Detection of *E. coli* in Cut Fruits Sold as Street Foods in Areas of Dhaka City and Assessing Their Antibiotic Resistance

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

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June 2025

Declaration

It is hereby declared that

1. The thesis submitted is my 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.

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Approval

The thesis/project titled "Detection of Enteric Pathogen *E. coli* in Street Food in Areas of Dhaka City" submitted by

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of Spring, 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of BS in Microbiology on 19 June, 2025.

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Ethics Statement

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

Abstract/ Executive Summary

This study investigated the prevalence and trend of antibiotic resistance of *Escherichia coli* in cucumber, hog plum, grapefruit, and guava fruits that are sold as cut fruits and fresh snacks as street foods in Dhaka, Bangladesh. Total 45 samples were obtained from different places in Dhaka city like, hospital areas in Shyamoli and congested areas around Gulshan lake. The results of the Xylose Lysine Deoxycholate (XLD) agar microbiological test and the PCR molecular confirmation showed that 48.89% (22/45) of the samples were contaminated with *E. coli*. Tests for antibiotic susceptibility using the Kirby-Bauer disk diffusion technique revealed high resistance to ampicillin (84.4%), clindamycin (86.7%), sulfamethoxazole (84.4%), and tetracycline (95.6%). Conversely, imipenem and meropenem were the most effective antibiotics, with sensitivity of 75.6% and 70%, respectively. The emergent development of multidrug resistance (approximately 90% among 22 isolates) among isolates bodes serious public health concerns regarding street foods. The findings call for the need for stricter hygiene practices, stricter food safety regulations, and continuous monitoring of antibiotic resistance among foodborne pathogens.

Keywords: XLD, PCR, AST

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List of Acronyms

AMR	Anti-Microbial Resistance
AST	Antibiotic Susceptibility Testing
DNA	Deoxyribonucleic Acid
MDR	Multi-Drug Resistance
MHA	Muller Hinton Agar
NA	Nutrient Agar
PCR	Polymerase Chain Reaction
TE	Tris-EDTA
UV	Ultra Violet
XLD	Xylose Lysine Deoxycholate
ZOI	Zone of Inhibition

Chapter 1

Introduction

1.1 Introduction:

Street food is an important part of the urban foodscape in most developing countries, including Bangladesh. It is a supplier of affordable, ready-to-eat, and convenient meals for millions of people daily, especially the poor, students, and day laborers (FAO, 2007). The informal and unorganized nature of street food vending, however, poses significant risks to food safety and public health. The vendors usually do not have access to clean water, sanitation, or waste disposal systems, and this increases the possibilities of environmental contaminants, unsafe handling, and microbial pathogens causing contamination (WHO, 2010; Alimi, 2016).

Raw salads and cut fruits are some of the most popular but hazardous categories of street foods. These foods are typically consumed raw without any pre-cooking, which does not provide an opportunity for microbial decontamination by heat. They are typically handled by peeling, cutting, and leaving them open to the air for several hours—steps that are highly likely to enhance the risk of microbial contamination (Barro et al., 2006). Several studies have noted the presence of pathogenic microbes in these foods, and one of the most prevalent contaminants discovered is *Escherichia coli (E. coli)*. (Islam et al., 2018).

E. coli is generally found in human and animal guts and is employed as a fecal contamination indicator microorganism for water and food. Most strains are harmless, but some pathotypes, such as Enterotoxigenic *E. coli* (ETEC) and Enterohemorrhagic *E. coli* (EHEC), can cause acute gastrointestinal disease (Kaper et al., 2004). Besides its role in being pathogenic, *E. coli* is increasingly a public health concern due to its expanding resistance against antibiotics commonly employed. Multidrug-resistant (MDR) strains have become prevalent globally, it

is becoming more difficult to treat, and the stakes are increased for severe health outcomes (Liu et al., 2016; WHO, 2020).

In Bangladesh, where regulatory control over the safety of street foods is yet to be strengthened, transmission of MDR *E. coli* through mass-consumed food items is a pressing but under-acknowledged hazard. It is especially of concern in densely populated urban settings like Dhaka, where demand for cheap street food is high. While microbial contamination of street-vended foods has been shown previously, antimicrobial resistance (AMR) patterns of the isolates have seldom been investigated (Sodagari et al., 2015).

By assessing the microbiological quality and antibiotic susceptibility patterns of *E. coli* recovered from cut fruits and salads sold on the street in a number of densely populated areas of Dhaka, including hospital zones (Suhrawardy Medical College, Dhaka Shishu Hospital, etc.) in Shyamoli and Gulshan Lake, this study sought to close that gap. 45 food samples, including cucumber, hog plum, grapefruit, and guava, were collected and examined.

Out of 45 samples, 22 samples (48.89%) were culture-positive for *E. coli* by colony characteristics on Xylose Lysine Deoxycholate (XLD) agar and confirmed molecularly using PCR with the 16S rRNA gene (585 bp amplicon). The antibiotic resistance pattern of the isolates was also examined with the Kirby-Bauer disk diffusion test and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

In recent years, the globe has been progressively troubled with the rise of AMR in foodborne pathogens, such as *E. coli*. MDR *E. coli* isolates have become resistant to a broad spectrum of commonly utilized antibiotics such as ampicillin, tetracycline, ciprofloxacin, and even last-resort antibiotics such as colistin and carbapenems (Liu et al., 2016; Van den Bogaard & Stobberingh, 2000). Since infection by MDR bacteria is difficult to treat, leading to longer disease duration, higher rate of mortality, and higher cost of healthcare, transmission and

development of resistant strains down the food chain is a major threat to the health of the people (WHO, 2020).

In Bangladesh, where street food is a common urban culture, poor hygiene, inefficient enforcement of food safety regulations, and inadequate awareness of food safety advice among street vendors increase the risk of microbial contamination and the transmission of AMR. In Dhaka and other urban centers, previous studies have reported high levels of microbial contamination, including the presence of pathogenic *E. coli* from street foods such as raw salads, fruit juices, and cut fruits (Sodagari et al., 2015; Islam et al., 2018). Nevertheless, limited scientific evidence has been reported on the antibiotic resistance patterns of *E. coli* isolates from such foods alone.

With this consideration, the present study aimed to analyze the microbiological safety of street-vended cut fruits and salads in selected high-traffic areas in Dhaka, Bangladesh. Specifically, it sought to isolate and identify *E. coli* from food samples gathered and determine their AMR patterns via standard laboratory techniques. By identifying the prevalence of MDR strains in ready-to-eat street foods, this study contributes to an understanding of the potential health risk to consumers and reinforces the urgency for improved food safety procedures and antimicrobial stewardship at the community level.

1.2 *E. coli:*

E. coli is a Gram-negative bacterium, facultative anaerobic and rod-shaped which can be found in the human intestine as part of normal flora. Although *E. coli* is usually harmless and is an important part of a healthy intestinal tract, sometimes they can cause illness.

1.3 Objective:

The primary objective of this study is to ascertain the prevalence of *Escherichia coli* in street-vended cut salads and fruits in Dhaka and to identify the antibiotic resistance pattern of the isolated strains. The research aims to determine the extent of microbial contamination of street foods ready for consumption and determine the multidrug resistance (MDR) pattern of the isolates, thus the potential public health risks and towards the formulation of an appropriate food safety and antimicrobial stewardship policy.

Chapter 2

Method and Materials

2.1 Flowchart:

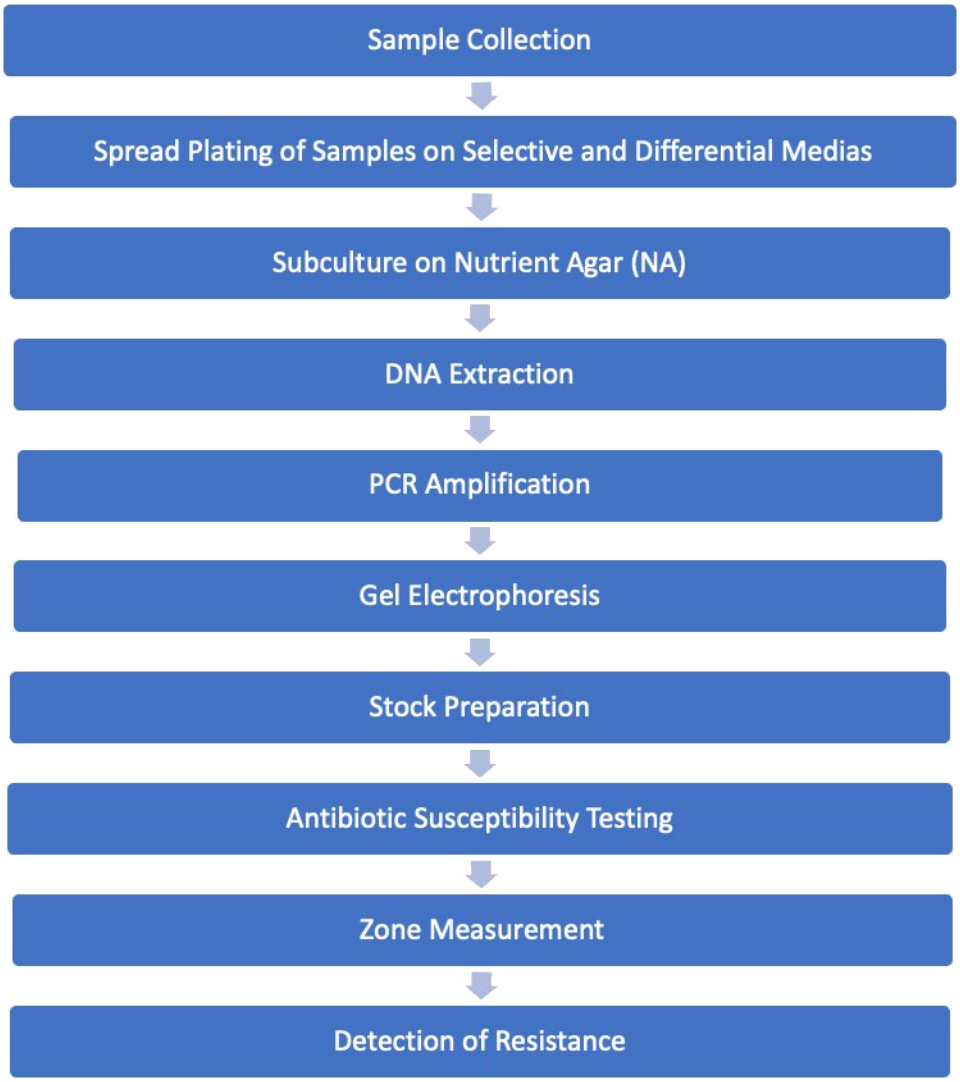

Figure 1: Work Flow of the Work Process

2.2 Site of Sample Collection:

Samples were collected from street vendors selling chopped fruits and salads in busy areas of Dhaka, namely the areas around the TB Hospital, Suhrawardy Medical College, and Shishu Hospital, as well as from busy wayside stands in the Shyamoli and Gulshan Lake areas. High customer demand and likely contamination risks from exposure to the environment and poor hygiene practices led to the selection of these locations. During busy vending hours, all samples were gathered and immediately transported to the laboratory while being stored in sterile collection bags with ice packs (Sodagari et al., 2015) and then processed by homogenization using a homogeniser.

2.3 Study Duration:

The duration of this study was between September 2024 to January 2025. samples were collected throughout the study period and the susceptibility test to antibiotics was conducted from January 2025.

2.4 Sample Size:

A total of 45 samples were collected throughout the study period. Mainly three types of fruits like, Guava, Hog Plum, Grapefruits and for salad, shredded cucumber were used as the samples. All samples were carried to the laboratory to conduct the tests.

2.5 Sample Processing:

Each sample was cut into thin slices using a sterile knife and then inoculated into autoclaved LB media and incubated at 37°C for 24 hours in a shaker incubator. The next day, each sample's 10 µL was mixed with 90 µL of autoclaved saline solution. After that samples were diluted in MCTs up to 10^{-4}. Then from the dilution number 4, approximately 90-100 µL was taken using a pipette and poured into XLD media (Szakál, D., & Pál, T. (2003). Using a glass spreader the sample was spread into the media and then placed into 37°C incubator for 24 hours. After 24 hours, yellow colonies were observed.

2.6 Bacterial identification:

For the detection of *Escherichia coli*, collected samples were spread onto Xylose Lysine Deoxycholate (XLD) agar, a selective and differential medium. After incubation at 37°C for 24–48 hours, suspected *E. coli* colonies were identified based on their characteristic yellow colonies, indicating lactose fermentation.

2.7 Bacterial DNA Extraction:

The confirmed isolates of *E. coli* were sub-cultured onto Nutrient Agar and incubated for 24 hours at 37°C. After 24 hours DNA was extracted by using the "Boiling method". This method was selected because of the efficiency, time and cost effectiveness. For that, 400 µl of TE buffer was taken in microcentrifuge tubes and a loopful of culture which was incubated for 24 hours at 37°C was suspended in the MCT (microcentrifuge tubes) tubes and vortexed for 15 seconds. After that the mixture was centrifuged at 13000 RPM for 10 minutes at 25°C. Then supernatant was discarded, and the pellet was again mixed with 400 µl of TE buffer and was heated at 95°C for 10 minutes using a dry heat block. After that the heated MCT tubes were centrifuged at 13,000 RPM for 10 minutes at 25°C. Then the supernatants were transferred into fresh MCT tubes and pellets were discarded. The collected supernatant was stored at -20°C.

2.8 PCR Amplification:

The Polymerase Chain Reaction (PCR) is used to amplify sequences of DNA. The steps of PCR include Denaturation, Annealing and Elongation. In the Denaturation step, the double stranded DNA template is converted to single stranded DNA sequences. Primers are then annealed to the single stranded sequences, leading to elongation of the target DNA. Primers are short, single stranded DNA sequences that are complementary to the ends of the target DNA sequence. They are required to begin the process of DNA synthesis, along with the thermostable enzyme DNA polymerase. The steps of PCR are repeated for a number of cycles, while the reaction takes place in a thermocycler (Gupta, 2019a). A working primer solution of 10 µM concentration was made through pipetting the material into sterile nuclease-free water starting from its original stocks. The primers were transferred to separate

tubes which used sterile microcentrifuge tubes to ensure purity without allowing contamination. The reactions for PCR analysis contained 13 µL of volume in separate sterilized PCR tubes for mixture preparation. The PCR reaction contained 2 µL template bacterial DNA from extraction and 6 µL PCR Master Mix, together with 3 µL nuclease-free water and 1 µL of each forward and reverse primer. Single-tube PCR amplification happens with Taq DNA polymerase and deoxynucleotide triphosphates (dNTPs) together with magnesium chloride ($MgCl_2$) inside an optimized reaction buffer to obtain maximum DNA amplification efficiency. The tubes received a quick spin before being introduced into the thermal cycler, where amplification occurred.

Primer	Primer Sequence	PCR Conditions	Number of Cycles	Amplicon Size	Reference
Escherichia coli	16 s-F: 5'-GACCTCGGTTTAGTTCACAG A-3' 16s-R: 5'-CACACGTGACGCTGACCA-3'	Initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 45s, annealing at 45°C for 45s and extension for 1 min followed by a final extension at 72°C for 5 min.	35	585 bp	(Thopireddy, 2023)

Table 1: Primer used for PCR

2.9 Agarose Gel Electrophoresis:

Agarose gel electrophoresis functioned as the analysis tool to study PCR products through DNA fragment separation made possible by using an electric field across an agarose matrix. The technique allows PCR-amplified products to be visible through ethidium bromide usage since this fluorescent dye binds DNA molecules and produces UV light-induced fluorescent signals. A 1.5% agarose gel prepared with 1X Tris-Acetate-EDTA (TAE) buffer functioned as the running buffer throughout this gel electrophoresis procedure. The gel contained 4 μL of ethidium bromide to allow DNA staining. The 100 bp DNA ladder served as a size marker for the PCR products by including it with the loaded PCR samples. The DNA fragments migrate through the gel matrix using 100 volts power for 45–60 minutes duration. The DNA gel went on the UV transilluminator for visualizing and documenting the bands that formed after the run was done.

2.10 Antibiotic Susceptibility Test:

The disc diffusion method was followed to conduct the test and the range of susceptibility and resistance was evaluated by CLSI guideline. By using the guideline, if an isolate was resistant or susceptible was found out. MHA agar was used to perform the Antibiotic Susceptibility Testing (AST). To conduct the test, a cotton swab was used to collect isolated colonies of *E. coli* that were mixed in 5ml saline and vortexed to mix it properly which was then matched to the McFarland 0.5 standard. The saline solution was then evenly spread through the MHA agar plate using sterile cotton swabs. After that antibiotic disks were put into the plate and the plates were incubated at 37°C for 24 hours and the result was observed.

Antibiotics	**Group Name**	**Disc Code**	**Disc Potency**	**Susceptible (mm) or more**	**Intermediate (mm)**	**Resistant (mm) or less**
Aminoglycoside	Gentamicin	CN10	10	15	13-14	12
	Amikacin	AK30	30	17	15-16	14
Carbapenem	Imipenem	IMP10	10	23	20-22	19
	Meropenem	MRP10	10	23	20-22	19
β-Lactamase	Ampicillin	AMP10	10	17	14-16	13
Penicillin & β-Lactamase inhibitor	Piperacillin Tazobactam	TZP110	10/100	21	18-20	17

Glycylcycline	Tigecycline	TGC15	15	17	15-16	14
Polymyxin	Colistin	CL10	10	18	11-17	10
Monobactam	Aztreonam	ATM30	30	21	18-20	17
Fluoroquinolone	Ciprofloxacin	CIP5	5	20	17-19	16
Cephalosporin	Cefepime	FEP30	30	24	20-23	19
Amphenicol	Chloramphenicol	C30	30	18	13-17	12
Lincomycin	Clindamycin	CD2	2	21	15-20	14
Sulfanilamide	Trimethoprim /Sulfamethoxazole	SXT25	25	16	11-15	10
Macrolide	Erythromycin	E15	15	17	14-16	13
Tetracycline	Tetracycline	TE30	30	15	12-14	11

Table 2: List of antibiotics used in the study

Chapter 3

Result

3.1 Spreading Result on XLD Media:

Out of 45 samples, 22 samples (48.89%) were positive for *E. coli.* The positive samples exhibited distinctive yellow colonies on Xylose Lysine Deoxycholate Agar (XLD Agar).

Figure 2: Yellow colonies on XLD Media

3.2 Result of PCR and Gel electrophoresis:

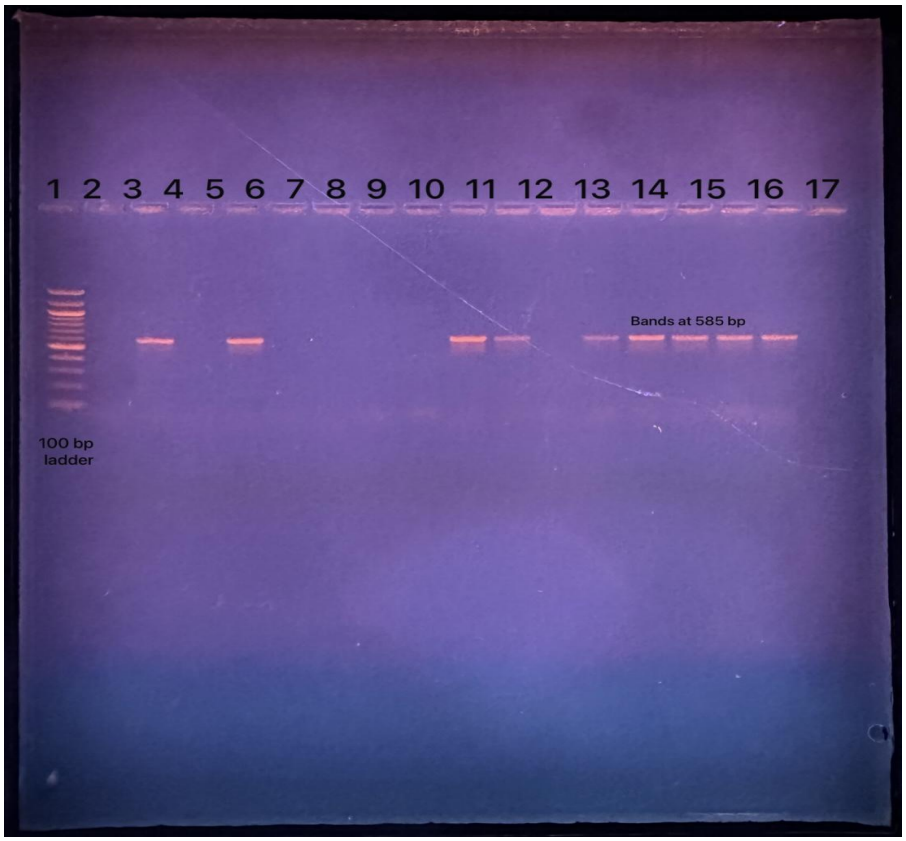

Figure 3: Agarose Gel electrophoresis of *Escherichia coli*, Lane number 1 is 100 bp Ladder, Lanes 2-17 contains the samples. Here lane 3,5,10,11,13-17 showed bands at 585 bp.

3.3 Antibiotic Susceptibility Pattern:

By using the Kirby-Bauer disk diffusion method, antibiotic susceptibility tests for all the confirmed *E. coli* isolates were done. The resistant, intermediate and susceptibility pattern was studied by comparing the value with the range of CLSI guidelines.

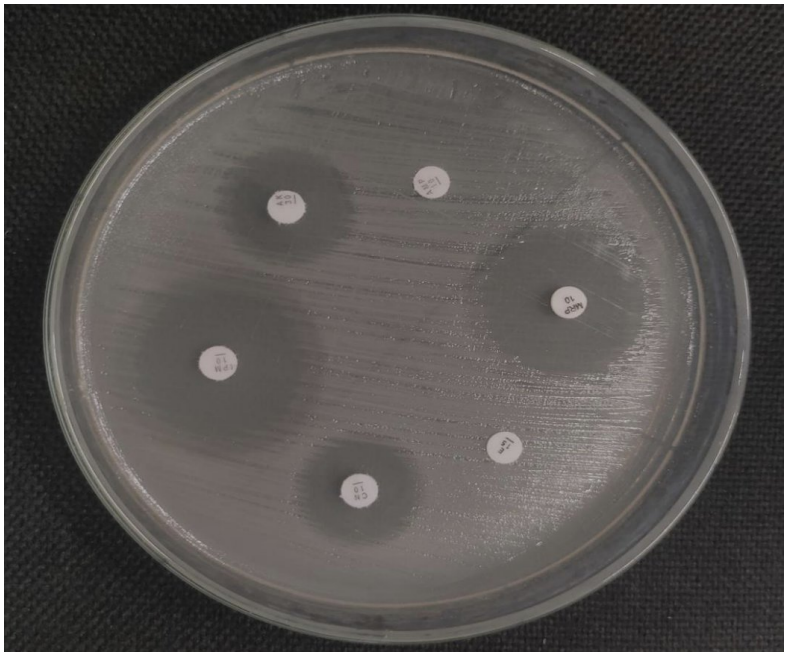

Figure 4: Antibiotic Susceptibility Test by using Kirby-Bauer disk diffusion method

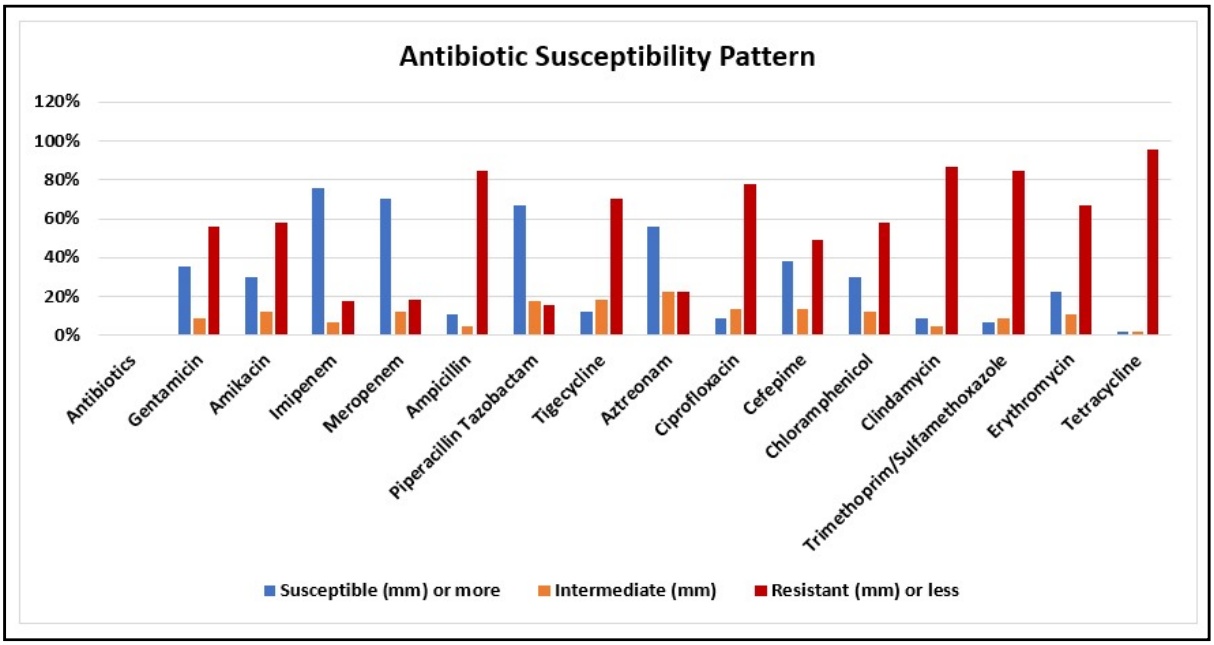

Figure 5: Antibiotic Susceptibility Profile of the Isolates

3.4 Multi Drug Resistant *E. coli*

The results indicated that an overwhelming proportion of *E. coli* isolates were resistant to several antibiotic classes, thereby testifying to the presence of MDR strains. Specifically, over 80% of the isolates were resistant to the most common antibiotics such as ampicillin (84.4%), clindamycin (86.7%), tetracycline (95.6%), and trimethoprim/sulfamethoxazole (84.4%). Additionally, a considerable number of isolates were also resistant to ciprofloxacin (77.8%) and chloramphenicol (58%). Based on the resistance pattern, more than 90% of the *E. coli* strains were MDR and had resistance to three or more antibiotic classes. The widespread presence of MDR *E. coli* among the street foods commonly consumed is a public health risk and demands prompt surveillance and control.

Chapter 4

Discussion

The presence of *E. coli* in 22 out of 45 street foods (48.89%) portrays an utmost level of microbial contamination in street foods like salads and fruits sold in Dhaka's densely populated areas. The presence of *E. coli* portrays fecal contamination, most likely on account of poor personal hygiene, poor sanitation, or improper handling and storage practices—factors consistently recognized in such studies (Sodagari et al., 2015).

In their 2009 study, Tambekar et al. reported that 43% of the food sold on Indian streets was contaminated with *E. coli*. Similarly, studies in Ghana and Nigeria found that *E. coli* was found in 45.8% and 52.4% of street food samples, respectively, supporting the idea that uncontrolled street food serves as a source of enteric infections (Mensah et al., 2002; Oranusi et al., 2013).

The isolates in this research showed antibiotic susceptibility patterns reflecting concerning trends of MDR. Resistance was high to ampicillin (84.4%), sulfamethoxazole (84.4%), clindamycin (86.7%), and tetracycline (95.6%). These findings are consistent with reports of resistance of *E. coli* in low and middle-income countries worldwide. For instance, Reuland et al. (2016) noted that more than 80% of the *E. coli* isolates from street foods were resistant to both ampicillin and sulfamethoxazole.

The most susceptible antimicrobials, however, were imipenem (75.6% susceptible) and meropenem (70% susceptible). This finding is consistent with that of Baral et al. (2018), who reported that *E. coli* carbapenem susceptibility patterns were consistent throughout Nepal. Carbapenem resistance is not yet fairly common in this group since the drug is typically stored and its efficacy is not compromised. Even low rates, however, are to be watched since they are concerning (17.8% for imipenem and 18% for meropenem, respectively).

Moreover, resistance to ciprofloxacin (77.8%) and cefepime (48.9%) reflects a growing level of prevalence of widely employed antibiotic ineffectiveness. Such resistance to ciprofloxacin has been reported in recent studies, with Hossain et al. (2021) reporting over 70% resistance in foodborne *E. coli* isolates in Bangladesh. Such high percentage resistance to fluoroquinolones and cephalosporins may be a result of their frequent and uncontrolled application in human medicine as well as agriculture.

It is important to note that 66.7% of the isolates were piperacillin-tazobactam sensitive, suggesting that β-lactam/β-lactamase inhibitor combos are still being used. However, the extensive resistance of 70% of tigecycline is quite concerning because it is a glycylcycline with broad-spectrum action.

The presence of multidrug-resistant *E. coli* in almost all of the positive samples suggests that street food may be a health concern. In addition to strict hygiene monitoring systems, more vendor education on food safety, and antibiotic stewardship initiatives, it urges consideration of the abuse of antibiotics.

Conclusion

The study revealed that 48.89% of the samples tested positive for *Escherichia coli*, showing a significant prevalence of the pathogen on street vendor fruits and salads in Dhaka. *E. coli* is an absolute indicator of fecal contamination and is most frequently preceded by unsafe water use, unhygienic conditions, and poor food handling. More ominously, however, the isolates were extremely drug-resistant to a series of drugs commonly utilized, i.e., tetracycline, ampicillin, clindamycin, and trimethoprim/sulfamethoxazole, indicating that there were new multidrug-resistant (MDR) strains in street-vended foods.

The high susceptibility to carbapenems (imipenem and meropenem) suggests that these antibiotics remain effective; however, their limited use in community settings must be preserved to prevent future resistance. These results highlight the urgent need for stronger regulatory oversight, proper training for food handlers, improved sanitation infrastructure, and routine surveillance of antimicrobial resistance in street foods to protect public health and combat the growing threat of antibiotic resistance.

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