Determination of Isolation & Antibiotic Susceptibility Pattern of *Salmonella* spp. from Fresh Vegetables Collected from Local Markets of Dhaka City

by

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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. We have acknowledged all main sources of help.

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Abstract

Many outbreaks of food-borne *Salmonella* have been connected to raw vegetables; however, very little information is known on *Salmonella* contamination of raw vegetables in Dhaka's local wet markets. In this research, isolation of Salmonella isolates & their antibiotic susceptibility pattern were investigated. In total, 50 samples of raw vegetables were collected from the various wet markets from Dhaka city. The samples with the highest level of contamination were tomato (18%, n=10), green chili (18%, n=10), and mint (18%, n=10). The antibiotic resistance patterns of the 55 Salmonella isolates recovered from those positive samples. After analyzing their antibiotic resistance profile, it is found that 55 of those isolates are resistant to at least one or more class of antibiotics, indicating that they are resistant to multiple antibiotics. Antibiotic resistance and multi-resistance of *Salmonella* spp. have developed significantly during the past decade, particularly in the developing nations where the use of antibiotics for human and animal therapy has been increased. The rate of resistance observed for Penicilline and Metronidazole is (100%) higher than all others. Followed by Clindamycin (98%), Erythromycin (95%), Tetracycline (31%), Amoxicillin (48%), Streptomycin (10%), Chloramphenicol (15%), Ceftriaxone (11%). Gentamycin, Co–Trimoxazole, and Imipenem has shown lower resistance to this third generation antibiotics, rather than showing more susceptibility to *Salmonella* spp. (98%, 93%, and 78%). This research had found that the contamination of *Salmonella* in raw vegetables offers a potential risk to public health.

Keywords: *Salmonella* spp.; Fresh vegetables; contamination; multi-drug resistance; sensitivity

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List of Acronyms:

XLD: Xylose Lysine Dexoycholate Agar

SS Agar: Salmonella Shigella Agar

MR: Methyl Red

VP: Voges-proskauer

TSI: Triple Sugar Iron

MHA: Muller Hinton Agar

MIU: Motility Indole Urease

AST: Antibiotic Susceptibility Test

µL: Micro-liter

mm: Millimeters

spp.: Species

WHO: World Health Organization

Chapter 1

Introduction

1.1.Background of the study

Salmonella is a group of bacteria belong to the family Enterobacteriaceae. It is a Gram-negative rod-shaped bacillus. *Salmonella* is one of the most dangerous food-borne illnesses in the world, and most people get it from eating food that has been contaminated (Kirk et al.,2015). Globally, Salmonella is responsible for 200 million to 1 billion infections each year, 93 million cases of gastroenteritis, 155,000 deaths, and 85 percent of food-borne illnesses. (Baiya et al.,2022)

Fresh vegetables are widely consumed around the world because they are an excellent source of dietary fiber, vitamins, and minerals, all of which are essential components of a healthy diet. In recent years, however, there have been reports of a growing number of human diseases and outbreaks linked to the ingestion of contaminated raw vegetables, particularly those caused by *Salmonella* (Callejon et al., 2015). Likewise, it is estimated that around 50% of *Salmonella* cases are caused by fruits and vegetables (Agnes et al.,2018). Raw vegetable eating has been associated to these illnesses. *Salmonella* can invade numerous plant species via their seeds, leaves, and fruits (Winthrop et al., 2003). According to statistics, it is the most common bacterial pathogen responsible for outbreaks of infections connected with fresh produce in developed countries, accounting for at least half of these outbreaks in the United States (53%) and the European Union (50%) combined (Sivapalasingam et al., 2004).

Salmonella is the pathogen that can cause infection, often known as salmonellosis. Ehuwa et al. (2021) investigated that *Salmonella* contamination of food & goods are connected to salmonellosis. The bacteria can causes illness by colonizing the digestive system once infective doses are consumed. Infected food & goods were a direct cause of the *Salmonella* epidemic that caused 1581 cases in Slovakia, Spain, and Poland. With the drive for ready-to-eat food products on a worldwide scale, it is becoming a serious challenge. The fact that this particular category of devices receives so little heating raises more serious safety concerns. The risk is further increased by the fact that they might be ingested without being subjected to extreme heat.

Again, salmonellosis is responsible for an estimated one and a half million cases of food-borne illness and more than four hundred deaths every year in the United States, as reported by the Centers for Disease Control and Prevention (CDC). *Salmonella* was found to be the most prevalent type of bacterial infection that was reported in 2007, according to the Surveillance Report from the Food Diseases Active Surveillance (FoodNet) program. Again, a study conducted in European Union and the European Economic Area shows that *Salmonella* is the second most frequent gastrointestinal infection and a major source of food-borne outbreaks. Out of the 53 of 169 cases of salmonellosis that were verified in the lab in 2020, 61 people lost their lives as a direct result of the infection (The European Surveillance System (TESSy,2021).

There are few reports in Bangladesh about microbiological contamination of vegetables sold in Dhaka city's local markets. *Salmonella* and other illnesses that spread through food are more common in wet markets, where there are also more contaminations. *Salmonella* is more likely to be found in wet markets than in grocery stores.

Salmonella can get into food by cross contamination. Cross-contamination is the physical movement or transfer of harmful bacteria from one person, object or place to another. Many stages in the production and distribution chain of fresh food can be contaminated with pathogens by direct contact with fecal waste during farming, such as wastewater irrigation and the use of bio-solids or animal manure as fertilizer (Mahbubur et al., 2022).

Food-borne diseases are those that are caused by substances that enter the body through the intake of food and can be contagious or poisonous. Anthrax, Tuberculosis, Brucellosis, Salmonellosis, Campylobacteriosis, and Leptospirosis are the most significant bacterial illnesses recorded in Bangladesh (Md. Najmol et al.,2019).

However prevention of cross contamination is the key factor of prevention food-borne illness. So, for prevention of *Salmonella* from cross contamination, eating raw or barely cooked eggs or meat should be stopped. According to Todd (2020), it is possible that customers value convenience and time savings over wholesome food preparation and handling. While most consumers are aware of the value of cross-contamination and heating in decreasing the development of food-borne disease, Fischeret AL research's demonstrated that this knowledge is not always transferred into behavior. Throughout the home food preparation process, potentially dangerous behaviors were seen including participant mistakes such letting raw meat fluids touch the finished dish. Declarative rather than procedural food safety knowledge was shown to be a superior predictor of effective bacterial reduction, according to the authors.

1.2. Aims & Objective:

1. This study could determine the detection and isolation of *Salmonella* from fresh vegetables in various local markets of Dhaka city

2. Due to the increasing prevalence of multidrug resistant organisms, this research also aimed to identify the antibiotic resistance profile.

3. Detect multidrug-resistant organisms among isolated bacterial contaminants.

Chapter 2

Materials & Methods:

2.1. Sample Collection Site:

For the purposes of this study, vegetable samples were collected from various wet markets located throughout the Dhaka city.

2.2. Duration& Source:

The study was conducted from January 2022 to May 2022 from different local wet vegetable markets in Dhaka city.

2.3. Sample Collection & numbers:

Every sample was wrapped in sterile zipper bags, given the appropriate labels, placed in ice packs to ensure that the cold temperature was maintained, and then transported to the laboratory for examination.

To avoid getting any of the samples contaminated, proper cautions was used at every stage, and hand gloves were worn whenever the samples were being handled.

Samples were: cabbages (n = 05), lettuces (n = 05), mint (n = 05), coriander (n = 05), capsicums (n = 05), tomatoes (n = 05), carrots (n = 05), cucumbers (n = 05), green chilies (n = 05) and spring onions (n = 05).

Total number of samples and areas are shown in table 1:

Season - Winter

AREA NAME	NUMBER OF SAMPLE
1.TONGI BAZAR	**10**
2.UTTARA KACHA BAZAR	**10**
3. MIRPUR 11 KACHA BAZAR	**10**
4. GULSHAN DCC MARKET	**10**
5.FARMGATE BAZAR	**10**
TOTAL :5	**TOTAL:50**

Table 1: Area name, number & Sample number

2.4. Sample preparation and inoculation of growth medium:

With sterilized knives, each of the samples was cut into little pieces and placed in a laminar airflow. For every one of the ten samples, a volume of 500µl of LB broth, also known as Luria-Bertani Broth, was prepared. LB broth is a nutrient-dense medium that encourages the growth of bacteria.

Each of the vegetable samples that had been weighed 5 g was placed in conical flasks that each contained 50 µl of LB broth. These flasks were then placed in a shaker incubator set to 37 °C for 24 hours. After done with incubation a 10^{-2} serial dilution was performed for each tube and transferring 200µl of overnight grown LB broth to 15 ml of distilled water test tubes.

2.5. Incubation on Xylose Lysine Deoxycholate agar (XLD Agar) Media:

After 10-2 serial dilutions, 30 µl was put onto XLD agar plates and the sample was circulated in the media with a sterile glass rod by pushing it back and forth. When the sample became sticky, the plate was flipped and incubated at 37°C for 24 hours. In this media, *Salmonella* decarboxylate lysine and breaks down thiosulfate to create hydrogen sulfide, which results in the development of red colonies with black centers in the media. Few presumptive colonies were chosen for additional biochemical and antimicrobial testing.

2.6. Incubation on Salmonella-Shigella (SS) media : The next step was to streak *Salmonella*-Shigella (SS) agar with the presumed colonies of a 24-hour bacterial culture obtained from the XLD agar and incubated at 37°C for 24 hours. Salmonella Shigella (SS) Agar is a differential medium with moderately selectivity for the isolation, culture, and differentiation of *Salmonella* spp. In this media, *Salmonella* will produce hydrogen sulfide (H2S) gas that result bacterial will form colorless colony with a black center.

2.7. Biochemical characteristics of *Salmonella spp.:* Biochemical identification was done of the selected 55 isolates of *Salmonella* spp. which include Catalase test , Oxidase test, Citrate utilization test, Methyl Red test , Voges-Proskauer test, Motility, Indole, Urease test, Triple sugar iron test for sugar fermentation. After that, the result was analyzed with the comparison of the Biochemical reference chart for more accuracy. After that, the tested *Salmonella* isolates were confirmed.

Biochemical tests	**Reaction**		
Catalase	+		
Oxidase	-		
Citrate	+		
Methyl Red test (MR)	+		
Voges-Proskauer test(VP)	–		
MIU(Motility , Indole production , Urease)	**Motility**	**Indole**	**Urease**
	+	-	-
TSI(Triple sugar iron test)	**Slant**	**Butt**	**H2S gas production**

	Red/ Yellow	**Black**	+

Table 2: The biochemical reactions carried out by all *Salmonella* spp.

2.8. Culture Preservation:

For short term preservation the 50 Salmonella spp. isolates were streaked into nutrient agar (NA) media and kept in the refrigerator at 4°Cfor 1-2 weeks.

For long term preservation 30% glycerol is used. A sterile inoculating loop was used to take bacteria from a culture plate and mix it with 5ml of Nutrient broth. Then it was left to sit for 24 hours at 37oC. The broth culture was then given of sterile glycerol and the ratio for both will be 250µl. Then, the eppendorf was put in a freezer at -20°C

2.9. Antibiotic Susceptibility Testing (AST):

For the purpose of determining the isolates' susceptibilities, the Kirby-Bauer disc diffusion method was used. Again, for determining which bacteria are susceptible to antibiotics and which are resistant, approximately 14 different antibiotic discs were used. The table below lists the antibiotics that were utilized in the research project.

Protocols for AST:

1. Standardized inoculum of 0.5 McFarland (approximate cell count density: 1.5x108) turbidity standard was prepared by taking 1-2 colonies of organisms.

2. Each of the test bacterial strains was lawn cultivated on correctly labeled Mueller Hinton Agar plates using sterile cotton swabs. The plates were then properly marked.

3. After the lawn culture, sterile forceps were used to gently pick out antibiotic disks from the stacks. These antibiotic disks were then very carefully placed on top of the lawn culture.

4. To avoid having inhibition zones that overlap with one another, care was taken to ensure that the disks have adequate spacing between them.

5. After incubation, antibiotic resistance was evaluated by calculating the diameter of the inhibitory zone in millimeters.

6. According to the Clinical and Laboratory Standards Institute, microorganisms are classified as being either resistant (R), intermediate (I), or susceptible (S) based on their zone of diameter (mm) values (CLSI) guidelines (CLSI, 2021) (Table 2)

SERIAL NO	ANTIBIOTIC	ANTIBIOTIC GROUP	DISC CODE	DISC POTENCY (µG)	RESISTANT (MM)	INTERMEDIATE (MM)	SENSITIVE (MM)
1	PENICILLIN	BETA-LACTAM	PEN	10	≤ 19	20-27	≥ 28
2	AMOXICILLIN		AML	10	≤ 13	14-17	≥ 18
3	STREPTOMYCIN	AMINOGLY-COSIDES	S	10	≤ 11	12-14	≥ 15
4	GENTAMYCIN		GEN	10	≤ 12	13-14	≥ 15
5	LEVOFLOXACIN	QUINOLONES	LE	5	≤ 18	19-27	≥ 28
6	CIPROFLOXACIN		CIP	5	≤ 20	21-30	≥ 31
7	ERYTHROMYCIN	MACROLIDES	E	15	≤ 13	14-22	≥ 23
8	TETRACYCLINE	TETRACYCLINE	TE	30	≤ 11	12-14	≥ 15
9	CHLORAMPHENICOL	PHENICOLS	C	30	≤ 12	13-17	≥ 18
10	CEFTRIAXONE	CEPHALOSPORIN	CTR	10	≤ 19	20-22	≥ 23
11	IMIPENEM	CARBAPENEM	IPM	10	≤ 19	20-22	≥ 23
12	CLINDAMYCIN	LINCOSAMIDES	CD	2	≤ 14	15-20	≥ 21
13	CO-TRIMOXAZOLE	SULFONAMIDES	COT	1.25	≤ 10	11-15	≥ 16
14	METRONIDAZOLE	NITROIMIDAZOLE	MT	5	≤ 15	16-18	≥ 20

Table 3: The antibiotic disks, their concentrations, and the sizes of the zones of inhibition (mm) for Enterobacteriaceae

Flowchart of the Study Design:

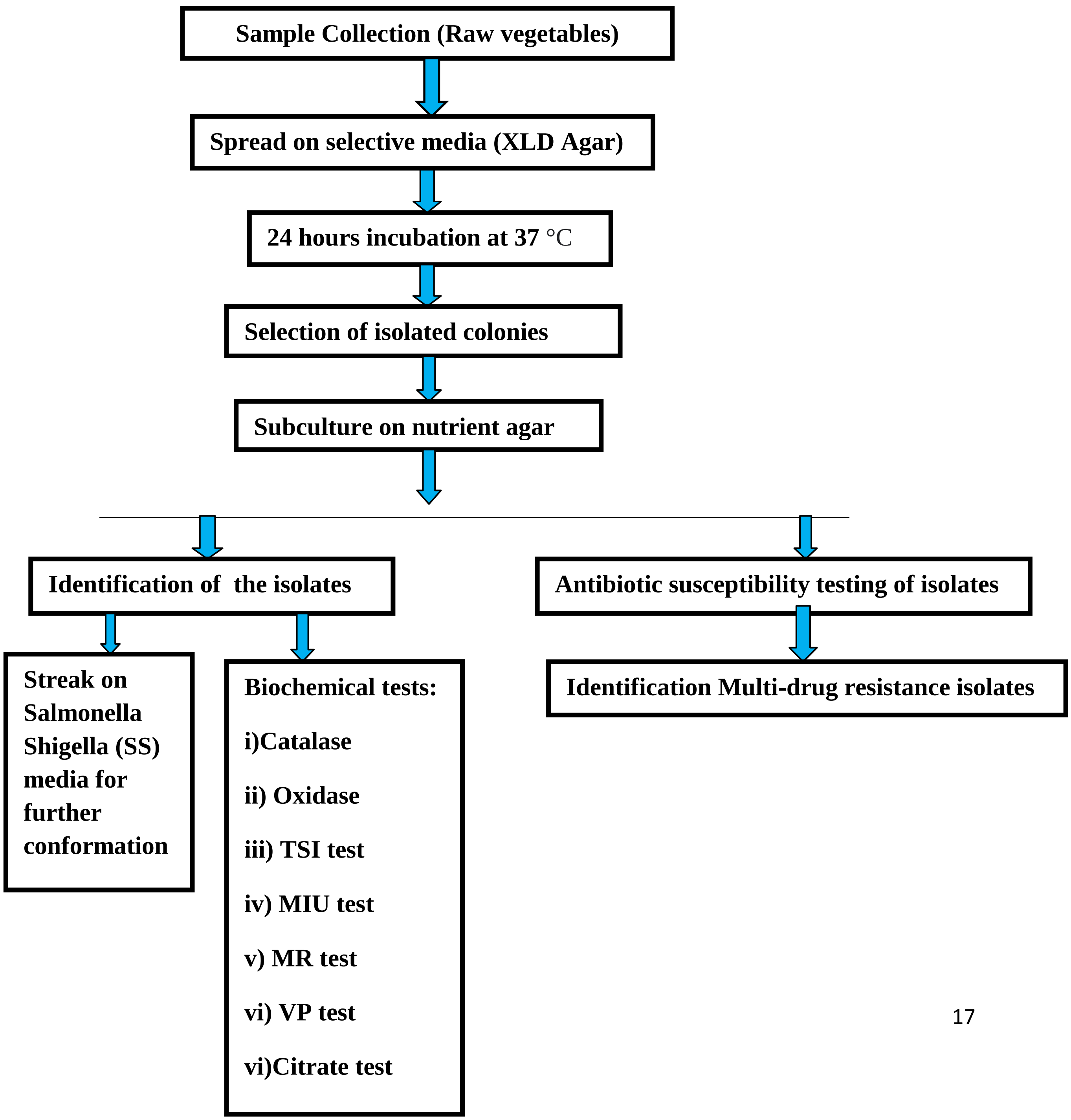


Figure 1: Flowchart of study design

Chapter 3

Result:

3.1. Bacterial Identification on XLD agar (Xylose Lysine Deoxycholate agar) and SS agar (Salmonella-Shigella Agar):

A total of 50 samples, were taken from different vegetable markets in different areas and 55 bacteria are isolated from those samples. In order to identify the organisms that were present in each sample, these samples were streaked onto a variety of agar media, including XLD and SS agar. After 24 hours of incubation at 37°C, all 55 isolates grew red colonies with black centers on XLD agar and colorless colonies with black centers on SS agar. This is how *Salmonella* spp. supposed to grow on those specific growth media.

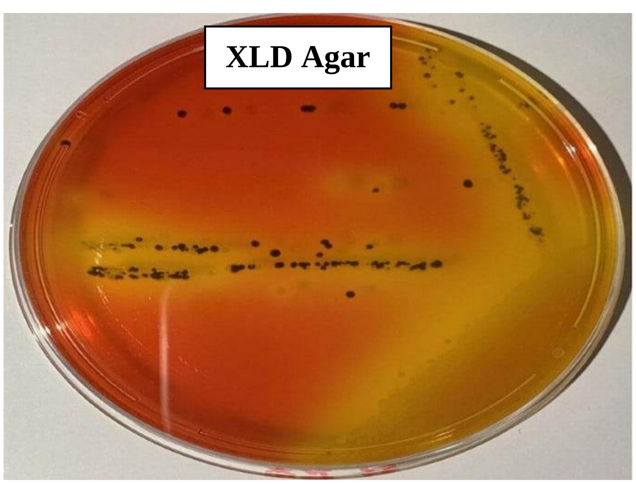


Figure 2: *Salmonella* growth on XLD (Xylose Lysine Deoxycholate agar) and SS agar (Salmonella-Shigella Agar)

3.2. Distribution of *Salmonella* spp. on different vegetables:

There were a total of 29 positive results for *Salmonella* spp. out of the 50 raw vegetable samples, and 55 possible bacteria were isolated from those 29 samples throughout this investigation. The highest positive rate for *Salmonella* spp. was identified in mint, green chili & tomato was(18%).Also, we found coriander, cabbage & capsicum has the positive rate (9%), cucumber (15%), lettuce (4%). The lowest positive rate was reported in both carrot &spring onion (0%).

VEGETABLES	NO. OF VEGETABLES	VEGETABLES POSITIVE FOR *SALMONELLA* SPP
GREEN CHILLI	05	05
TOMATO	05	05
CUCUMBER	05	05
MINT	05	04
CAPSICUM	05	03
CORIANDER	05	03
CABBAGE	05	02
LETTUCE	05	02
CARROT	05	0
SPRING ONION	05	0
TOTAL = 10	TOTAL=50	TOTAL =29

Table 4:Number of positive samples for *Salmonella* spp.

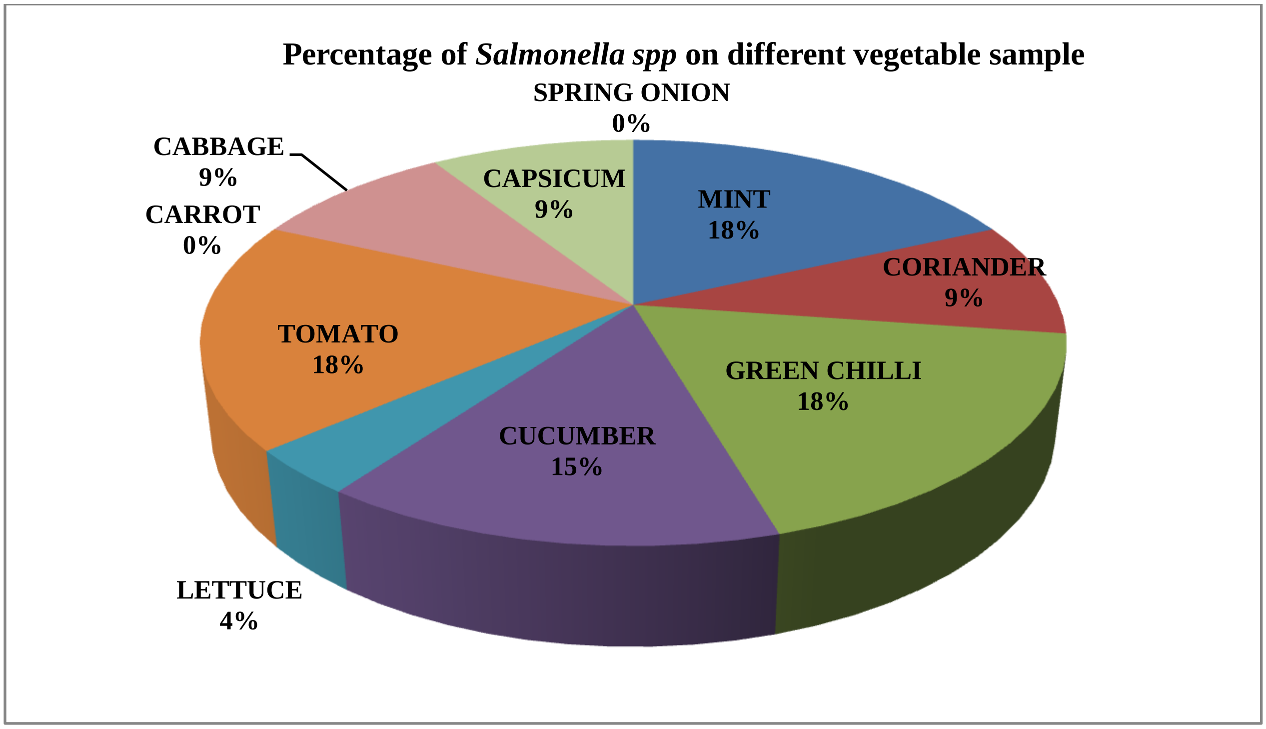
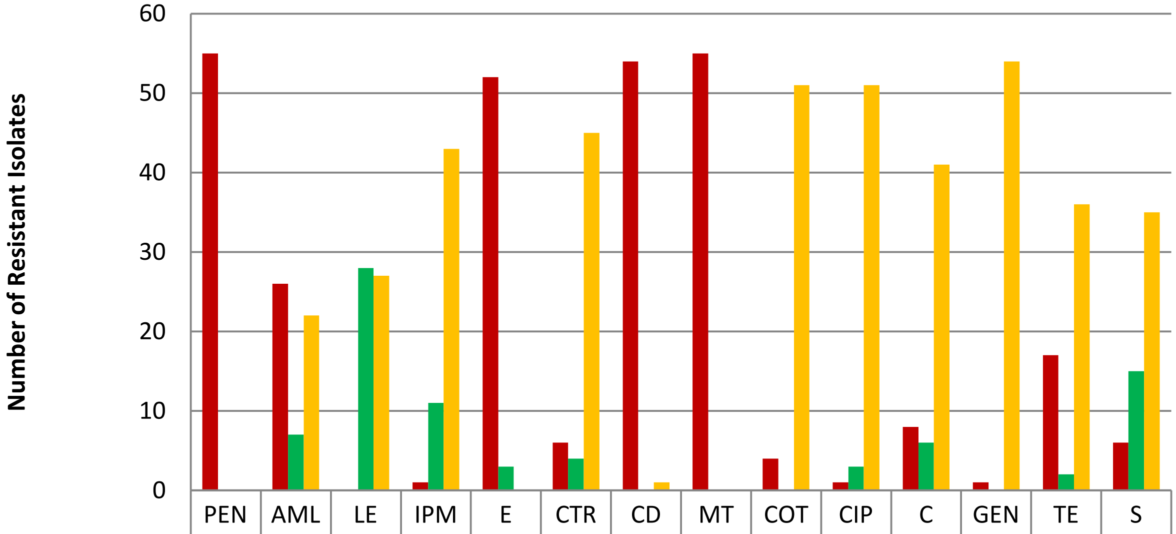


Figure 3: Percentage (%) of *Salmonella* spp. on different vegetables

3.3. Antibiotic resistance profile of 55 isolates:

Bacterial isolates were chosen for an antibiotic susceptibility test after their identification and confirmation. There were approximately fourteen different antibiotics used for each of the fifty five different strains that were identified from the vegetable samples. The susceptibility of the isolates to these antibiotics, as well as their patterns of resistance analyzed. Here we found the antibiotic susceptibility of all the isolates (100%) were resistant to Penicilline and Metronidazole(100%); Followed by Clindamycin (98%)Erythromycin (95%), Tetracycline (31%), Amoxicillin (48%), Streptomycin (10%), Chloramphenicol (15%), Ceftriaxone (11%),Co – Trimoxazole (7%),Levofloxain (0%), Gentamycin , Ciprofloxacin, and Imipenem (1%)respectively. In contrast, the susceptibility of *Salmonella* spp. to the antibiotics Gentamycin, Co–Trimoxazole, and Imipenem was found to be 98%, 93%, and 78% correspondingly in this study. These antibiotics were found to be the most efficient against *Salmonella* spp. The chart for antibiotic susceptibility pattern is shown below:

After measuring the zone of inhibition (mm), the organisms were classified as Resistant (R), Intermediate Resistant (I), or Sensitive per CLSI (2021) guidelines (S)



	PEN	AML	LE	IPM	E	CTR	CD	MT	COT	CIP	C	GEN	TE	S
Resistant	55	26	0	1	52	6	54	55	4	1	8	1	17	6
Intermediate	0	7	28	11	3	4	0	0	0	3	6	0	2	15
Sensitive	0	22	27	43	0	45	1	0	51	51	41	54	36	35

Figure 4: Number of (R) Resistant, (I) Intermediate, (S) Sensitive for tested 14 antibiotics

3.4. Identification of the Multidrug resistant isolates:

Antibiotic resistance patterns showed that many of the organisms were resistant to three or more drugs. Each and every one of the isolates had a MAR Index of 0.28 or above, those are shown in table below: Here, we found that Mint-4 from area 4 has shown a high resistance pattern. Among all 14 antibiotics it is resistant to 12 antibiotics which made it high resistance bacteria among those 55 tested isolates of *Salmonella* spp.

SERIAL NO	**AREA NO**	**SAMPLES OF SALMONELLA SPP.**	**RESISTANT ANTIBIOTICS (A)**	**TOTAL TESTED ANTIBIOTICS (B)**	**MAR (A/B)**	**LEVEL OF RESISTANT**
1	4	MINT-3	4	14	0.285	HIGH
2	4	GC-4	6	14	0.428	HIGH
3	4	MINT-1	6	14	0.428	HIGH
4	4	GC-6	7	14	0.5	HIGH
5	4	GC-11	5	14	0.357	HIGH
6	4	GC-8	5	14	0.357	HIGH

7	**4**	**MINT-4**	**12**	**14**	**0.857**	**HIGH**
8	4	TOM-2	4	14	0.285	HIGH
9	4	TOM-3	4	14	0.285	HIGH
10	4	CU-5	4	14	0.285	HIGH
11	2	CORI-1	6	14	0.428	HIGH
12	2	LET-1	6	14	0.428	HIGH
13	2	TOM-2	5	14	0.357	HIGH
14	2	GC-11	5	14	0.357	HIGH
15	2	CAP-1	4	14	0.285	HIGH
16	2	TOM-1	4	14	0.285	HIGH
17	2	MINT-1	4	14	0.285	HIGH
18	2	CU-1	5	14	0.357	HIGH
20	2	TOM-5	4	14	0.285	HIGH
21	2	TOM-6	4	14	0.285	HIGH
22	3	GC-2	5	14	0.357	HIGH
23	3	TOM-4	5	14	0.357	HIGH
24	3	GC-5	6	14	0.428	HIGH
25	3	TOM-1	5	14	0.357	HIGH
26	3	CAB-1	6	14	0.357	HIGH
27	3	CAP-1	5	14	0.428	HIGH
28	3	CAB-2	5	14	0.357	HIGH
29	3	CAB-4	6	14	0.285	HIGH
30	3	CU-1	5	14	0.357	HIGH
31	3	MINT-2	4	14	0.428	HIGH
32	3	CAB-3	5	14	0.357	HIGH
33	3	MINT-1	6	14	0.571	HIGH
34	3	MINT-3	5	14	0.571	HIGH
35	3	CU-3	4	14	0.285	HIGH
36	3	CU-4	4	14	0.285	HIGH
37	1	MINT-2	8	14	0.571	HIGH
38	1	MINT-3	8	14	0.285	HIGH
39	1	MINT-5	8	14	0.5	HIGH
40	1	CORI-2	4	14	0.285	HIGH
41	1	MINT-1	7	14	0.357	HIGH
42	1	GC-11	4	14	0.287	HIGH
43	1	CORI-1	5	14	0.357	HIGH
44	1	TOM-4	4	14	0.285	HIGH
45	5	LET-1	4	14	0.285	HIGH
46	5	CORI-1	4	14	0.285	HIGH
47	5	CAP-3	5	14	0.357	HIGH
48	5	CAP-2	5	14	0.357	HIGH
49	5	CAP-1	5	14	0.357	HIGH
50	5	GC-3	6	14	0.428	HIGH
51	5	CORI-2	6	14	0.428	HIGH
52	5	GC-2	5	14	0.357	HIGH
53	5	CAB-4	5	14	0.357	HIGH
54	5	CU-6	4	14	0.285	HIGH
55	5	CU-1	4	14	0.285	HIGH

Table 5: Multiple Antibiotic Resistance (MAR) Index of the 55 isolates.

Chapter 4

Discussion:

In this study, a total of 29 out of 50 raw vegetable samples taken from local markets in five different districts of Dhaka city tested positive for *Salmonella* spp. After examining the culture and physical traits of bacterial isolates in various selective and differential medium and carrying out the standard biochemical assays, the isolates had been presumptively identified as Salmonella. In Citrate test, maximum isolates out of the 55 isolates tested citrate positive. Enteric fever, which includes fever, prostration, and septicemia, is often used to describe a kind of typhoid brought on by non-typhoidal *Salmonella* infections brought on by *S. enteric* subspecies (Bush et al., 2023). Nevertheless, presumptive tests are not always conclusive, thus extra verification tests could be necessary.

Among the 55 bacterial isolates selected from 29 positive samples in several areas of Dhaka city, the percentage of *Salmonella* spp. was highest in mint, green chili and tomato (18%) followed by cucumber (15%), coriander , capsicum and cabbage (9%) and lettuce (4%), carrot (0%) and spring onion (0%) respectively. Hence, it was noted that there was no *Salmonella* in the carrot and spring onion samples. The microbiological quality of vegetables is significantly influenced by the bacterial burden. A high total viable count (TVC) indicates a risky situation and, consequently, the potential for contamination. Vegetable-related TVCs varied depending on the location (Uddain, 2022).

The 55 bacteria isolated from fresh vegetables were evaluated for their antibiotic susceptibility against 14 antibiotics from 11 different groups after being presumptively identified *Salmonella* spp. which are Aminoglycoside, Beta–Lactam, Lincosamide, Macrolide, Nitroimidazole, Quinolone, Sulfonamide, Phenicols, Carbapenem, Cephalosporin, and Tetracycline. Here we found the antibiotic susceptibility of all the isolates (100%) were resistant to Penicilline and Metronidazole(100%); Followed by 98% of the isolates (n= 54) were resistant to Clindamycin(CD) , 95% of the isolates (n= 52) were resistant to Erythromycin (E), 31% of them (n=17) were resistant to Tetracycline (T), 48% of them (N=26) were resistant to Amoxicillin (AML/AMX). 10% of them (N=5) were resistant to Streptomycin(S) ,15% of them (N=8) were resistant to Chloramphenicol (C), 11% of them (N=6) were resistant to C Ceftriaxone (CTR),), 7% of them (N=4) were resistant to Cotrimoxazole (COT), 1% of all the isolates (N=1) were resistant to Gentamycin(GEN), Ciprofloxacin(CIP) and Imipenem(IMP) Respectively and lastly 0% of all the isolates (N=0) were resistant to Levofloxacin(LE) . In contrast, the susceptibility of *Salmonella* spp. to the antibiotics Gentamycin, Co–Trimoxazole, and Imipenem was found to be 98%, 93%, and 78% correspondingly in this study. These antibiotics were found to be the most efficient against Salmonella spp.

In our study, we found 100% of the isolates tested positive for antibiotic resistant to penicillin and metronidazole; 95% of the isolates were resistant to erythromycin (E), while 98% of them were sensitive to clindamycin (CD). Another study explains that *Salmonella* isolates from samples of fresh produce were frequently resistant to a number of clinically significant antibiotics, such as ampicillin, erythromycin, co-amoxiclav, and cephalothin (Rahman et al., 2021)

Our study researched, 98% of all the isolates were susceptible to Gentamycin and the lowest MAR Index was 0.28. In another study, all isolates of *Salmonella* were 100% susceptible to cephalothin and Streptomycin (66.6%) had the greatest rate of antibiotic resistance, and *Salmonella* serovars' multiple antibiotic resistance (MAR) index ranged from 0.08 to 0.83 (Abatcha et al., 2018)

According to our study, 55 isolates of fresh vegetables were resistant to 14 different antibiotics which are Penicillin(P), Metronidazole(MT), Clindamycin(CD) , Erythromycin (E), Tetracycline

(T), Amoxicillin (AML/AMX), Streptomycin(S) , Chloramphenicol (C), Ceftriaxone (CTR), Cotrimoxazole (COT), Gentamycin(GEN), Ciprofloxacin(CIP) Respectively Our research analyzed that Penicillin and Metronidazole were 100% resistant of 55 isolates where another search investigated 100% of all isolates were penicillin- and vancomycin-resistant. Salmonella spp. found in vegetable and soil samples have been found to exhibit similarantibiotic resistance to penicillin and vancomycin (Wadamori et al., 2016).

The MAR index was calculated by dividing the number of antibiotics that each isolate was resistant to by the total number of antibiotics tested. Indicators larger than 0.2 were considered high risk, indicating that the isolates were multidrug resistant and that they originated from a source of extensive antibiotic usage, as previously described (Mthembu et al., 2019).The isolate in sample Mint-04 was resistant to 12 of 14 antibiotics (85.7%) with the exception of Levofloxacin (LE) and Imipenem (IMP) and the highest MAR Index was 0.8, and the lowest MAR Index was 0.28 which was expressed 36% (n=20) of the total 55 isolates. Thus, every bacteria isolated from fresh vegetables are tested. According to reports, vegetables were shown very susceptibility to cross-contamination with foods contaminated with *Salmonella* and showing a high level of antibiotic resistance.

The spread of antibiotic resistance in *Salmonella enterica* species is greatly aided by the horizontal transmission of resistance genes. These resistance genes can be discovered in bacterial chromosomes or resistant plasmids. The most effective way to transfer resistance is by the horizontal transmission of genes via plasmids, which happens often and involves multiple resistance genes at once. The resistant genes that plasmids, integrons, or transposons acquire have the potential to spread to different strains or species (Nair et al., 2018). Contaminated irrigation water and manure spread on agricultural fields may have the ability to transfer antimicrobial resistant bacteria to fresh food. When fruits and vegetables are eaten without first being washed, the customer is most at risk. *Salmonella* in fresh fruit can be avoided by using good agricultural practices (GAPs) on farms and appropriate handling procedures in households and on farms (Kilonzo et al., 2018).

Chapter 5

Conclusion:

Generally, Spring onion was free of *Salmonella* spp. Nevertheless, samples of lettuce, tomatoes and cabbage only seldom included *Salmonella* spp., but samples of mint, green chilies, cucumbers, coriander, and capsicum frequently did. *Salmonella* spp. being present in the frequently consumed fresh veggies raises questions about the hygiene and security of eating them and other raw vegetables. Food can harbor a variety of illnesses that could be harmful to the consumer health. So, to reduce the risk of bacterial infection, vulnerable populations including the elderly, patients with weak immune system, and pregnant women should refrain from eating raw vegetables. Food emergency response laboratories should select the most acceptable technique or a combination of methods having the appropriate analytical qualities to address food emergency and safety needs. Discovering therapeutic targets and new compounds that can combat the emergence of antibiotic resistance and treat *Salmonella* infections may be made possible by contemporary technology and molecular techniques.

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