Isolation, Identification, and Antibiogram Studies of *Escherichia coli* from Salad Vegetable Samples Sold in Dhaka City

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences

Brac University

February 2023

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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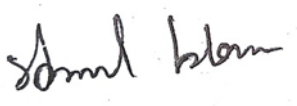

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Approval

The thesis/project titled “Isolation, identification, and antibiogram studies of *Escherichia coli* from salad vegetable samples sold in Dhaka city” submitted by,

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Ethics Statement

For completion of this study, samples from selected venues were collected following all the necessary precautions. All the experiments were done in BRAC University Laboratory. It should also be noted that no animal or human models were used or harmed in this study.

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Abstract/ Executive Summary

The purpose of the study was to investigate the microbial composition of typical salad ingredients and their potential to serve as a source of antibiotic-resistant bacteria. Six different marketplaces in the city of Dhaka were used to gather samples. This study "Isolation, Identification, And Antibiogram Studies of *Escherichia Coli* from Salad Vegetable Samples Sold in Dhaka City" was done with aim of surveying the amount of resistance among strains of *Escherichia coli* isolated via Salad items. In this present study, 200 *E. coli* samples were collected from salad items of six different areas of Dhaka city. These positive 200 samples of *Escherichia coli* were then taken for the antibiotic susceptibility testing which was performed using Kirby-Bauer disk diffusion method. This study shows percentage of sensitivity and resistance among 200 salad samples. Streptomycin (S) 94% and Imipenem (IMP) 97% shows most sensitivity where Amikacin (AMK) 90.5% and Ampicillin 97% shows most resistance among these antibiotics. Number and Percentage of isolate resistant to one antibiotic is 100% where number and Percentage of isolate resistant to more than one antibiotic is 98% among all the 200 samples. From the findings, it is essential to prepare salads according to hygienic standards and that salads may serve as key reservoirs for several antibiotic-resistant bacteria.

Keywords: Antibiotic resistant bacteria, Microbiological quality, Media, Broth, Salad, Water, Antibiogram.

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List of Acronyms

AST	Antibiotic Susceptibility Test
MHA	Muller Hinton Agar
FDA	Food and Drug Administration (Federal agency)
MDR	Multidrug-resistant
CDC	Centers for Disease Control and Prevention (Government agency)
MDRO	Multidrug-resistant Organisms
ECDC	European Centre for Disease Prevention and Control
EMB	Eosin methylene blue

Chapter 1

Introduction

Introduction

A frequent commensal bacterium in the gut is *Escherichia coli*. However, some *E. coli* strains were found to have- mobile genetic elements, such as plasmids, transposons, bacteriophages, and pathogenicity islands and these properties have led to the deleterious pathogenicity of *Escherichia coli* (Sethabutr et al., 1993). It is a significant foodborne pathogen that infects people and causes gastroenteritis. Since 1885, *E. coli* has been acknowledged both as a diverse pathogen and a benign commensal (Bower et al., 1999). The fecal-oral route is how *Escherichia coli* is transferred. It is very frequently found in water, soil, and food due to its flexibility and versatility (Kljujev I et al., 2012).

The risk of bacterial contamination increases when raw manure is used as fertilizer. The United States, Germany, and other nations consider *Escherichia coli* to be a significant foodborne pathogen since it can cause human gastroenteritis (Luna-Gierke et al., 2014; Buchholz et al., 2011; Lee et al., 2012). Statistics on food poisoning show that from 2008 to 2011, pathogenic strains of *E. coli* were responsible for 10.2–16.2% of all outbreaks in Korea (KFDA, 2012). Meldrum et al., (2009) reported that two large outbreaks in the United Kingdom demonstrated the significant health problems that could arise from consumption of contaminated salads. The most recent romaine lettuce outbreak occurred in Germany in 2011, where enterohaemorrhagic *E. coli* (EHEC) infection claimed the lives of 14 people.

When it comes to *E. coli* levels in common market-sold items, such as salad vegetables, the majority of developing nations, particularly Bangladesh, often lack effective control and inspection. Given the general lack of knowledge about this issue, this might be harmful. Some researchers propose that, fresh fruits and vegetables included pathogenic *E. coli* (Hong et al., 2012). Salad is a dish that often consists of raw leafy green vegetables that have been mixed with additional raw or cooked vegetables, fruit, cheese, or other components.

Salads are a common addition to meals and snacks in Bangladesh. However, it has been said that food and water, in particular, serve as vectors for the spread of microbial infections, including those brought on by coliforms (Ifediora et al., 2006). *Salmonellae, Shigellae*, and enteropathogenic *Escherichia coli* are a few noteworthy enteric pathogens among the coliforms. In terms of human health and illness, *Escherichia coli O157:H7* and *Salmonella spp.* are the most harmful food-borne bacterial pathogens (Olsen et al. 2000). As a result, research should be done to determine the presence of pathogenic *E. coli* in salad fruits and vegetables

offered in Dhaka's traditional markets and grocery shops, as well as the extent of this contamination.

Antibiotic-resistant pathogenic bacteria will be prevented and spread using the same techniques that are used to prevent and control the transmission of pathogenic bacteria through food. Additional controls for antimicrobial resistant bacteria may be required because antimicrobial resistance in foodborne pathogens and commensals poses a distinct public health risk. In order to reduce morbidity, death, and the financial impact of bacterial infections, antibiotics have long been employed in human and veterinary medicine.

The public health community is now concerned because *E. coli* has become resistant to one or more antibiotics. The widespread and increasing usage of antibiotics is associated with the prevalence of germs that are resistant to treatment. Antimicrobials are utilized in the food production process to treat and prevent diseases in livestock, promote growth, and boost feed efficiency (NARMS, CDC.2022). For instance, feeding animals these antibiotics at low doses over an extended period of time may select for and disseminate antibiotic resistance to other bacteria in the food chain (Lima et al., 2017).

However, plant-based foods, especially salads, constitute a major factor in the spread of antibiotic resistance and are a growing source of concern. Major public health concerns have been raised by the isolation of multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL) generating *E. coli* from raw meat, vegetable salad, egg surface, unpasteurized milk, raw fish, and water (shivakumar et al., 2021, Silva et al., 2019). Studies on pathogenic *E. coli* serotypes in RTE meals must be continued to assure total food safety.

Numerous foods, especially those with animal origins and those exposed to sewage pollution, have been identified as vectors for the transmission of infections to people (“Microorganisms in foods,”2006). Because drug-resistant strains of *E. coli* are becoming more common, treating infections with them has become more challenging globally. The health of consumers is seriously threatened by the growing resistance identified in *E. coli* strains to the majority of antibiotics (Trojan et al., 2016).

In order to effectively assess the risk of foodborne outbreaks, the current study set out to determine the prevalence of pathogenic *E. coli* in fresh vegetable products that are commercially available in Dhaka, such as vegetable salad mix, sprouts, baby leaf vegetables, and unpasteurized fruit and vegetable juices. It also sought to characterize any pathogenic *E. coli* by virulence groups. The objective of this study was- Isolating, identifying, and

characterizing *Escherichia coli* in salad products in Dhaka City, Bangladesh. Nevertheless, *E. coli* was isolated from different salad items was also tested for antimicrobial sensitivity using 11 regularly used antibiotics.

1.1 Antibiotics and Antibiotic Resistance

The development of antibiotic resistance in hospitals, communities, and the environment in tandem with their usage has long been hailed as one of the greatest scientific discoveries of the 20th century. The extraordinary genetic abilities of microbes have benefited from man's overuse of antibiotics, allowing them to exploit every potential source of resistance gene and every method of horizontal gene transfer to develop multiple mechanisms of resistance for each and every antibiotic that has been used in clinical settings, in agriculture, or in any other context.

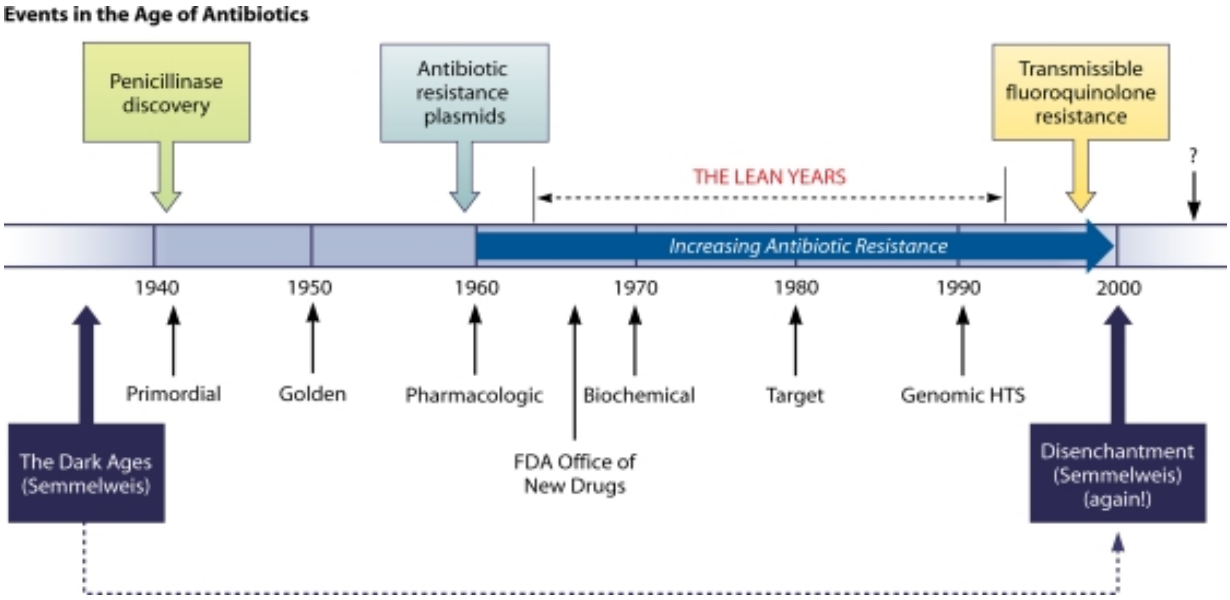

Figure 1.1: History of antibiotic discovery and development of antibiotic resistance

This timeline displays the first year of reported antimicrobial resistance history, as well as the year that various antibiotics were first introduced (Figure 1). The dark ages when no antibiotics was discovered, the golden era when most of the antibiotics that are currently in use was identified and the lean years depict that the discovery of potential antimicrobial agents are declining yet the number of resistance development is on the rise (Figure 1).

Now, different antibiotics have different mode of actions and this variation leads to the emergence of different types of antimicrobial resistance mechanisms in bacteria. Albeit, the antibiotic was initially synthesized by analyzing the bacterial pathogenesis. Some common modes of antibiotics are- antibiotic target alteration, antibiotic target replacement, antibiotic efflux pump, et cetera (Table 1).

Table 1.1: Modes of action and resistance mechanisms of commonly used antibiotics

Antibiotic class	Example(s)	Target	Mode(s) of resistance
β-Lactams	Penicillins (ampicillin), cephalosporins (cephamycin), penems (meropenem), monobactams (aztreonam)	Peptidoglycan biosynthesis	Hydrolysis, efflux, altered target
Aminoglycosides	Gentamicin, streptomycin, spectinomycin	Translation	Phosphorylation, acetylation, nucleotidylation, efflux, altered target
Glycopeptides	Vancomycin, teicoplanin	Peptidoglycan biosynthesis	Reprogramming peptidoglycan biosynthesis
Tetracyclines	Minocycline, tigecycline	Translation	Monooxygenation, efflux, altered target
Macrolides	Erythromycin, azithromycin	Translation	Hydrolysis, glycosylation, phosphorylation, efflux, altered target
Lincosamides	Clindamycin	Translation	Nucleotidylation, efflux, altered target

Antibiotic class	Example(s)	Target	Mode(s) of resistance
Streptogramins	Synercid	Translation	C-O lyase (type B streptogramins), acetylation (type A streptogramins), efflux, altered target
Oxazolidinones	Linezolid	Translation	Efflux, altered target
Phenicols	Chloramphenicol	Translation	Acetylation, efflux, altered target
Quinolones	Ciprofloxacin	DNA replication	Acetylation, efflux, altered target
Pyrimidines	Trimethoprim	C_1 metabolism	Efflux, altered target
Sulfonamides	Sulfamethoxazole	C_1 metabolism	Efflux, altered target
Rifamycins	Rifampin	Transcription	ADP-ribosylation, efflux, altered target
Lipopeptides	Daptomycin	Cell membrane	Altered target
Cationic peptides	Colistin	Cell membrane	Altered target, efflux

INHIBIT		CLASIFICATION		ANTIBIOTICS			
		Beta-Lactamase inhib.	Sulbactam	Clavulanic Acid	Tazobactam	Avibactam	
		Penicillins	***Penicinillase – Sensible***				
			Aminopenicillins (broad spectrum)	Ampicillin Amoxicillin			
			Natural Penicillins (narrow spectrum)	Penicillin G: Na, K, Procainic, Benzathine (IV, IM) Penicillin VK: VO			
			Penicinillase – Resistant *(very narrow spectrum)*				
			Nafcillin	Oxacillin	Dicloxacillin		
			Antipseudomonal *(extended spectrum)*				
			Carboxipenicillins	Ticarcillin	Carbenicillin		
			Ureidopenicillins	Piperacillin	Azlocillin	Mezlocillin	
		Cephalosporins	1° Generation	Cephalexine	Cefazolin	Cefadroxil	
				Cephradrine			
			2° Generation	Cefuroxime	Cefprozil	Cefaclor	
				Cefoxitin	Cefotetan	Loracarbef	
			3° Generation	Cefoperazone	Ceftriaxone	Cefixime	
				Cefpodoxime	Ceftizoxime	Cefotaxime	
				Cefdinir	Ceftibuten	Ceftazidime	
				Cefditoren			
			4° Generation	Cefepime	Cefpirome *		
			5° Generation	Ceftaroline	Ceftolozane		
		Carbapenems	Meropenem	Ertapenem	Doripenem	Imipenem + Cylastatine	
		Monobactams	Aztreonam				
	No lactam	***Glycopeptides***	Vancomycin	Telavancin	Dalbavancin	Oritavancin	
		Other	Colistin	Polymyxin B	Daptomycin	Isoniazid	
Protein Synthesis	***30S***	***Amino-glycosides***	Amikacin	Gentamicin	Tobramycin		
			Streptomycin	Neomycin			
		Tetracyclins	Doxycycline	Minocycline	Tigecyclin		
			Tetracyclin	Demeclocylin*			
	50S	***Oxazolidonones***	Linezolid	Tidezolid			
		Streptogramins	Quinupristin/Dalfopristin				
		Cloramphenicol					
		Macrolides	Erythromycin	Azithromycin	Clarithromycin		
		Lincosamides	Clindamycin	Lincomycin			
DNA topoisomerases		***Fluorquinolones***	Ciprofloxacin	Moxifloxacin	Levofloxacin	Gemifloxacin	
			Norfloxacin	Ofloxacin	Enofloxacin	Sparfloxacin	
		Quinolones	Nalidixic Acid				
Folic Acid Synthesis		***Sulfonamides***	Sulfamethoxazole (SMX)	Ag Sulfadiazine	Sulfasalazine	Sulfisoxazole	
		DHFR inhibitors	Trimethoprim (TMP)	Pyrimethamine			
DNA (damage)		***Nitroimidazoles***	Metronidazole	Tinidazole			
mRNA synth.		Rifampim					

Figure 1.2: Classification of antibiotics

Similar group of antibiotics are classified into an antibiotic group based on their functionalities. Some antibiotics confer resistance by inhibiting protein synthesis, inhibiting folic acid synthesis, damaging DNA, inhibiting mRNA synthesis, et cetera (Figure 1.2).

Antibiotic Resistance: One of the most important developments in medical history is the creation of antibacterial agents. Before it was known that diseases were brought on by bacteria, viruses, fungi, or prions, the word "antibiotic"—which is derived from the Greek words anti- (against) and bios (life)—was used to describe drugs that treat infections. As a result, a medicine known as an antibiotic was thought to be something that could destroy any living thing. Antimicrobial resistance occurs when bacteria, fungi, and other microorganisms learn to resist the medications meant to kill them. That implies that the germs survive and develop. Treatment for resistant infections can be challenging and perhaps impossible.

Antimicrobial resistance poses a serious danger to global public health, causing at least 1.27 million deaths globally and approximately 5 million fatalities in 2019. Each year, more than 2.8 million illnesses in the US are resistant to antibiotics. The 2019 Antibiotic Resistance (AR) Threats Report from the CDC estimates that more than 35,000 people pass away as a result.

Multi-drug resistance: Multidrug resistance is a result of the use of antimicrobial drugs as growth promoters in animal feeds intended for human consumption, mutations, the acquisition of new genetic material, exposure to cells with new genetic material, and other factors that favor antimicrobial resistance. However, improper usage of antimicrobial agents has resulted in the post-antibiotic era, which is a reality in many parts of the world today.

In literal terms, Multidrug resistance (MDR) means ‘resistant to more than one antimicrobial agent'. Multidrug-resistant organisms (MDRO) are predominantly bacteria, that are resistant to one or more classes of antimicrobial agents (Tenney J., 2018). It is commonly known that *Escherichia coli (E. coli)*, a prevalent food-borne pathogen, is MDR and has fluoroquinolone resistance. Similar to this, a recent study showed that *E. coli* isolates isolated from urine samples had high levels of resistance to -lactam and fluoroquinolone drugs (Ray J., 2015). The selective pressure of antibiotics, which is heightened by their excessive or improper usage, is one of the primary risk factors linked to the emergence of multidrug-resistant (MDR) bacteria (Concia et al.,2017).

1.2 *Escherichia coli*

The gram-negative, rod-shaped, facultatively anaerobic bacteria *Escherichia coli* lives in the digestive tracts of warm-blooded animals. Although most strains of coli are benign, some of them are pathogenic to humans and other animals. Some *E. coli* strains that are resistant to a broad range of antimicrobial drugs have been reported to have emerged and spread widely in recent years (Figure 3) (Wright, Bartoloni and Sahm DF et al., 2001).

Since there are fewer, or often no, effective antimicrobial medicines available for illnesses caused by these bacteria, the emergence of resistance to numerous antimicrobial drugs in pathogenic bacteria has grown to be a serious concern to public health. (Oteo et al., 2001). Multi-drug resistant (MDR) is defined as being non-susceptible to at least one agent in three or more antimicrobial categories by the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC) respectively4. MDR bacteria are the main reason why treatments for infectious diseases fail, increasing the length and severity of morbidity, and increasing the risk of death (Howard DH et al., 2003).

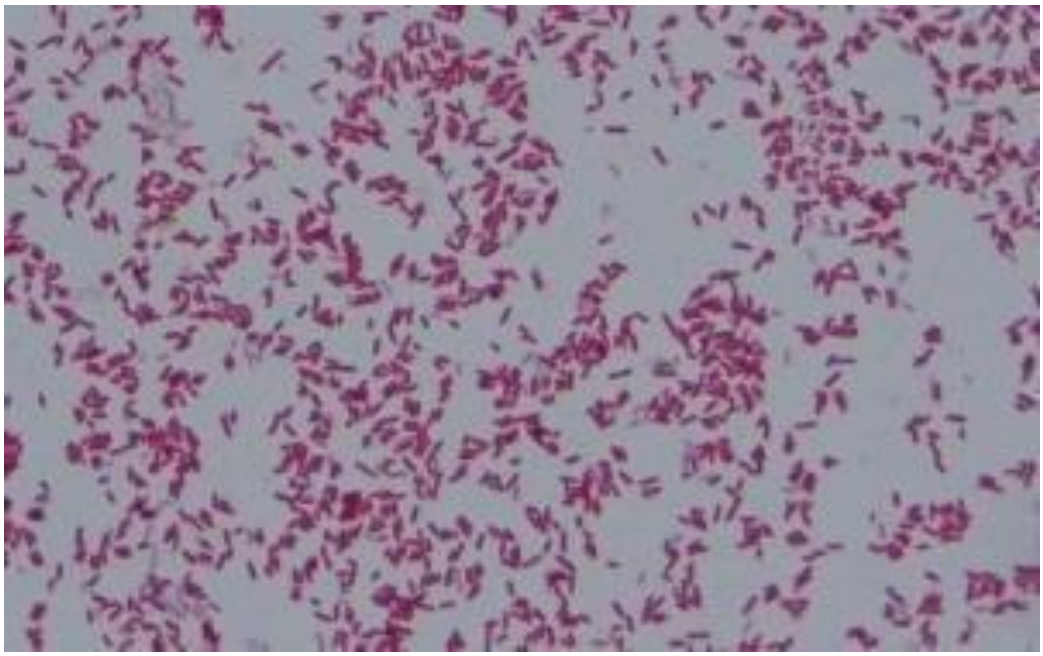

Figure 1.3: Gram negative, pink colored, small rod shape *E. coli* under light microscope. (100x)

As opportunistic infections, *E. coli* from the normal intestinal flora often pose no threat to the host. They only pose a threat to regular people on very rare occasions. This primarily occurs in people whose immune systems are compromised and are unable to keep these commensals in check in their natural environment, or after a traumatic breach of the natural barriers separating

the gut from other ordinarily sterile parts of the body or following surgical procedures. When the host's local defenses are compromised by primary pathogens, they can also be a component of mixed infections.

Both humans and animals can spread the harmful *E. coli* bacteria. These are the principal methods of spread, a) eating infected, uncooked fruits and vegetables eating undercooked or raw meat, b) consumption of unpasteurized milk, c) consuming or swimming in tainted water, d) touch with an unclean person who doesn't routinely wash their hands, e) contact with infectious animals.

***E. coli* infections:** Human illnesses caused predominantly by *E. coli* include intestinal disorders, newborn meningitis, and urinary tract infections. These circumstances depend on a particular set of harmful (virulence) characteristics that the organism possesses.

Chapter 2
Materials and Method

2.1 Sample Collection:

Study area: In order to complete this thesis, six distinct places in the Dhaka area were examined. All samples were taken outside in various Dhaka regions. The laboratory study was done at BRAC University's Microbiology Research Laboratory, which is part of the Department of Mathematics and Natural Sciences.

Study period: Between November 2022 and February 2023, a total of 60 salad vegetable samples were gathered from street sellers, grocery stores, and traditional markets throughout Dhaka city.

Table 2.1: Names of the gathered vegetables sample

Common Name	Scientific Name
Tomato	*Lycopersican esculentum*
Carrot	*Daucas carota*
Capsicum	*Capsicum Fruits scence*
Cucumber	*Cucumis sativas*
Coriander	*Coriandrum sativum*
Lettuce	*Lactuca sativa*
Mint (Pudina)	*Mentha arvensis*
Green chilli	*Capsicum annuum*
Cabbage	*Brassica oleracea var. capitata*
Spring onion	*Allium fistulosum*

The number of samples per area: 10 of these commonly used salad vegetable samples were collected from each area of Dhaka city by visiting multiple stores per area.

Raw vegetable samples were collected in the sterile sample collection polyethene bags from different sources of a certain area.

Culture media:

Sample collection on EC broth: EC Broth is a selective medium for the differentiation of fecal coliforms and the confirmatory test for *Escherichia coli* from food and environmental samples. Collected raw salad samples were poured into EC broth medium in sterile conical flasks and put in the shaker incubator for 24 hours at 37°C for bacterial enrichment.

Sample collection on MacConkey Agar: A selective and differential culture medium, MacConkey also functions as an indicator. The MacConkey medium is made to separate Gram-negative and enteric bacilli based on how they ferment lactose. It has bile salts and crystal violet, which prevent the growth of other gram-positive bacteria while promoting the selection and expansion of gram-negative bacteria that can ferment lactose.

Figure 2.1: *E coli* colonies on MAC media

Isolation of *Escherichia coli* from salad samples: The collected samples then processed through serial dilution and spread as quickly as possible on the surface of the plate in MacConkey agar (MAC) media with a sterile spreader. The plates were kept in an incubator at 37°C for 24 h. followed by 24-hour incubation. Then the plates were observed for single colony.

Collection of samples on Eosin Methyl Blue Agar: Eosin Methylene Blue (EMB) agar is a differential microbiological medium that gives a color indicator to distinguish between organisms that digest lactose (like *E. coli*) and those that do not. It somewhat limits the development of Gram-positive bacteria (e.g., Salmonella, Shigella). Holt-Harris, Teague, and Levine are credited with creating the original EMB agar, which they later modified. As a result, it combines the Levine, Holt-Harris, and Teague formulae. It comprises two carbohydrates in addition to the phosphate and peptic digest of animal tissue described by Holt-Harris and Teague. In medical labs, the medium is crucial for quickly differentiating gram-negative harmful microorganisms.

Identification of suspected *Escherichia coli* isolates: Also, for identification those colonies were inoculated Eosin methylene blue (EMB) is a selective stain for Gram-negative bacteria. EMB contains dyes that are toxic to Gram-positive bacteria. EMB is the selective and differential medium for coliforms. After incubation for another 24 hours at 37°C the *Escherichia coli* colonies were black colonies with a metallic green sheen caused by large quantities of acid that is produced and that precipitates out the dyes onto the growth surface (Berry C.W. et al., 1984). The bacterial isolates were then identified following standard microbiological procedures based on cultural, morphological and biochemical characteristics

***E coli* Confirmatory PCR**:

DNA Extraction: With little modification, crude DNA was extracted from the isolates using the boiling method (Queipo-Ortun et al., 2008). The organisms were briefly grown at 37°C on EMB agar overnight.

A medium-sized colony was taken using sterile tips for incubation and combined with 200 l of deionized water. The combination was next boiled for 10 minutes in boiling water, after which it was submerged in ice for 10 minutes, and finally centrifuged at 10,000 rpm for 10 minutes. DNA-containing supernatant was collected. Before use, the DNA sample was stored at -20°C.

Using primers specific to the *E. coli* 16S rRNA gene, the isolated organisms' preliminary identification as *E. coli* were verified. With a minor adjustment, the PCR was carried out in accordance with the method outlined by Schippa et al., 2010.

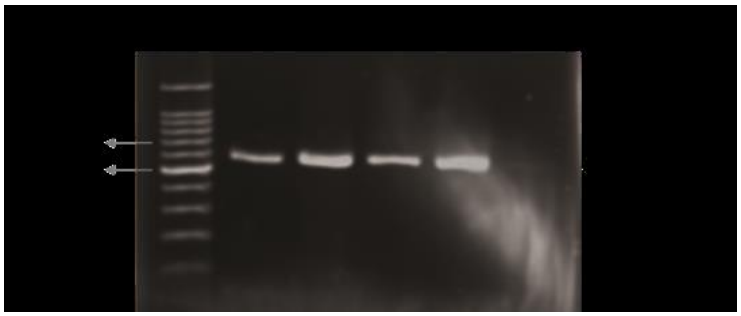

Fig 2.2 Representative photograph of the PCR of E. coli using the primer ECO-1 and ECO-2 targeting 16S rRNA gene. M = 100 bp DNA Ladder, PC = Positive control, T1-T3 = Test samples, NC = Negative control.

Table 2.2: Confirmatory PCR details

Target Gene	Primer name	Sequence	PCR condition	Product size	Reference
16SrRNA gene	ECO-1	GACCTCGGTTTAGT TCACAGA	• 25µl reaction containing PCR master mix. • 10pmol of primer was prepared. After initial incubation at 95°C for 3 min • 30-cycle amplification protocol was followed as 94°C for 45s, 58°C for 45 s and 72°C for 60 s, and a final extension step of 72°C for 3 min.	585bp	Schippa et al., 2010
	ECO-2	CACACGCTGACGCT GACCA			

Electrophoresis of the PCR products was done using 2% agarose gel. After electrophoresis, the gel was stained for 10 minutes in ethidium bromide for visualization.

Microbial culture of the samples: all the samples were incubated for 24 hours at 37°C for growth. The next day after incubation selective colonies were taken from the media and inoculated on nutrient agar and preserved for further purpose.

2.3 Antibiotic susceptibility testing (AST)

Disk Diffusion Method: For the salad samples, Kirby-Bauer disk diffusion method was used to determine the antimicrobial specialized susceptibility profiles of the *E. coli* isolates. These antimicrobial specialists were chosen due to their importance in treating human infections and their ability to portray different antimicrobial operator classes with a range of perspectives.

Muller-Hinton Agar: Mueller-Hinton agar has been employed for a variety of antimicrobial activity tests for ages. mostly due to the medium's non-selective and non-differential nature. This implies that nearly every creature plated on this surface will grow. It has starch in it. Toxins generated by bacteria are known to be absorbed by starch, preventing them from interfering with antibiotics.

Additionally, it controls how quickly the medicines diffuse through the agar. It is a granular agar. Unlike most other plates, this enables better antibiotic diffusion. A more appropriate zone of inhibition results from improved diffusion. For susceptibility testing, MHA has acceptable

batch-to-batch repeatability. Thymidine and thymine, which are inhibitors of sulfonamide, trimethoprim, and tetracycline, are in low concentration in MHA.

Inoculation of the Muller Hinton Agar (MHA) plates: The test organisms were extracted from a 24-hour nutrient media culture plate, and autoclaved cotton swabs were then dipped into the bacterial suspensions. To remove any extra fluid after the dip, the swab was pushed on the test tube wall. The next step was to repeatedly swipe the swab at various angles to ensure that the bacteria were distributed evenly. To remove any extra liquids, the swab was used to clean the plate's rim. Before adding the antibiotic, the plate was given time to soak in the suspension.

Using tweezers, the discs were carefully positioned. The discs were gently placed on the lawn and then pressed against it, ensuring total contact with the lawn. The tweezers were first dipped in alcohol and then burned. •Once all the discs were inserted, the plate was covered by the lid, not inverted, and the forty lawns were constructed and named properly. After that, the plates were transported for a 24-hour incubation period at 37 degrees Celsius.

Measurement of the zone of inhibition: Bacterial growth around each disc was seen after incubation. If a particular antibiotic is effective against the test organism, a region of "no growth" will be seen. After 18 to 24 hours of incubation, the plates were measured to determine the widths (in millimeters) of the acceptable development restraint zones surrounding the antimicrobial disks. The zone of inhibition was calculated using a standard ruler (Clinical and Laboratory Standards Institute, 1997, 1999).

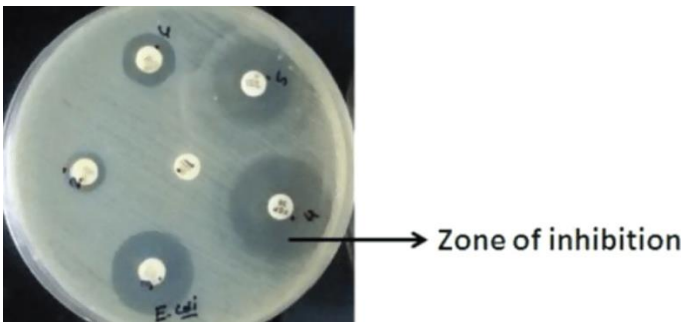

Figure 2.3: Zone of inhibition on MHA plate

It was determined whether a condition was resistant, intermediate, or susceptible using the understanding table (supplied by Oxoid Limited, England). The National Antimicrobial Resistance Monitoring System recommended using break points to determine if *E. coli* was resistant or susceptible. The data were organized in a Microsoft Excel document for making graphs and charts to visualize the results in more of a factual manner.

Table 2.3: Concentrations and diffusion zones of the antibiotics tested in this study.

Antibiotic	Disk Concentration	Diffusion zones (mm)		
		Resistant	Intermediate	Sensitive
Ampicillin (AMP)	10 µg/disk	≤13	14-16	≥17
Ciprofloxacin (CIP)	5 µg/disk	≤15	16-20	≥21
Amikacin (AMK)	30 µg/disk	≤14	15-16	≥17
Gentamycin (GEN)	10 µg/disk	≤12	13-14	≥15
Kanamycin (K)	30 µg/disk	≤13	14-17	≥18
Streptomycin (S)	10 µg/disk	≤11	12-14	≥15
Erythromycin (E)	15 µg/disk	≤13	14-22	≥23
Chloramphenicol (CL)	30 µg/disk	≤12	13-17	≥18
Tetracycline (TE)	30 µg/disk	≤11	12-14	≥15
Imipenem (IMP)	10 µg/disk	≤13	14-15	≥16
Meropenem (MRP)	10 µg/disk	≤19	20-22	≥23

Different antibiotics have different zones of antibiotic diffusion. Based on the diffusion information measured using a millimeter scale- resistant, intermediate and sensitive are classified (Table 2.2).

The workflow of the methodology as follows.

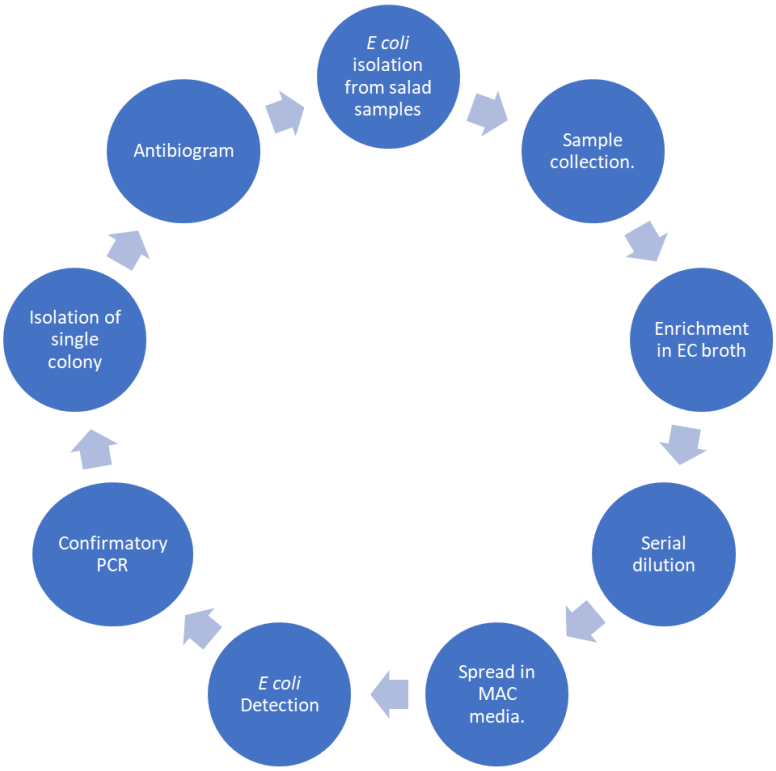

Figure 2.4: Workflow of methodology

Here (Figure 4), *E. coli* sample was collected from different salad vegetables and different methods and media was used to achieve the antibiogram data on the analyzed samples. Firstly, the bacteria present in the samples were allowed to grow in the media and target organism *E. coli* was detected based on the aforementioned criteria. Singe colonies of the target bacteria was used for antibiogram and the antibiogram data have been discussed in the later.

Chapter 3

Results & Discussion

Antibiotic susceptibility test

Antibiogram of *E. coli* strains from salad samples collected from six distinct areas of Dhaka city. Total 200 strains of *E. coli* were tested for antibiotic susceptibility. The number and percentages of resistance and sensitivity are presented below.

Table 3.1: Antimicrobial susceptibility results for 200 *E. coli* isolates from salad samples,

Antibiotic	Sensitive	Percentage	Intermediate	Percentage	Resistant	Percentage
Ampicillin (AMP)	1	0.5%	4	2%	195	97.5%
Ciprofloxacin (CIP)	153	76.5%	16	8%	31	15.5%
Amikacin (AMK)	0	0	19	9.5%	181	90.5%
Gentamycin (GEN)	154	77%	15	7.5%	31	15.5%
Kanamycin (K)	8	4%	124	62%	68	34%
Streptomycin (S)	191	95.5%	3	1.5%	6	3%
Erythromycin (E)	172	86%	10	5%	18	9%
Chloramphenicol (CL)	177	88.5%	11	5.5%	12	6%
Colistin (C)	128	64%	57	28.5%	25	12.5%
Tetracycline (TE)	173	86.5%	17	8.5%	10	5%
Imipenem (IMP)	194	97%	3	1.5%	3	1.5%
Meropenem (MRP)	175	87.5%	24	12%	1	0.5%

According to the table Ampicillin shows 195 or 97.5% resistant cases among 300 patients which is the highest among all the antibiotics. Amikacin and Kanamycin also shows most resistant among other antibiotics that were used.

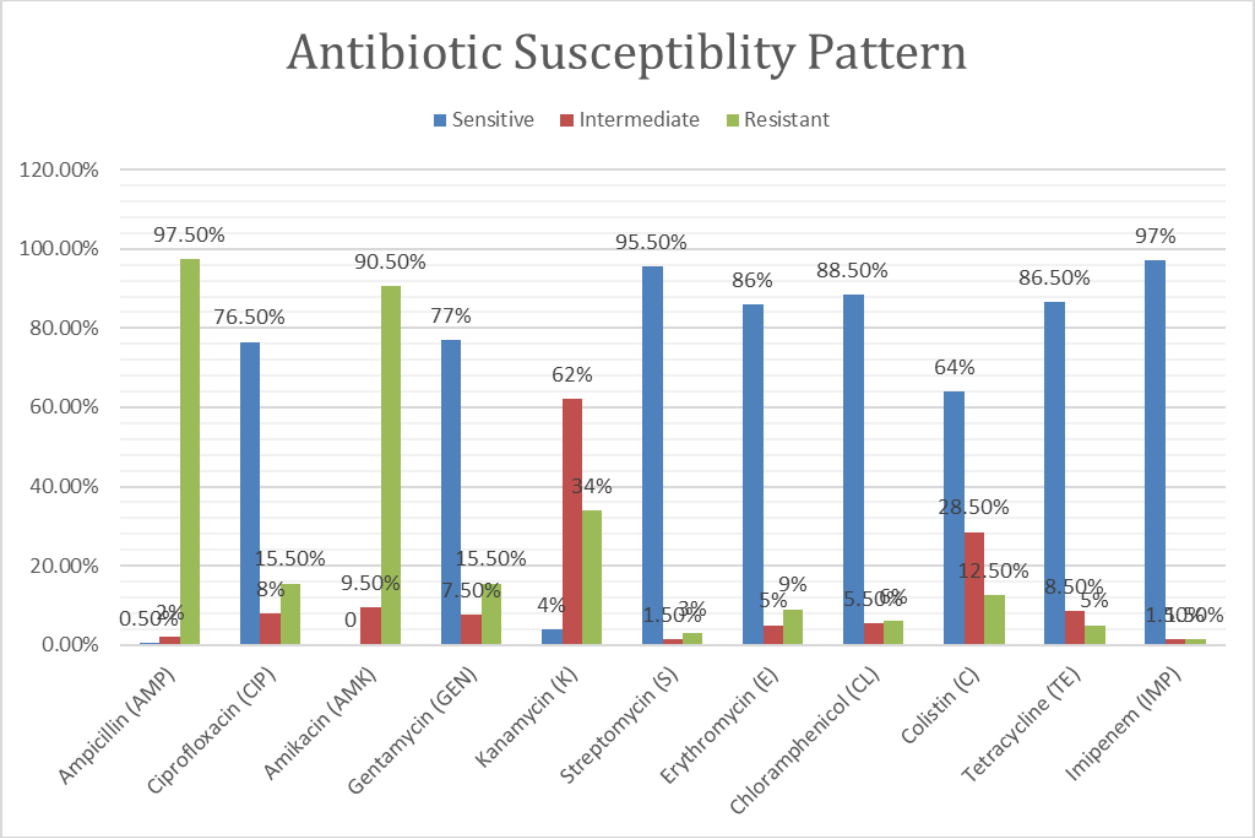

Figure 3.1: Antibiotic susceptibility pattern of 300 *E. coli* isolates from Salad samples

This chart shows percentage of sensitivity and resistance among 200 salad samples. Streptomycin (S) 94% and Imipenem (IMP) 97% shows most sensitivity where Amikacin (AMK) 90.5% and Ampicillin 97% shows most resistance among these antibiotics. Most of the other antibiotics are relatively sensitive.

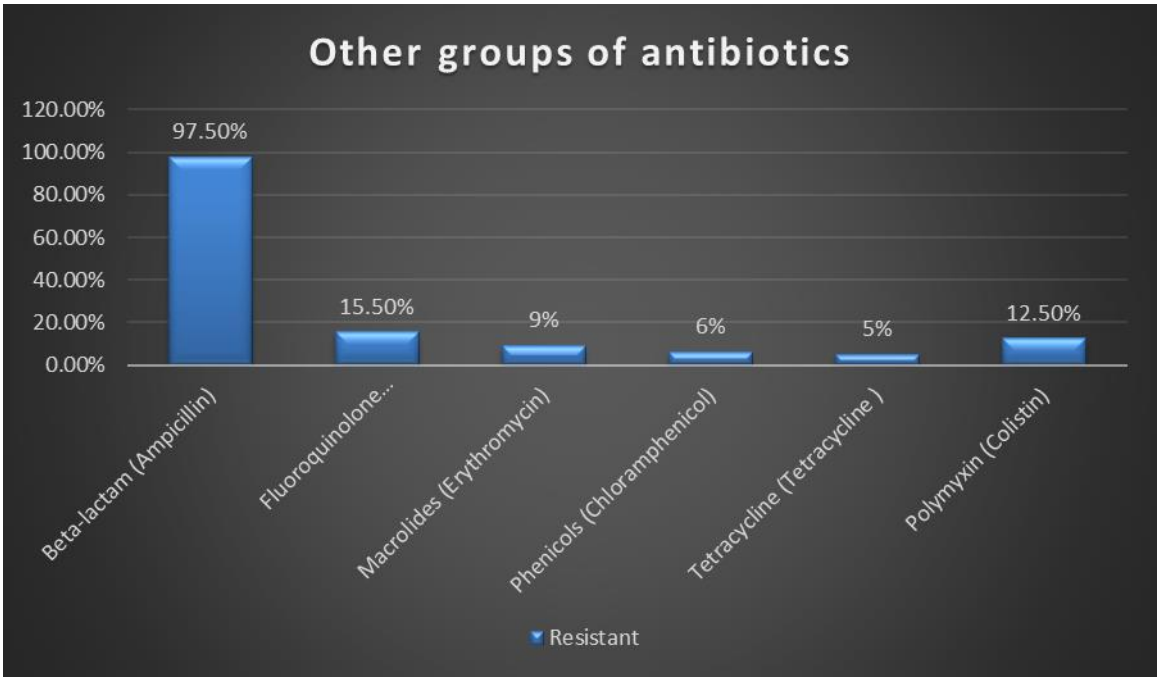

Figure 3.2: Resistance pattern of the groups of Antibiotics

This graph shows how the groups of antibiotics are resistant among the selected antibiotics that were choose for this experiment. The purpose of this chart was to find out the Multidrug resistance among the isolated strains. Acquired resistance to at least one agent in three or more antimicrobial categories is known as multidrug resistance (MDR). Bacterial isolates that are extensively drug resistant (XDR) are only susceptible to one or two antimicrobial categories, and are therefore no susceptible to at least one agent in all but two or fewer antimicrobial categories.

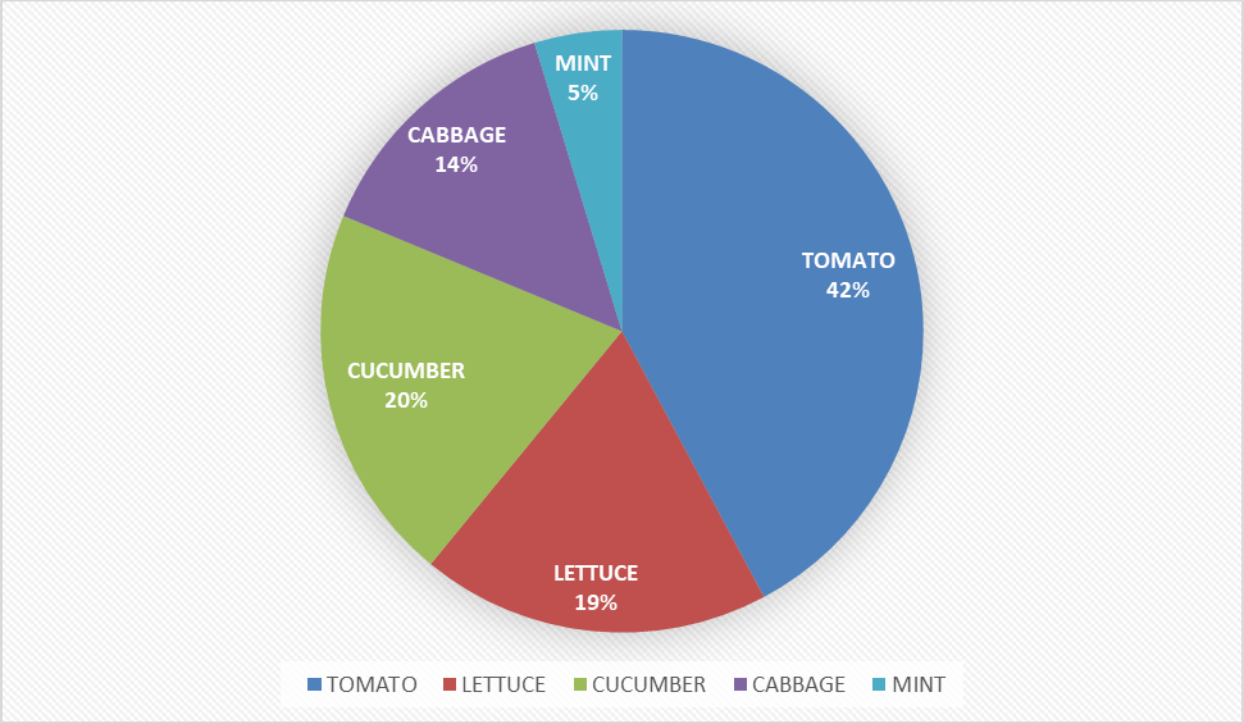

Figure 3.3: Resistance pattern among the samples

Here is showcases the resistance pattern among the ten vegetable samples that were gathered. Out of those ten samples these 5 samples showed most resistance. Most was Tomato followed by cucumber, lettuce, cabbage and mint. These all are part of ready to eat food items that we consume almost on daily basis without or less processing or cooking.

Discussion:

The accessibility of these typical salad sample ingredients is crucial to city inhabitants' daily lives. Foods, if processed without according to hygienic standards, they can transmit a range of infections and cause a variety of food-borne illnesses, especially with samples. Public health is significantly impacted by unsafe and unhygienic food handling, which leads to a range of chronic and non-chronic illnesses. Due to a lack of understanding, awareness, and adherence to the food rules, food contamination and food-borne illnesses are quite prevalent in Bangladesh.

In the results we can see that almost all the areas of Dhaka city except had salad items that are multidrug resistant. This means the antimicrobial resistance is far heavier already than we can imagine. Increased antibiotic resistance among *E. coli* isolates is demonstrated by the high rate of resistance to several antibiotics. The growth of multidrug resistance in *E. coli* has brought attention to the need to increase public awareness, educate doctors and veterinarians, and take the necessary steps to reduce antibiotic use without restriction.

This experiment also in an ongoing process, meaning there are further more studies to be done with these samples. For instance, pathogenicity detection and pathogenic strains identification are to be decided after more steps of the experiment.

Conclusion:

Increased antibiotic resistance among *E. coli* isolates is demonstrated by the high rate of resistance to several antibiotics. The growth of multidrug resistance in *E. coli* has brought attention to the need to increase public awareness, educate doctors and veterinarians, and take the necessary steps to reduce antibiotic use without restriction.

According to the report, salad food products are a severe problem for public health. Public food establishments must enhance hygienic and good manufacturing practices in order to reach a safer level of *E. coli* in salad items sold for human consumption. Additionally, the MDR *E. coli* found in these items pose grave risks to public health.

To conclude I would like to add that case studies like this happen regularly on developed countries like US or UK and that’s why we see most outbreaks like this from that regions. So in future if our country can have more surveys like this we may get to know about similar or more severe outbreaks that are already happening,

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Appendix A.

Composition of the media used:

MacConkey agar

Bacto peptone 17.0 g

Protease peptone 3.0 g

Lactose 10.0 g

Bile salt 1.5 g

NaCl 5.0 g

Agar 15.0 g

Neutral red 0.03 g

Crystal violet 0.001 g

Distilled water 1000 ml

PH 7.2

Sterilized at 121ºC under 15 lbs/in2 pressure for 15 minutes

Mueller-Hinton Agar

Beef extract 2.0 g

Acid hydrolysate of Casein (technical) 17.5 g

Starch 1.5 g

Agar 17.5 g

Distilled water 1000 ml

pH 7.3

Sterilized at 121ºC under15 lbs/in2 pressure for 15 minutes

APPENDIX B.

Composition of chemicals and reagents

Normal saline

NaCl 1.50g

Distilled water 2000 ml