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

By

Umme Habiba Mishu

ID: 19226011

Rubia Archi Lubna

ID: 19226012

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

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing a 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.

Student's Full Name & Signature:

Umme Habiba Mishu
19226011

Rubia Archi Lubna
19226011

Approval

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

1. Umme Habiba Mishu (19226011)

2. Rubia Archi Lubna (19226012)

of Spring, 2024 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on September 19, 2024.

Examining Committee:

Supervisor:
Fahim Kabir Monjurul Haque, PhD
Associate Professor
Department of Mathematics and Natural Sciences BRAC University

Program Coordinator:_____ Dr. Nadia Sultana Deen
Associate professor
Department of Mathematics and Natural Sciences
BRAC University

Departmental Head:_____ Dr. Firoze H Haque

Professor and chairperson

Department of Mathematics and Natural Sciences
BRAC University.

Abstract/ Executive Summary

Escherichia coli (E.coli) is a gram-negative, rod-shaped bacterium in the family Enterobacteriaceae. *E.coli* is an organism of bacteria that can be identified living in a variety of conditions, such as warm blooded animals' and humans' gastrointestinal tracts, where it is a component of the gut microbiota and frequently released into the environment through wastewater effluent or diarrhea. The most common way to develop *E.coli* is by consuming contaminated food. Therefore, the purpose of the study was to find out the microbial composition of typical salad components and their potential to serve as a source of antibiotic-resistant bacteria. For the collection of samples, six different market places of Dhaka city were focused. This study "Isolation, Identification, and Antibiogram Studies of *E.coli* from Salad Vegetable Samples Sold in Dhaka City" was done with the target to survey the amount of resistance among *Escherichia coli* strains isolated from Salad items. In this present study, 200 *E. coli* samples were collected from salad items of six different areas of Dhaka city. After the extraction of these 200 positive *Escherichia coli* samples, the Kirby-Bauer disk diffusion method had been performed to evaluate the pathogenic organisms that are susceptible to antibiotics. From these 200 samples, the current study explained a combination of susceptibility and resistance. Amikacin (AMK) 90.5% and Ampicillin 97% indicate the greatest amount of resistance between these various types of antibiotics, whether streptomycin (S) 94% and imipenem (IMP) 97% indicate the greatest sensitivity. The fraction of isolated resistance to a single antibiotic is 100%, whilst the amount of isolated resistance to different antibiotics is 98% among these 200 samples. The outcome shows that Salads should always be well cleaned as these salads might be an essential safe place for various types of antibiotic resistant pathogens.

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

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Umme Habiba Mishu
Rubia Archi Lubna

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List of Acronyms

AST= Antibiotic Susceptibility Test

MHA= Muller Hinton Agar

MDR= Multidrug-resistant

CDC= Centers for Disease Control and Prevention (Government agency)

ECDC= European Centre for Disease Prevention and Control

EMB= Eosin methylene blue

Chapter 1

Introduction

Introduction

Escherichia coli (*E. Coli*) is a common resident bacteria in the intestines. Still, it was discovered that certain strains of *Escherichia coli* have mobile genetic components, including plasmids, transposons, bacteriophages, and pathogenicity islands. These characteristics have contributed to the harmful pathogenicity of *Escherichia coli*. (Sethabutr et al., 1993). This pathogen can impact humans and generates gastroenteritis. The majority of *E. coli* strains are safe but a few can cause serious food-borne illnesses. Enteroaggregative (EAEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), and diffuse adherent (DAEC) *E. coli* strains cause gastroenteritis in humans. A complex cell wall surrounds *Escherichia coli* cells, consisting of two concentric lipid bilayers, the outer membrane and the cytoplasmic membrane, with a periplasmic distinction in between. This cell wall provides a variety of purposes including defense, transport, motility, sensing, detoxification, and energy production. In the opinion of Bower et al. (1999), *Escherichia coli* has been identified as a harmless residential bacteria and an assortment of pathogens since 1885. Because of its adaptability and versatility, it can also be found more regularly in water, soil and food (Kljujev I et al., 2012). The bacterial contamination increases when raw feces is used as fertilizer. The US, Germany, and other nations have classified *Escherichia coli* as an essential dangerous pathogen because it can cause gastroenteritis in humans. Two significant epidemics in the United Kingdom, as reported by Meldrum et al. (2009), outlined the potentially dangerous health effects of eating contaminated salads. In 2011, a lettuce-related epidemic developed in Germany which brought about 14 deaths because of an enterohemorrhagic *Escherichia coli* (EHEC) contamination.

In Bangladesh, salads are usually an appetizer or dessert. On the contrary, it has been claimed that certain kinds of microbes, such as coliforms, may spread through food and water especially (Ifediora et al., 2006). The most hazardous food-borne pathogenic bacteria for people's health and sickness are *Salmonella spp*. and *Escherichia coli* O157:H7 (Olsen et al. 2000). Therefore, research must be done to determine whether and how much pathogenic *E. coli* is present in salad fruits and vegetables sold in Dhaka's traditional markets and convenience stores. There is a correlation between the emergence of pathogens that are resistant to drugs and increasing usage of antibiotics. Through the process of making food, antibiotics are utilized to treat and prevent infections in cattle as well as promote development and enhance the efficiency of feed (NARMS, CDC.2022). For example, giving these

medicines to animals repeatedly in very small doses may promote the emergence of antibiotic resistance in other types of microorganisms across the food chain. (Lima et al., 2017). The isolated existence of extended-spectrum (ESBL) and multidrug-resistant (MDR) *E. coli* in raw meat, vegetable salad, egg surfaces, unpasteurized milk, raw fish, and water has caused important healthcare issues to arise. (Shivakumar et al., 2021, Silva et al., 2019).

The current study set out to find out 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, in order to properly evaluate the risk of foodborne outbreaks. Additionally, it aimed to describe all pathogenic *E. coli* according to their virulence groups. This study was out to isolate, identify, and characterize *Escherichia coli* found in salad items in Bangladesh's capital of Dhaka. Nevertheless, 11 commonly used antibiotics were employed to assess the antimicrobial susceptibility of *E. coli* that was isolated from various salad products.

1.1 Antibiotics and Antibiotic Resistance

One of the biggest scientific discoveries of the 20th century has long been the emergence of antibiotic resistance in hospitals, communities, and the environment in parallel with their consumption. Because of man's overuse of antibiotics, microbes have developed extraordinary genetic abilities that have helped them develop multiple mechanisms of resistance for every antibiotic that has been used in clinical settings, agriculture, or any other context. These mechanisms have been developed by taking advantage of every possible source of resistance gene and every method of horizontal gene transfer. Currently, the modes of action of many antibiotics vary, and this variety causes various forms of antimicrobial resistance mechanisms in bacteria to evolve. However, the antibiotic was first created by studying the pathophysiology of the bacterium.

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 Translation	Reprogramming peptidoglycan biosynthesis Monooxygenation, efflux, altered target
Tetracycline	Minocycline,		

s	tigecycline		
Macrolides	Erythromycin, azithromycin	Translation	Hydrolysis, glycosylation, phosphorylation, efflux, altered target
Lincosamides	Clindamycin	Translation	Nucleotidylation, efflux, altered target

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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 **Sulfonamides**	Trimethoprim Sulfamethoxazole	C_1metabolism C_1metabolism	Efflux, altered target Efflux, altered target

Rifamycins **Lipopeptides**	Rifampin Daptomycin	Transcription Cell membrane	ADP-ribosylation, efflux, altered target Altered target
Cationic peptides	Colistin	Cell membrane	Altered target, efflux

Table 1.1: Modes of action of commonly used antibiotics

Antibiotic Resistance: The development and use of antibacterial agents is one of the most important discoveries in the history of medicine. The phrase "antibiotic" (derived from the Greek words anti- (against) and bios (life)) was employed to refer to medications that treat infections until it became apparent that diseases were triggered by bacteria, viruses, fungus, or prions. Thus, the idea that an antibiotic could eradicate every organism that exists originated from this drug. Antimicrobial resistance is the consequence of bacteria, fungi, and other microbes gaining a tolerance to the drugs aimed at eliminating them. That means the bacteria thrive and multiply. It can be complicated, if not unattainable, to combat infections that are resistant.

With at least 1.27 million deaths worldwide and around five million reported deaths in 2019, antimicrobial resistance is an important threat to public health internationally. In the US, illnesses resistant to antibiotics contribute to around 2.8 million occurrences per year. In accordance with the CDC's 2019, According to the Threats Assessment, this is expected to result in over 35,000 deaths.

Multi-drug resistance: Multidrug resistance is caused by a variety of factors, including genetic alterations, the acquisition of new genes, contact with cells containing novel genetic

materials, the use of antibiotics as growth promoters in animal feed intended for human consumption, and other circumstances that encourage antimicrobial resistance. However, the incorrect use of antimicrobial drugs has resulted in the post-antibiotic age in many parts of the world today.

The overwhelming majority of organisms that are multidrug-resistant are microorganisms that express resistance to one or more antimicrobial agent categories (Tenney J., 2018). A frequently occurring food-borne infectious agent, *E. coli* has been demonstrated to be multidrug resistant (MDR) and to be resistant to fluoroquinolones. In a comparable direction, a newest study found that major quantities of antibiotic resistance to -lactam and fluoroquinolone antibiotics were seen in *E. Coli* samples extracted from samples from urine (Ray J (2015). Microorganisms turned into Multidrug-resistant (MDR) due to a number of causes and one of which is the selective effectiveness of antibiotics, which increases by the overuse or misuse of these antibiotics (Concia et al., 2017).

1.2 *Escherichia coli*

The rod-shaped, facultatively anaerobic, gram-negative bacterium *Escherichia coli* is found in the digestive systems of animals that have warm blood. While the majority of coli strains are harmless, some can be harmful to people and other animals. There have been reports of the emergence and widespread transmission of some *E. coli* strains in recent years that are resistant to a wide variety of antimicrobial drugs. (Wright, Bartoloni and Sahm DF et al., 2001).

The establishment of resistant pathogenic bacteria against various antimicrobial drugs has become a significant threat to public health. Since 2001, Oteo et al. The US Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC), correspondingly, characterize multi-drug resistant (MDR) as non-susceptible to at least a single substance in three or more antimicrobial categories. Drugs for infectious conditions are mostly inadequate because of MDR bacteria.(Howard DH et al., 2003)

Since *E. coli* produces opportunistic infections in the gut, generally it cannot be considered a

dangerous bacterium. In rare cases, sometimes it can attack people who have a weak immune system. Primary pathogenic organisms may additionally be an integral component of combination infections when they undermine the host's localized resistance.

The dangerous *E. coli* microorganisms can be transmitted by humans as well as animals. These are the primary mechanisms that how the virus spreads: a) by means of ingesting poorly cooked or still-raw meat; b) by way of taking unprocessed dairy products; c) by way of coming in contact with polluted people who neglect to clean their hands; and d) by way of interactions with transferable wildlife.

***E. coli* infections**: Gastrointestinal difficulties, infant meningitis, and infections in the urinary tract represent some of the human conditions mostly triggered by *E. coli*. All of these conditions are predicated upon the organism exhibiting a specific combination of damaging (virulence) features.

Chapter 2

Materials and Method

2.1 Sample Collection:

Study region: In order to conduct this thesis, Six different locations in Dhaka city were explored. Every sample was collected on the outside market of numerous areas of Dhaka city. This study originated in The Microbiological Research Lab of the Department of Mathematics and Natural Sciences of BRAC University.

Study period: A total of 60 salad samples were collected from the street vendors, convenience stores, and local marketplaces of Dhaka city between October 2022 to February 2023.

Table 2.1: Names of the gathered vegetables sample

Tomato
Carrot
Capsicum
Cucumber
Lettuce
Mint (Pudina)
Green chili

Spring Onion
Cabbage
Coriander

The number of samples per area: These ten frequently used vegetable salad samples were collected by looking through many retailers in each area from every part of Dhaka. Raw vegetable samples were collected in sterile polythene samples acquiring bags from several locations within a designated area.

Culture media:

Sample collection on EC broth: This broth is a selective medium utilized for collecting samples from food and the environment for the verification test for *Escherichia coli* and for the identification of fecal coliforms. Collected unprepared salad samples were placed into sterilized conical flasks with EC broth medium, and the shaker incubator was turned up for a 24-hour bacterial enhancement time at 37°C.

Sample obtaining with MacConkey Agar: MacConkey is a selective and differential medium for growth that provides an indicator on its own. According to how microorganisms metabolize lactose, the MacConkey medium was developed to allow differentiation between enteric and Gram-negative bacteria. It comprises crystal violet and bile salts, which restrict the expansion of other gram-positive bacteria while supporting the emergence of gram-negative bacteria that are accustomed to degrading lactose.

Figure 2.1: *E coli* colonies on MAC media

Isolation of *Escherichia coli* from salad samples: *Escherichia coli* is extracted from salad samples by periodic dilutions of the samples, which are then promptly distributed to the plate's surface employing a sterilized spreader and MacConkey agar (MAC) media. After an incubation time of 24 hours, the plates had been kept at 37°C in an incubator. Afterwards, the plates were analyzed for a single colony.

Samples collection on Eosin Methylene Blue Agar: A colored indicator called Eosin Methylene Blue agar, or EMB, is used as a differential microbiological medium that differentiates between organisms (such as *E. coli*) that metabolize lactose alongside those that aren't capable. It prevents the proliferation of Gram-positive bacteria, such as Shigella and Salmonella, to some level. The initial EMB agar had been developed by Holt-Harris, Teague, and Levine; they afterwards added revisions to it. Therefore, it unifies the Levine, Holt-Harris, and Teague calculations. It incorporates two carbohydrates along with the animal tissue digested phosphate and peptic according to Holt-Harris and Teague. The medium serves a function in medical testing facilities for promptly spotting threatening gram-negative bacteria.

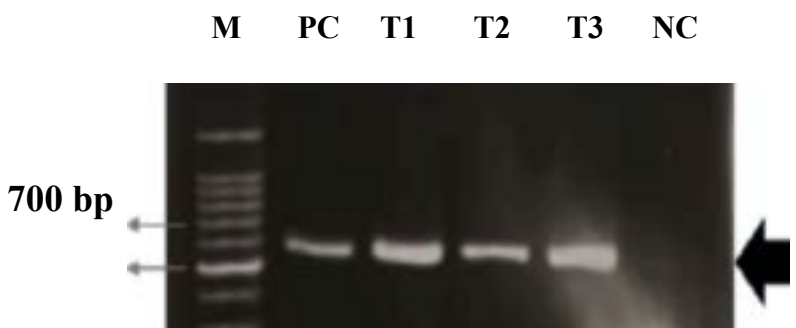
Identification of suspected *Escherichia coli* isolates: A unique stain for Gram-negative bacteria is eosin methylene blue (EMB). Gram-positive bacteria are unable to survive the

lethal dyes found in EMB. For coliforms, EMB serves as the selective and differential medium. The *Escherichia coli* colonies appeared black with a green metallic sheen while being incubated for an extra 24 hours at 37°C. This was a consequence of the high generation of acid, which precipitates the dyes into the growth exterior (Berry C.W. et al., 1984). Later then, isolated bacteria were detected using traditional microbiological approaches based solely on their morphological, biochemical, and cultural features.

E coli Confirmatory PCR:

DNA Extraction: DNA extraction has been done following the boiling technique on isolates of bacteria requiring few modifications (Queipo-Ortun et al., 2008). Staying the night on EMB agar, the organisms had been grown for a specific amount of time at 37°C.

Using sterile tips, a medium-sized colony was collected for incubation and injected into 200 liters of deionized water. The entire mixture was dipped in hot water and allowed to boil for 10 minutes. It then went through a ten-minute, 10,000 rpm centrifugation before it was placed in an ice bath. The DNA-containing supernatant was extracted. The extracted DNA sample had been stored at -20°C before use. Using 16S rRNA gene-specific primers, the originally identified E. coli was confirmed.



500 bp **585 bp**

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			

Sample microbial culture: In order to enhance growth, every single sample was placed in an incubator for 24 hours at 37°C. After the incubation procedure, specified colonies have been eliminated from the culture medium the next day, implanted on nutrient agar, and ultimately kept for usage afterwards.

2.3 Antibiotic susceptibility testing (AST)

Disk Diffusion Method: An antibiotic sensitivity test is utilized to determine the most effective method of treatment for bacterial illness. All antibiotics have a unique range of susceptibility that is evaluated by CLSI. By using the susceptibility range, we can recognize the infections that are resistant and carry out additional steps. The procedure required, spectrophotometer and Mc-Farland 0.5 standard and Kirby-Bauer method was used.

Mueller-Hinton Agar: For several years, Mueller-Hinton agar was frequently utilized to perform several kinds of antimicrobial activity testing. the non-selective and non-differential character of the medium constituting the underlying cause. This indicates that the majority of species deposited on such a surface will reproduce. It comprises starch. Although starch removes bacterial toxins, antibiotics cannot interact with the toxins manufactured by this bacterium. The single colony which was a 24-hours incubated sub-cultured isolate was inoculated in 5ml saline. Optical density was measured, here 0.5 McFarland was used as standard which is OD= 0.1. After confirming the desired value, the Kirby-Bauer method was carried out. Clear zone of inhibition confirms that the particular bacteria is susceptible to that particular antibiotic and no zone of inhibition means the bacteria is resistant to that particular antibiotic.

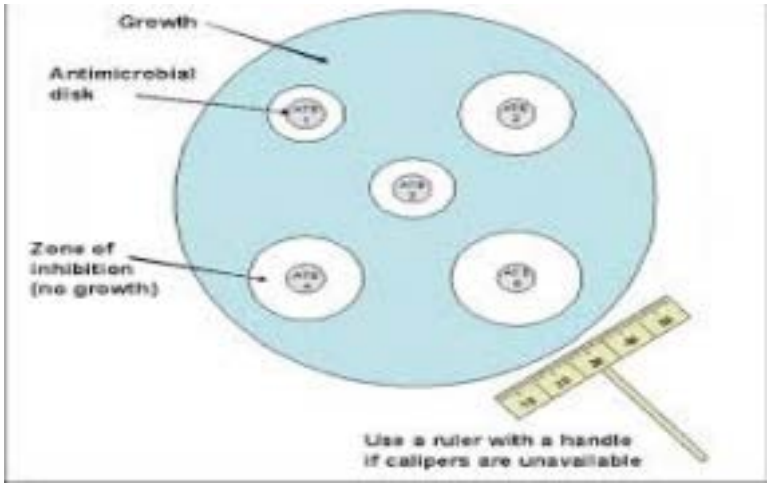


Figure 2.3: Zone of inhibition on MHA plate

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
Gentamicin (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

Methodology of workflow:

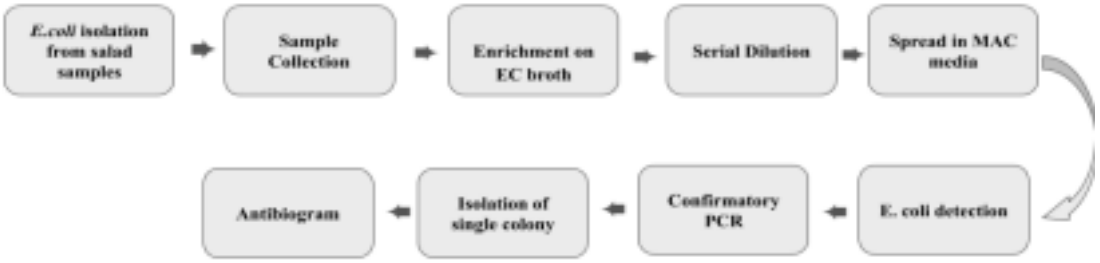


Figure 2.4: Methodology of Workflow

To get the antibiogram data for the tested samples, a sample of *E. coli* was collected from salad vegetables using different techniques and mediums. First, the bacterial samples provided the opportunity to survive and grow in the media and based on the previously established standards, the target organism *E. coli* was found. For the antibiogram, a single colony of the target bacteria were selected and the results of the antibiogram will be discussed next.

Chapter 3

Results & Discussion

Antibiotic susceptibility test

Salad samples collected from six different sites of the Dhaka city were used to generate an antibiogram of *E. coli.* 200 different strains of *E. Coli* were tested for antibiotic susceptibility. The percentages and numbers for both resistance and sensitivity are shown 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%
Gentamicin (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%
Chloramphenic ol (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%

The data for ampicillin indicates that out of 300 patients, 195 or 97.5% of the cases are resistant, making it the most resistant antibiotic. From the other used antibiotics, amikacin and kanamycin show the most resistance.

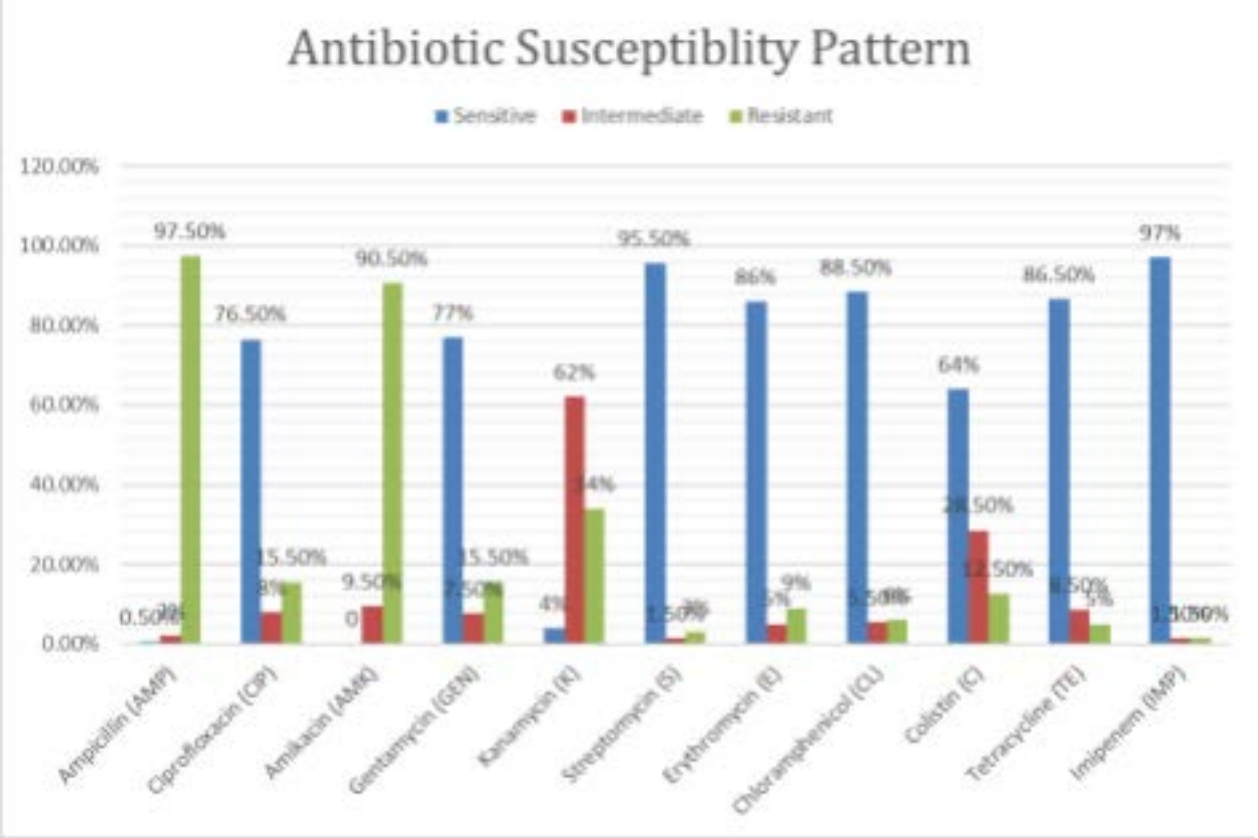


Figure 3.1: Antibiotic susceptibility pattern of 200 *E. coli* isolates from Salad samples

From these antibiotics, Streptomycin (S) shows the greatest sensitivity at 94% and Imipenem (IMP) around 97%, Amikacin (AMK) at 90.5% and Ampicillin (97%) gives the most resistance. The vast majority of the remaining antibiotics have different levels of sensitivity.

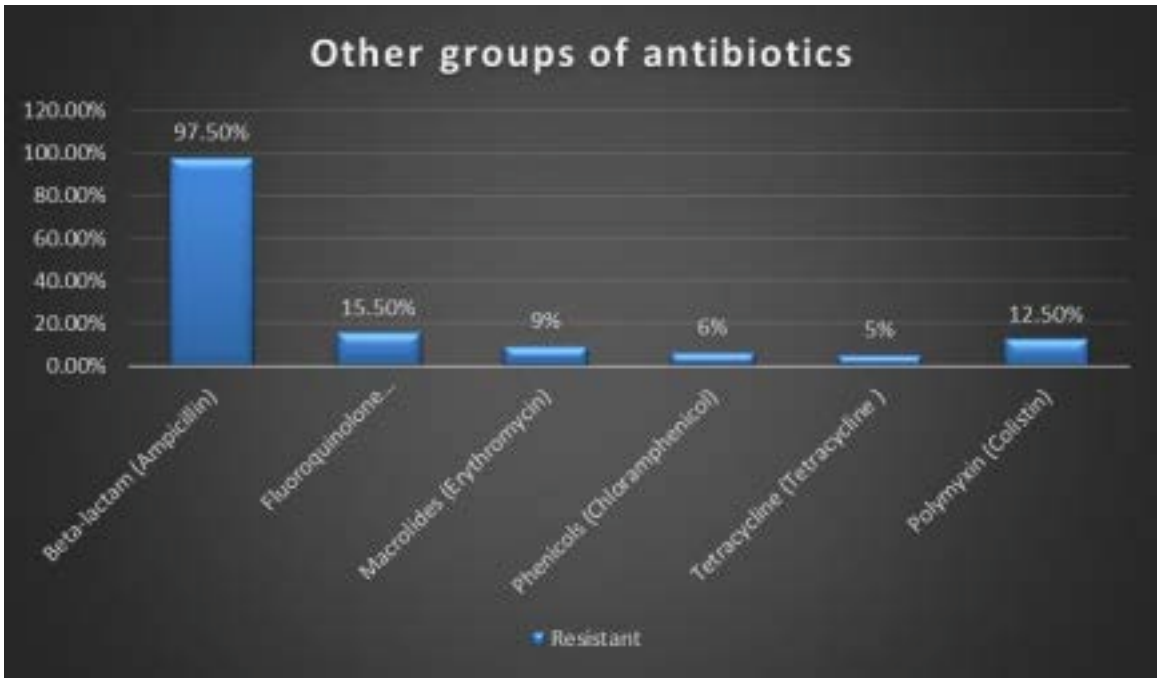


Figure 3.2: Resistance pattern of the groups of Antibiotics

This graph illustrates the resistance patterns of the chosen antibiotic groups for the current study. Determining the isolated strains' multidrug resistance was the purpose of this chart. Multidrug resistance (MDR) is defined as developed resistance to at least a single antibiotic in three or more antimicrobial groups. Extensively drug-resistant (XDR) bacterial isolates are only sensitive to one or two antimicrobial groups, indicating they cannot be susceptible to at least one agent in any other antimicrobial group except for the last two or fewer.

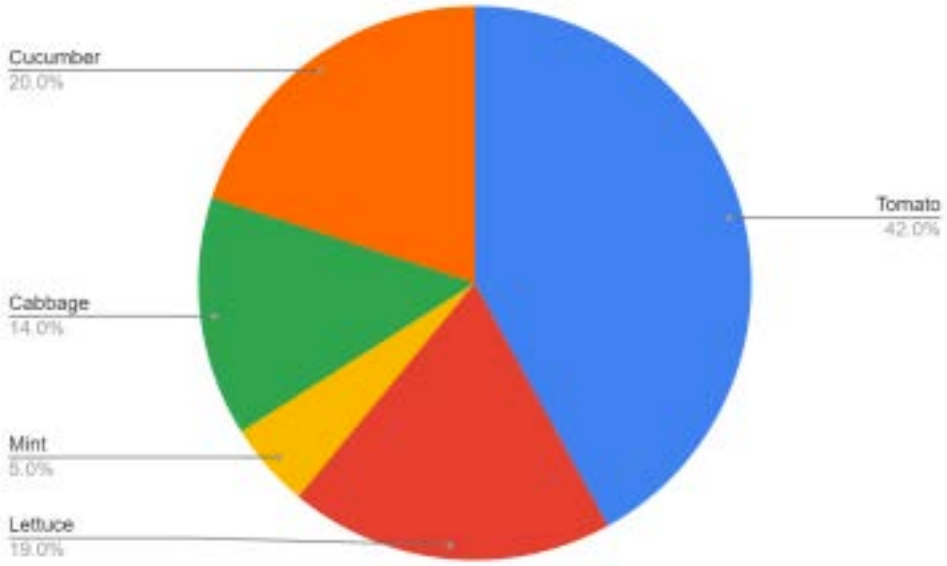


Figure 3.3: Resistance pattern among the samples

This chart shows the resistance characteristic among the collected 10 vegetable samples. These five samples showed the biggest resistance out among the 10 samples. Tomatoes remained the most prevalent vegetables, then cucumbers, lettuce, cabbage, and mint.

Discussion:

The bacteria *Escherichia coli* (*E.coli*) was first identified by Theodor Escherich, a German pediatrician in 1885 after isolating it from infant feces. *E. coli* is a gram-negative, non-sporulating, rod-shaped, facultatively anaerobic, and coliform bacteria that often lives in the environment, food, and the lower stomach of warm-blooded animals. Although the majority of *E. coli* strains are harmless, some serotypes can cause diarrhea when taken through contaminated food or drink, while others may result in urinary tract infections (UTIs), anemia, respiratory or kidney infections. To gain virulence factors, some strains of *E.coli* have transformed into harmful ones by employing plasmids, transposons, bacteriophages, or pathogenicity islands. Antibiotic resistance genes have been generated in many gram-negative bacteria and *E.coli* is not an exception. These bacteria have developed different mechanisms that grant them antibiotic resistance. *E.coli* is capable of producing extended-spectrum beta-lactamase (ESBL) which makes the bacteria resistant to beta lactams. On the other hand, *E.coli* that produce carbapenemase, have genes that provide carbapenem resistance.

The daily lives of city dwellers depend heavily on the availability of these common salad example factors. When foods are prepared improperly, especially when it comes to sampling, they can spread a number of pathogens and result in a wide range of food-borne disorders. Food handling that is hazardous and unclean has an enormous impact on public health and can result in a variety of chronic and non-chronic diseases. In Bangladesh, foodborne infections and food contamination are commonplace due to a lack of knowledge, awareness, and compliance with food regulations. Relative to salad items, the data indicates that multidrug resistance is present in almost all areas of Dhaka city. This suggests that antibiotic resistance is already significantly stronger than we would have thought. The high prevalence of resistance to several drugs among *E. coli* isolates indicates increased resistance to antibiotics. The emergence of multidrug resistance in *E. Coli* points out the need for more public awareness, medical and veterinary education, and the implementation of suitable steps to restrict the widespread consumption of antibiotics. Furthermore, as the study is still in progress, more investigation needs to be done by using these resources.

Conclusion:

The high prevalence of antibiotic resistance seen in *E. coli* isolates is evidence of increased antibiotic resistance. The necessity to raise public awareness, educate medical professionals and veterinarians and take the necessary steps to control antibiotic usage without restriction has been pointed out by the emergence of multidrug resistance in *E. coli.* Antibiotic resistance is a significant issue that is becoming worse every day. The treatment of infections with appropriate antibiotic doses, which will ultimately fail due to resistance pattern changes, has an impact on every part of the world. If circumstances stay like that, it will be difficult to provide the right treatments to patients. Multidrug resistance *Escherichia coli* has grown into an alarming issue because it is inherently susceptible to almost every clinically important antimicrobial drug.

According to the study, Salad food items create a serious threat to public health. To reduce the amount of *E. coli* in salads marketed for human consumption, public food providers must improve their hygienic and excellent manufacturing standards. Also, there is an extreme danger to public health caused by the MDR *E. coli* discovered in these food items.

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