Salmonella spp.* are 52.5% and 37.5% respectively. The highest prevalence rate of both *E. coli* (90%) and *Salmonella spp.* (80%) found on Dead duck Intestinal Content and the lowest prevalence of both *E. coli* (20%) and *Salmonella spp.* (10%) was found in live duck liver sample.

The isolates of *Escherichia coli* and *Salmonella spp*.were tested for antibiogram against five antibiotics from different groups that are often used in fields and found on the market. Ciprofloxacin and Gentamycin are highly sensitive against *E. coli* and *Salmonella spp.*. All the isolated bacteria were resistant to ampicillin, amoxicillin, and tetracycline.

Based on the outcomes of this study, it may be concluded that:

- *Escherichia coli* and *Salmonella spp.* successfully isolated by different bacteriological media and biochemical tests.
- *E. coli* and *Salmonella spp.* are more prevalent in dead bird intestinal contents compared to others.
- All the identified bacteria were confirmed to be pathogenic to humans or to have zoonotic significance.
- Most of the isolates showed multi-drug resistance, but sensitive to ciprofloxacin and gentamycin, resistant to amoxicillin and ampicillin.

Further research on the following topics could be conducted in relation to the current study:

- Antibiogram analysis using a specific antibiotic resistance gene and a microdilution approach with specific antibiotics.
- Molecular characterization by PCR of those bacteria.
- In vivo study of pathogenic effects of each bacterium.
- Notice to higher authorities, policymakers, and consumers about the importance of public health.

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APPENDIX I

COMPOSITION OF DIFFERENT MEDIA

1. Nutrient broth

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

2. Nutrient Agar

Peptone	5.00 gm
Sodium chloride	5.00 gm
HM peptone B#	1.50 gm
Yeast extract	1.50 gm
Agar	15.00 gm
Final pH (at 25°C)	7.4 ± 0.2

3. MacConkey Agar

Peptones (meat and casein)	3.00 gm
Pancreatic digest of gelatin	17.00 gm
Lactose monohydrate	10.00 gm
Bile salts	1.50 gm
Sodium chloride	5.00 gm
Crystal violet	0.001 gm
Neutral red	0.03 gm
Agar	13.50 gm
pH after sterilization(at 25°C)	7.1 ± 0.2

4. Eosin Methylene Blue Agar

Peptic digest of animal tissue	10.00 gm
Dipotassium phosphate	2.00 gm
Lactose	5.00 gm
Sucrose	5.00 gm
Eosin – Y	0.40 gm
Methylene blue	0.065 gm
Agar	13.50 gm
Final pH (at 25°C)	7.2 ± 0.2

5. Brilliant Green Agar Medium

Peptone	5.00 gm
Tryptone	5.00 gm
Yeast extract	3.00 gm
Lactose	10.00 gm
Sucrose	10.00 gm
Sodium chloride	5.00 gm
Phenol red	0.08 gm
Brilliant green	0.0125 gm
Agar	20.00 gm
pH after sterilization (at 25°C)	6.9 ± 0.2 gm

6. Salmonella-Shigella agar

Proteose peptone	5.00 gm
Lactose	10.00 gm
Bile salts mixture	8.50 gm
Sodium citrate	8.50 gm
Sodium thiosulphate	8.50 gm
Ferric citrate	1.00 gm
Brilliant green	0.00033 gm
Neutral red	0.025 gm

Agar	13.50 gm
Final pH (at 25°C)	7.0 ± 0.2

7. Phosphate buffer saline

Sodium chloride	8.00gm
Disodium hydrogen phosphate Potassium chloride	
Potassium hydrogen phosphate	
Distilled water to make	

8. Mueller Hinton Agar

HM infusion B from	300.00 gm
Acicase	17.50 gm
Starch	1.50 gm
Agar	17.00 gm
Final pH (at 25°C)	7.4 ± 0.1

9. Methyl Red Indicator

Methyl red	0.20 gm
Ethyl alcohol	60.00 ml
Distilled water	40.00 ml

10. Voges–Proskauer (MR-VP) broth

Buffered peptone	7.00 ml
Dextrose	5.00 gm
Dipotassium phosphate	5.00 gm
Final pH (at 25°C)	6.9 ± 0.2