CHARACTERIZATION OF PATHOGENIC *ESCHERICHIA COLI* ISOLATED FROM FRESH VEGETABLES OF DHAKA CITY

By

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A thesis submitted to the Department of Mathematics and Natural Science in partial fulfillment of the requirements for the degree of Masters of Science in Biotechnology

Department of Mathematics and Natural Science
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September 2022

Declaration

It is hereby declared that

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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.
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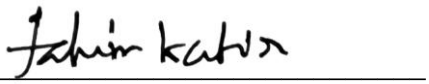

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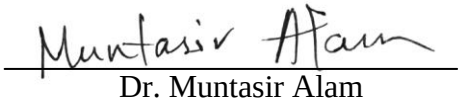

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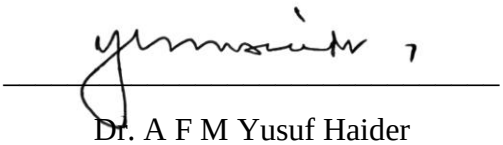

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Ethics Statement

No human or animal model was used in this study.

Abstract

Escherichia coli (E. coli) is generally harmless bacteria that resides in the intestines of humans and animals and maintains intestinal health. This benign intestinal resident is also a highly adaptable and frequently lethal infection. Vegetables are susceptible to *E. coli* contamination from pre-harvest through post-harvest. This study aims to determine the frequency of the virulence gene and the antibiotic resistance of *E. coli* in fresh vegetables gathered from various areas of Dhaka. Of 250 vegetable samples, 101 (40.4%) included E. *coli*. Dhanmondi had the most positive isolates (12.87%) and followed by Rampura (11.88%), Farmgate (9.9%), Uttara (9.9%), Gulshan-1(7.92%), Gulshan-2 (7.92%), Banani (7.92%), Khilgoan (7.92%), Mirpur (6.94%), Mohakhali (5.94%), Mohammadpur (5.94%), and Bashundhara (4.95%). The highest percentage of positive isolates in Tomato was 52.0%. Respectively, followed by Capsicum (51.85%), Carrot (51.35%), Coriander (47.61%), Cabbage (44%), Cucumber (40.9%), Green Chili (36.84%), Mint (31.81%), Spring Onion (21.42%), Lettuce (20.83%). From the antibiotic susceptibility test, 95.04% of isolates showed multi-drug resistance (MDR).

Moreover, the pathogenicity of 40 bacteria out of 101 isolates was evaluated through PCR, and 26 (65%) of the 40 isolates were confirmed positive for pathogenicity. According to these findings, there is a considerable cause for worry for the residents of the Dhaka region since there is a significant threat to human health. As a realization of these findings, it became clear that there is a compelling need to raise public awareness regarding the hygienic practices that should be followed while selling and purchasing fresh produce.

Keywords: *Escherichia coli;* Antibiotic susceptibility; Pathogenicity; Vegetables; Isolates; MDR; XDR; PCR; Primer; Gene; Prevalence.

Dedication

Dedicated to My Parents

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Unmilita Das Moon

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List of Acronyms

E. coli	*Escherichia coli*
MDR	Multi-drug resistance
PCR	Polymerase chain reaction
XDR	Extensively drug-resistant
EC broth	*Escherichia coli* Broth
MIU	Motility Urease test
MHA	Mueller–Hinton agar
Bp	Base pair
DNA	Deoxyribonucleic acid
EPEC	Enteropathogenic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
EHEC	Enterohemorrhagic *Escherichia coli*
Min	Minute
Sec	Second
TAE	Tris-acetate-EDTA

Glossary

MDR:	Multidrug-resistant (MDR) is acquired resistance to at least one antimicrobial agent from three or more antimicrobial groups.
XDR:	Extensive drug-resistant, or XDR, is characterized as resistance to at least one antimicrobial agent in all but two or fewer antimicrobial categories
PCR:	Polymerase chain reaction is a laboratory technique for amplifying millions to billions of copies of a given section of DNA in a short period of time.
Isolation:	Bacterial isolation is the process of isolating one species of bacteria from a mixed culture of bacteria using various plating methods such as pouring, spreading, streaking, and serial dilution.
Pathogenicity:	Pathogenicity is the characteristic or state of being pathogenic, or the propensity to cause illness, whereas virulence is the capacity of an organism to cause disease, or its degree of pathogenicity within a group or species.

Chapter 1

Introduction

A healthful diet should emphasize the consumption of fresh vegetables. They are an excellent source of essential vitamins, minerals, fiber, and other elements necessary for maintaining a healthy body. Despite the nutritional and health benefits of vegetables, there has been an upsurge in occurrences of human diseases associated with consuming fresh vegetables over the last several years [1,2]. Pathogenic *Escherichia coli* (*E. coli*) is a harmful bacteria found in fresh vegetables and is a severe foodborne pathogen linked with gastroenteritis globally [3]. *E. coli*, which is often present in the stomach, is a commensal bacterium. However, some *E. coli* have become dangerous because they picked up virulence factors from mobile genetic elements like plasmids, transposons, bacteriophages, and pathogenicity islands [4]. Pathogenic *E. coli* strains include Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enterohemorrhagic *E. coli*, Enteroaggregative *E. coli* (EAEC), and Enteroinvasive *E. coli* (EIEC) [5]. Each of these pathotypes has virulence elements like producing toxins, hemolysins, proteases, or having a hydrophobic cell surface etc. that, when put together, make them dangerous and play a big part in infection [6,7]. In addition to the impact of virulence gene acquisition and its influence on pathogenicity, resistance gene acquisition plays a significant role in therapeutic failure and the rise in the death rate. By 2050, the failure to manage the growth of multi-drug (MDR), extensive drug (XDR), and pan-drug resistance would raise the death rate by 10 million [8]. Misuse and overuse of antibiotics in human and veterinary medicine and as growth promoters in livestock have led to resistance to several antimicrobial classes, including β-lactam, cephalosporins, tetracyclines, macrolides, and polymyxins [9]. The most recent *E. coli* epidemic occurrence was reported by Centers for Disease Control and Prevention (CDC) in August of 2022, with 37 confirmed cases in four states of the United States of America, although the outbreak's source is unknown [10]. In May

2021, 17 people in Arizona got *E. coli* O157:H7 infections from pasteurized yogurt, according to the Washington State Department of Health [11]. In most developing nations, such as Bangladesh, there is typically inadequate regulation and inspection of *E. coli* infection rate in market-sold commodities, such as fresh vegetables and fruits. More awareness needs to be raised, especially when the populace's understanding of the issue is inadequate. Multiple papers have documented the presence of pathogenic *E. coli* in fresh produce [12]. For this reason, it is essential to carry out researches on the detection and level of contamination of pathogenic *E. coli* in salad vegetables sold in traditional grocery stores and markets in Bangladesh in order to characterize any pathogenic *E. coli* by virulence groups in order to provide information for the effective risk assessment of foodborne outbreaks [3,12]. This study aimed to assess the availability of virulence genes in vegetable samples from several regions of Dhaka, as well as their antibiotic resistance profile.

Chapter 2

Methods and Materials

2.1 Samples Collection

Between March to June of 2022, 250 samples were collected from various traditional vegetable markets, grocery shops, and street vendors located around Dhaka. The vegetable samples were collected from 12 different areas of Dhaka city which included Gulshan-1, Gulshan-2, Banani, Mohakhali, Farmgate, Rampura, Khilgaon, Dhanmondi, Uttara, Mohammadpur, Mirpur, and Bashundhara. The samples were brought to the laboratory in insulated ice box on the same day they were purchased from the various vendors.

2.2 Sample Type

In this study the following vegetables samples were collected from different local markets and street vendors of Dhaka city: Lettuce (*Lactuca sativa*), Tomato (*Solanum lycopersicum L.*), Capsicum (*Capsicum annuum*), Cucumber (*Cucumis sativus*), Cabbage (*Brassica oleracea L. var. capitata*), Spring Onion (*Allium fistulosum*), Carrot (*Daucus carota*), Coriander (*Coriandrum sativum*), Mint (*Mentha spicata subsp. spicata*), and Green Chili (*Capsicum frutescens*). The vegetable samples were chosen based on the criteria that are often eaten raw as salad.

2.3 Preparation of Samples

The *E. coli* strains were needed to be enriched and for this purpose the collected vegetable samples were chopped into tiny pieces, and 25 g of each sample was combined with 250 mL of EC broth (Hi-media, India) (**Figure 1**), which was then incubated for 18–24 hours at 37°C.

Figure 1: Vegetable samples inoculated in EC Broth for enrichment

2.4 Isolation and Identification

The enriched cultures were diluted and spread over MacConkey (Hi-media, India) agar and incubated at 37°C for 18–24 hours. At least five putative single positive colonies with *E. coli* characteristic color (pink) (**Figure 2)** were chosen from the plates. The isolated colonies were sub-cultured multiple times to measure colony homogeneity. Bacteria were identified using colony characteristics, Gram's staining, and a series of biochemical tests, including the Citrate test, the Motility, Urease (MIU) test. Methyl red, Indole test, Voges-Proskauer (V-P), Catalase and Coagulase test [13]. Moreover, PCR was conducted to confirm *E. coli* by targeting *Eco* gene using *ATCC* gene as positive control by the following primer (**Figure 6)** (ECO-1: 5'-GACCTCGGTTTAGTTCACAGA-3'; ECO-2: 5'-CACACGCTGACGCTGACCA-3'; 585 bp). The isolated *E. coli* were stored for further usage with 30% glycerol at −20°C.

2.5 Antimicrobial Susceptibility Test

An antimicrobial susceptibility test was performed on Mueller–Hinton agar (MHA) (HiMedia, India) [14] by using the Kirby–Bauer disk diffusion method with the mentioned antibiotic disks: Amikacin (30 µg), Gentamycin (10 µg), Kanamycin (30 µg), Streptomycin (10 µg), Imipenem (10 µg), Meropenem (10 µg), Ampicillin (10 µg), Ciprofloxacin (5 µg), Erythromycin (15 µg), Chloramphenicol (30 µg), Colistin (10 µg), and Tetracycline (30 µg) (**Table 1**). The turbidity of bacterial suspension was set to 0.5 McFarland by suspending

overnight colonies in 1 mL of 0.9% NaCl solution. A comparison was made between the zone sizes of the bacteria and the requirements for resistance and sensitivity established by the Clinical and Laboratory Standards Institute (CLSI) (M100-28th ed.) [15]. Furthermore, multidrug resistant and extensive drag resistant pattern had been analyzed. Multidrug-resistant (MDR) is acquired resistance to at least one antimicrobial agent from three or more antimicrobial groups. Extensive drug-resistant, or XDR, is characterized as resistance to at least one antimicrobial agent in all but two or fewer antimicrobial categories [16]. All XDR falls into MDR but all MDR does not falls into XDR category. **Table 1:** List of antibiotics used in this study

Antibiotic Group	Antibiotic Name	Disc code	Disc potency (µg)	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Aminoglycosides	Amikacin	AK	30	≥ 17	15-16	≤ 14
	Gentamicin	GEN	10	≥ 15	13-14	≤ 12
	Kanamycin	KAN	30	≥ 18	14-17	≤ 13
	Streptomycin	S	10	≥ 15	12-14	≤ 11
Carbapenem	Imipenem	IMI	10	≥ 23	18-22	≤ 19
	Meropenem	MEM	10	≥ 23	18-22	≤ 19
β-lactam	Ampicillin	AMP	10	≥ 17	12-16	≤ 13
Fluoroquinolone	Ciprofloxacin	CIP	5	≥ 21	16-20	≤ 15
Macrolides	Erythromycin	E	15	≥ 23	14-22	≤ 13
Phenicols	Chloramphenicol	C	30	≥ 18	13-17	≤ 12
Polymyxin	Colistin	CT	10	≥ 17	12-16	≤ 11
Tetracycline	Tetracycline	TE	30	≥ 15	12-14	≤ 11

Table 1: List of antibiotics used in this study

2.6 DNA Extraction

Overnight culture was used for DNA extraction and then it was centrifuged at 13,000–16,000 × g for 2 minutes to pellet the cells and after that the supernatant was removed. After that DNA was extracted by using the Wizard Genomic DNA Purification Kit from Promega Corp [17]. In this procedure 1ml of an overnight culture is added to a 1.5ml microcentrifuge tube and then it is centrifuged at 13,000–16,000 × g for 2 minutes to pellet the cells and the supernatant removed. After that 600µl of Nuclei Lysis Solution was added and gently pipette until the cells are resuspended. Then the microcentrifuge tubes were incubated at 80°C water bath for 5 minutes to lyse the cells; then cooled to room temperature. Again 3µl of RNase Solution was added to the cell lysate and inverted the tube 2–5 times to mix. After that the microcentrifuge tubes were incubated at 37°C water bath for 15–60 minutes and cooled the sample to room temperature. Next, 200µl of Protein Precipitation Solution added to the RNase-treated cell lysate and vortexed vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution with the cell lysate. After that the microcentrifuge tubes were incubated on ice for 5 minutes and centrifuged at 13,000–16,000 × g for 3 minutes. Then the supernatant containing the DNA was transferred to a clean 1.5ml microcentrifuge tube containing 600µl of room temperature isopropanol and gently mixed by inversion until the thread-like strands of DNA form a visible mass. Again the microcentrifuge tubes were centrifuged at 13,000–16,000 × g for 2 minutes and carefully poured off the supernatant and drained the tube on clean absorbent paper. After that 600µl of room temperature 70% ethanol was added and gently inverted the tube several times to wash the DNA pellet. Then centrifuged at 13,000–16,000 × g for 2 minutes and carefully aspirated the ethanol. Next, drained the tube on clean absorbent paper and allowed the pellet to air-dry for 10–15 minutes. Finally, 100µl of DNA Rehydration Solution was added to the tube and rehydrated the DNA by incubating at 65°C for 1 hour and then stored the DNA at 2–8°C for further study purposes.

2.7 Targeted Genes and Primers

Certain genes were selected to test the availability to *Escherichia coli*'s pathogenic strains in the vegetable samples which includes: *Stx* gene for detecting Shiga toxin (STEC), *EaeA* gene for detecting Enteropathogenic *E. coli* (EPEC), *IpaH* gene for detecting Enteroinvasive *E. coli* (EIEC), heat labile *EltB* toxin gene for detecting Enterotoxigenic *E. coli* (ETEC), *FliC* gene for detecting Enterohemorrhagic *E. coli* or *E. coli* O157:H7 (EHEC) (**Table 2**).

Target Gene	Primer	Sequence	Amplicon Size	Conditions	Reference
Stx (STEC / Shiga Toxin)	*stx1*	F- 5'-GGGATAGATCCAGAGGAAGG-3'	622 bp	5 min at 95ºC; 1 min at 95ºC; 1 min at 60ºC; 1 min at 72ºC; 5 min at 72ºC; 20 Cycle.	[18]
		R-5'-CCGGACACATAGAAGGAAACTC-3'			
	stx2	F-5'-CTGGCGTTAATGGAGTTCAG-3'	381 bp		
		R-5'-CCTGTCGCCAGTTATCTGAC-3'			
EaeA (EPEC)	*eaeA*	F-5'-CCCGAATTCGGCACAAGCATAAGC-3'	629 bp	5 min at 94ºC; 40 sec at 95ºC; 20 sec at 65ºC; 45 sec at 72ºC; 5 min at 72ºC; 35 Cycle.	[19]
		R-5'-CCCGGATCCGTCTCGCCAGTATTC-3'			
	EAE157	F-5'- CAGGTCGTCGTGTCTGCTAAA-3'	1087 bp	5 min at 94ºC; 1 min at 94ºC; 1 min at 55ºC; 2 min at 72ºC; 5 min at 72ºC; 35 Cycle.	[20]
		R-5'-TCAGCGTGGTTGGATCAACCT-3'			
IpaH (EIEC)	*ipaH*	F-5'-AGGTTAATCTTTGCAGGGCT-3'	425 bp	3 min at 95ºC; 1 min at 94°C; 1 min at 55°C; 1 min at 72ºC; 10 min at 72°C; 35 Cycle.	[21]
		R-5'-CAACAACCAGCTTACTGCCT-3'			
EltB (ETEC)	*eltB*	F-5'-TCTCTATGTGCATACGGAGC-3'	322 bp	5 min at 95ºC; 30 sec at 95°C; 45 sec at 60°C; 1 min at 72ºC; 10 min at 72°C; 25 Cycle.	[22]
		R-5'-CCATACTGATTGCCGCAAT-3'			

fliC (EHEC)	*fliCh7*	F-5'-GCGCTGTCGAGTTCTATCGAGC-3' R-5'-CAACGGTGACTTTATCGCCATTCC-3'	625 bp	5 min at 94ºC; 45 sec at 94°C; 30 sec at 54°C; 45 sec at 72ºC; 10 min at 72°C; 40 Cycle.	[23]

Table 2: List of primers and PCR conditions for this study

2.8 PCR

Molecular detection of pathogenic *E. coli* was carried out by Singleplex PCR with the help of the PCR machine (The Applied Biosystems *2720 Thermal Cycler)*. To conduct the procedure 25µl PCR mixture was prepared of every isolated bacterium of each primer which contained 5µl Nuclease free water, 2µl Forward primer, 2µl Reverse primer, 12µl PCR Master Mix (EmeraldAmp), 4µl Template DNA which was vortexed for 3-5sec to mix properly. Then PCR was performed by maintaining the PCR conditions for each targeted gene (**Table 2**). After completing PCR, all the PCR products needed to be sorted at - 20°C for further analysis.

2.9 Agarose Gel Electrophoresis

5µl of 50 bp ladder (BioLab) were used to conduct agarose gel electrophoresis and the electricity was run for 50 minutes at 100 V. 1.5% gel agarose were used and the gel was stained with MIDORI Green and DNA band was observed under ultraviolet light. TAE (Tris-acetate-EDTA) buffer was used to prepare the gel as well as running buffer.

Chapter 3

Results

3.1 Area-wise Distribution of *Escherichia coli* Positive Samples

A total of 101 (40.4%) **(Table 4)** *E. coli*-positive samples were detected (**Figure 2)** among the 250 vegetable samples taken from various local vegetable markets around Dhaka city. Dhanmondi (12.87%) had the most positive isolates, followed by Rampura (11.88%), Farmgate (9.9%), Uttara (9.9%), Gulshan-1(7.92%), Gulshan-2 (7.92%), Banani (7.92%), Khilgoan (7.92%), Mirpur (6.94%), Mohakhali (5.94%), Mohammadpur (5.94%), and Bashundhara (4.95%). **(Table 3 and Figure 3)**.

Figure-2: *E. coli* colonies in MacConkey (Hi-media) Agar

Area Name	Positive Samples n (%)
Gulshan-1	8 (7.92%)
Banani	8 (7.92%)
Mohakhali	6 (5.94%)
Farmgate	10 (9.9%)
Rampura	12 (11.88%)
Khilgaon	8 (7.92%)
Dhanmondi	13 (12.87%)

Gulshan-2	8 (7.92%)
Mohammadpur	6 (5.94%)
Uttara	10 (9.9%)
Mirpur	7 (6.94%)
Bashundhara	5 (4.95%)
Total	101 (100%)

Table 3: *E. coli* positive samples based on area

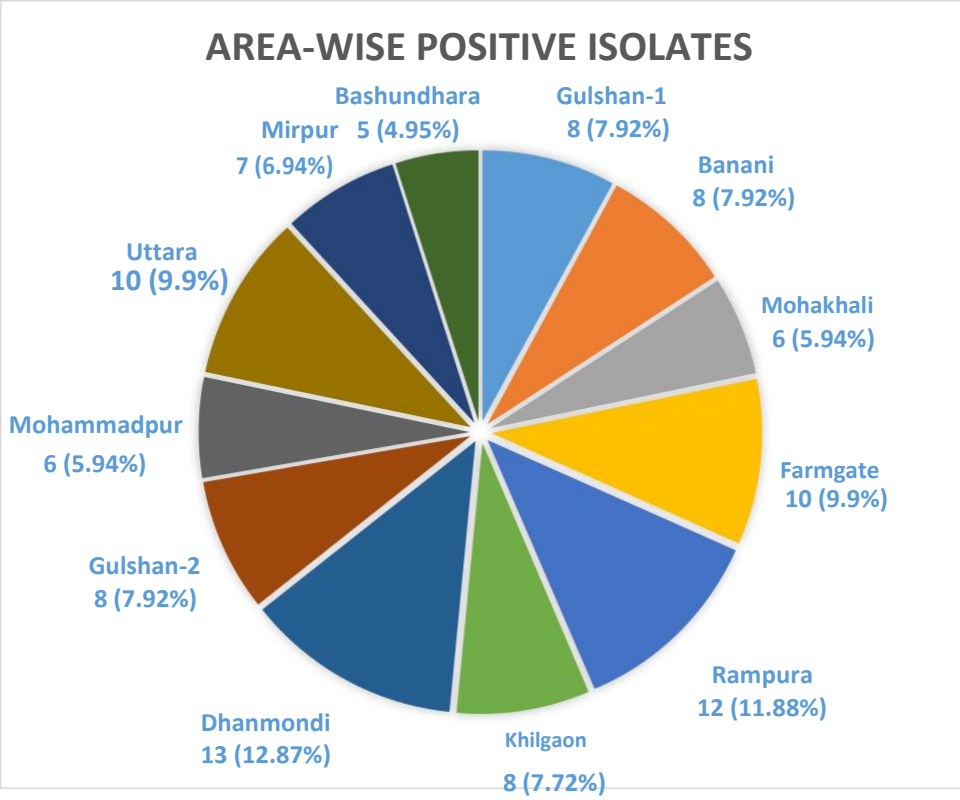

Figure 3: Pie chart of *E. coli* positive samples based on area

3.2 *Escherichia coli* Positive Isolates Distribution Per Sample

There were a total of 101 positive isolates, which indicated 40.4% of the total, and among these, the number of positive isolates in Tomato was the highest, at 52.0%. Respectively followed by Capsicum (51.85%), Carrot (51.35%), Coriander (47.61%), Cabbage (44%), Cucumber

(40.9%), Green Chili (36.84%), Mint (31.81%), Spring Onion (21.42%), Lettuce (20.83%) **(Table 4 and Figure 4)**.

Sample Name	Number of Samples	Positive Isolates Quantity n (%)
Lettuce	24	5 (20.83%)
Tomato	25	13 (52%)
Capsicum	27	14 (51.85%)
Cucumber	22	9 (40.9%)
Cabbage	25	11 (44%)
Spring Onion	28	6 (21.42%)
Carrot	37	19 (51.35%)
Coriander	21	10 (47.61%)
Mint	22	7 (31.81%)
Green Chili	19	7 (36.84%)
Total	250	101 (40.4 %)

Table 4: *E. coli* isolates from different vegetable samples

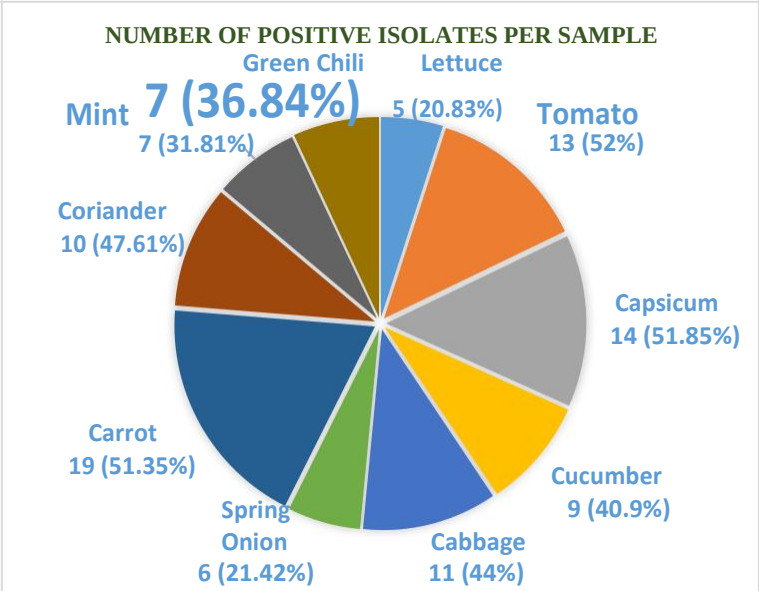

Figure 4: Pie chart of *E. coli* isolates from different vegetable samples

3.3 Antibiotic Susceptibility Test Analysis

In this analysis, among 101 isolates, all strains were resistant to Ampicillin and Erythromycin, and 93.07% were resistant to Colistin. In contrast, 99% of strains were sensitive to Gentamicin, 98.01% to Ciprofloxacin, 97.02% to Meropenem, and 93.06% to Amikacin. In addition, Chloramphenicol and Tetracycline exhibited 86.13% and 85.14% sensitivity, respectively. The patterns of antibiotic resistance demonstrated by other isolates were quite varied **(Table 5)**. Furthermore, 95.04% of the isolates had multidrug resistance (MDR) patterns, and 6.93% of the MDR isolates had extended drug resistance (XDR) **(Figure 5)**.

Antibiotic Group	Antibiotic Name	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Aminoglycosides	Amikacin	94 (93.07%)	4 (3.96%)	3 (2.97%)
	Gentamicin	100 (99%)	0	1 (0.99%)
	Kanamycin	52 (51.48%)	32 (31.68%)	17 (16.83%)
	Streptomycin	24 (23.76%)	33 (32.67%)	44 (43.56%)
Carbapenem	Imipenem	77 (76.23%)	6 (5.94%)	18 (17.82)
	Meropenem	98(97.02%)	3 (2.97)	0
β-lactam	Ampicillin	0	0	101 (100%)
Fluoroquinolone	Ciprofloxacin	99 (98.01%)	2 (1.98%)	0
Macrolides	Erythromycin	0	0	101 (100%)
Phenicols	Chloramphenicol	87 (86.13%)	0	14 (13.86%)
Polymyxin	Colistin	0	7 (6.93%)	94 (93.07%)
Tetracycline	Tetracycline	86 (85.14%)	0	15 (14.85%)

Table 5: Antibiotic Susceptibility profile of *E. coli* positive isolates

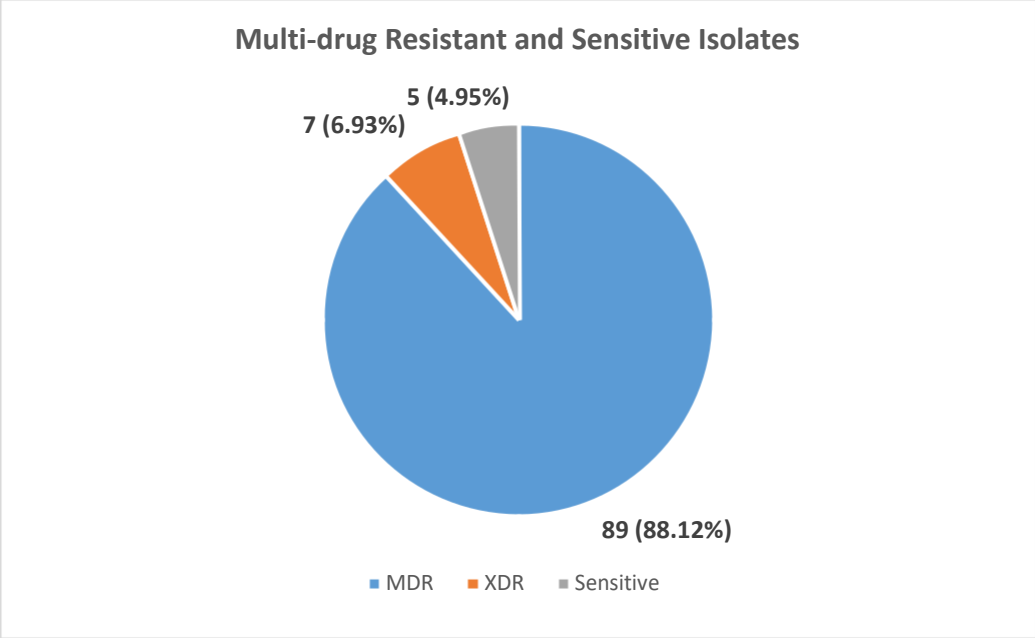

Figure 5: Multi-drug resistant and sensitive isolates

3.4 Genotyping

After performing PCR for *E. coli* confirmation to detect *the eco* gene, the expected band line was detected in the gel agarose electrophoresis test (**Figure 6)**. Next, 40 strains out of 101 isolates were subjected to PCR analysis to check the availability of virulence genes. 26 (65%) of the 40 isolates **(Table 6)** exhibited positive PCR test findings for pathogenicity. 15 (57.69%) of the 26 positive virulence gene isolates were EPEC positive. 11 (42.30%) and 4 (15.38%) of these isolates were identified with the primers *eaeA* and *EAE157*, respectively. The *IpaH* (EIEC) positive gene was detected in four (15.38%) samples: carrot and spring onion. *FliC* (EHEC) genes were detected in 3 (11.53%) cabbage and lettuce isolates. Both the *Stx* (STEC) and *EltB* (ETEC) genes were detected in 2 (7.7%) isolates of cucumber, coriander, mint, and cabbage. In addition, area-wise analysis of the 26 pathogenic strains revealed that the most significant proportion of positive virulence isolates was obtained in Khilgaon (19.23%), followed by Farmgate (15.38%), Rampura (11.53%), and Gulshan-2 (11.53%) **(Table 7)**. Furthermore, it was observed that among the 65% virulence

gene *eaeA* was the highest (4.23%), and it was found in Coriander, Cucumber, Tomato, Carrot, Spring onion, Green chili, and Mint.

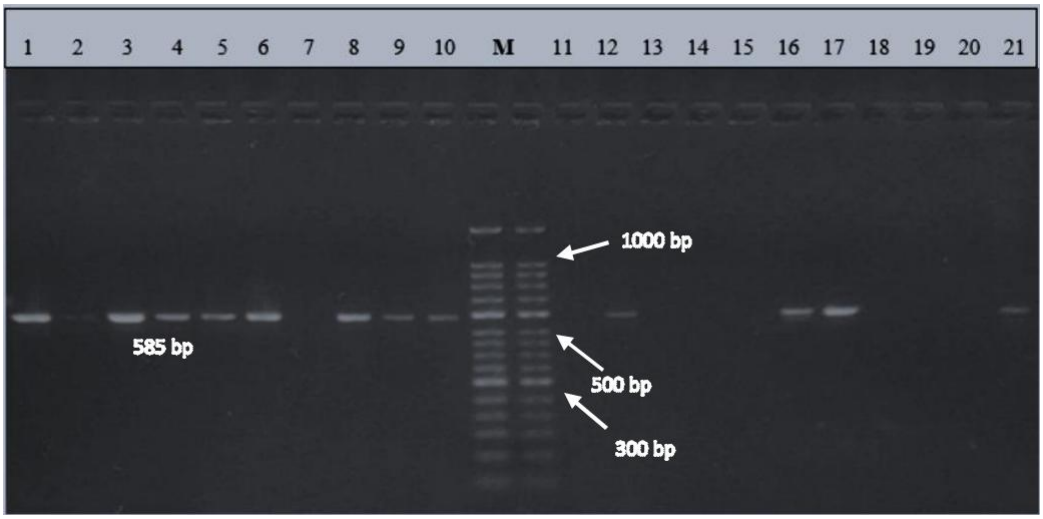

Figure 6: *E. coli* confirmatory PCR result. [Here M was a 50 bp DNA marker. Lane 1-19 different samples were loaded, 20 was negative control and 21 was positive control (*ATCC*). Amplicon size 585 bp.]

Isolates	Presence of Virulence Gene and Primer						
	stx1	***stx2***	***eaeA***	***EAE157***	***ipaH***	***eltB***	***flich7***
S-5			(+)				
S-40					(+)		
S-44					(+)		
S-51			(+)				
S-41							
S-21			(+)				
S-74			(+)				
S-79			(+)				
S-23							
S-95				(+)			
S-70							(+)
S-100			(+)				
S-45			(+)				
S-24						(+)	

S-31							(+)
S-47		(+)					
S-52						(+)	
S-97							
S-91							
S-72			(+)				
S-37							
S-38				(+)			
S-32				(+)			
S-27			(+)				
S-30							
S-17							
S-49					(+)		
S-20		(+)					
S-22							
S-62				(+)			
S-87							
S-69							
S-60					(+)		
S-73							(+)
S-55							
S-59							
S-48							
S-6			(+)				
S-15			(+)				
S-12							
Total	0	2(7.7%)	11(42.3%)	4(15.3%)	4(15.3%)	2 (7.7)	3(11.5%)

Table 6: Summary of pathogenic *E. coli* isolates. [Here S indicates isolates and the numerical value represent the serial number of isolates.]

Area Name	Isolates	Presence of Virulence Gene							Quantity of Virulence Gene n (%)
		stx1	***stx2***	***eaeA***	***EAE157***	***ipaH***	***eltB***	***flich7***	
Gulshan-1	S-5			(+)					2 (7.7%)
	S-6			(+)					
Banani	S-15			(+)					1 (3.84%)
Mohakhali	S-20		(+)						2 (7.7%)
	S-21			(+)					
Farmgate	S-27			(+)					4 (15.38%)
	S-31				(+)				
	S-32							(+)	
	S-24						(+)		
Rampura	S-38				(+)				3 (11.53%)
	S-40					(+)			
	S-44					(+)			
Khilgaon	S-51			(+)					5 (19.23%)
	S-45			(+)					
	S-47		(+)						
	S-52						(+)		
	S-49					(+)			
Dhanmondi	S-60					(+)			2 (7.7%)
	S-62				(+)				
Gulshan-2	S-70							(+)	3 (11.53%)
	S-72			(+)					
	S-73							(+)	
Mohammadpur	S-79			(+)					2 (7.7%)
	S-74			(+)					
Uttara	N/A								0
Mirpur	S-95				(+)				1 (3.84%)
Bashundhara	S-100			(+)					1 (3.84%)
Total									26 (65%)

Table 7: Positive virulence isolates based on area. [Here S indicates isolates and the numerical value represent the serial number of isolates.]

Chapter 4

Discussion

Food-borne infections are a crucial public health concern that demands the attention of both authorities and the general public. This analysis showed the concerning occurrence of pathogenic *E. coli* within the most common and widely consumed fresh vegetables and the general environmental state different of the fresh vegetable market in Dhaka City. The purpose of this study was to determine the characterization and antibiotic-resistant profile of the *E. coli* virulence gene. This experiment obtained 40.4% of *E. coli* containing the sample from 250 vegetables. Among 101 isolates, all strains were resistant to Ampicillin and Erythromycin, and 93.07% were resistant to Colistin. In contrast, 99% of strains were sensitive to Gentamicin,

98.01% to Ciprofloxacin, 97.02% to Meropenem, and 93.06% to Amikacin. This indicated that β-lactam and Macrolides group antibiotics might not be effective for treatment against these virulence genes. Moreover, 95.04% of the isolates exhibited multidrug resistance (MDR) patterns, with 6.93% having extended drug resistance (XDR), a serious concern as drug-resistant organisms are a global public health problem with a high fatality rate. As Gentamicin, Ciprofloxacin, Meropenem, and Amikacin showed high susceptibility against these virulence genes of *E. coli*; these antimicrobials can be utilized to treat diseases caused by these pathogenic strains. Furthermore, 40 isolates from the 101 *E. coli* isolates were tested for pathogenicity using PCR, and 65% of the isolates tested positive, which indicates the high-level availability of *E. coli* virulence genes in fresh produce that people commonly consume.

In addition, *the stx2* gene was detected here, which can produce Shiga toxin. Infection with STEC typically manifests as gastrointestinal illness, such as mild or bloody diarrhea and hemorrhagic colitis (HC), and may proceed to the life-threatening hemolytic uremic syndrome (HUS) [24]. 15 (57.69%) of the 26 positive virulence gene isolates were EPEC positive. In developing countries, infections caused by EPEC are a significant cause of diarrhea in infants

[25]. Also, EIEC, EHEC, and ETEC positive virulence gene presence, respectively 15.38%, 11.53%, and 7.7% found through PCR analysis. The pathogenic strains can cause various disease syndromes, such as wound infections, meningitis, septicemia, atherosclerosis, hemolytic uremic syndrome, and immunological disorders like reactive and rheumatoid arthritis. These outcomes of the study can help to assume the concerning situation of pathogenic *E. coli* gene presence in fresh vegetables. Nonetheless, this study provided a basic overview of the microbiological quality of fresh vegetables in Dhaka, which will aid in taking the necessary steps to ensure hygienic conditions during the vegetable market. Further research is required to assess the presence of pathogenic *E. coli* in commonly consumed fresh vegetables to ensure public health safety and guarantee public health protection.

Chapter 5

Conclusion

Over the past decade, there has been a significant increase in foodborne outbreak cases associated with fresh produce caused by pathogenic *E. coli*. These outbreaks of pathogenic *E. coli* not only resulted in significant monetary losses and severely impacted human health, including mortality. Through the development of novel antimicrobial drugs and vaccines, significant efforts have been made to treat pathogenic *E. coli* and its associated symptoms. Nevertheless, only in the short term, as drug therapy and antibacterial chemicals in the environment and food are only effective temporarily. The best way to stop outbreaks of pathogenic E. coli and other diseases that are spread by contaminated food may be to clean the environment and maintain personal hygiene. The analysis of this study offered a general overview of the microbiological quality of fresh vegetables in Dhaka, which would assist in taking the required actions to maintain sanitary conditions during the vegetable market and increase awareness of practicing hygiene.

Chapter 6

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