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Research Article

PREVALENCE AND VIRULENCE PROFILING OF MULTIDRUG-RESISTANT *SALMONELLA* FROM RETAIL VEGETABLES ADJACENT TO POULTRY MARKETS IN SYLHET

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Abstract

Salmonella spp., members of the *Enterobacteriaceae* family, are major cause of diarrheal illnesses global and resides in the gastrointestinal tracts of animals. Multidrug-resistant *Salmonella* can easily contaminate vegetables sold close to the poultry markets, as poultry animals are the primary reservoir of *Salmonella*. This study investigated the prevalence of *Salmonella* spp. in 75 fresh vegetable samples (Cucumber, Tomato, Carrot) collected from different retail locations across Sylhet city. *Salmonella* was isolated and identified using conventional culture techniques on selective *Salmonella-Shigella* (SS) agar, Gram staining, and biochemical profiling. The resulting isolates were examined for antibiotic resistance against seven antibiotics widely used in poultry industry (amoxicillin, ciprofloxacin, oxytetracycline, erythromycin, gentamycin, colistin, nalidixic acid) via the disk diffusion assay. A total of 43 isolates (57.33%) obtained from local markets were confirmed positive for *Salmonella* with a prevalence of 75.44%, whereas *Salmonella* was absent in the samples taken from departmental shops. The highest abundance of *Salmonella* was observed in the Cucumbers (64.285%), followed by Tomatoes (62.962%) and Carrots (40%). Antimicrobial resistance profiling revealed that 51.16% of the isolates exhibited multidrug resistance with MAR index ranging from 0.28 to 0.71. All isolates demonstrated susceptibility to gentamycin but resistant to amoxicillin and erythromycin. PCR screening of virulence-associated genes (*invA*, *fimA*, *sipB*) confirmed their presence and indicated a notable correlation with the multidrug-resistance. The findings demonstrated that the proximity of open poultry stall to a vegetable vending space poses a substantial public health risk, exposing consumers to virulent, antibiotic-resistant foodborne pathogen.

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Introduction

Globally, foodborne infections cost the lives of over 33 million people annually, accounting for 1 in 10 cases of illness (Pires et al., 2021). The most prevalent illnesses brought on by contaminated food are diarrheal infections, which affect 550 million people annually, including 220 million children younger than five years old. One of the four main causes of diarrheal illnesses worldwide is *Salmonella* (WHO, 2018). It is the most prevalent bacterial pathogen responsible for fresh produce-associated diseases in developed countries, causing approximately 53% outbreaks in United States and 50% in European Union (Callejón et al., 2015; Sivapalasingam et al., 2004). American scientist Salmon made the discovery of the *Salmonella* species more than a century ago. They belong to the family *Enterobacteriaceae* and is characterized as facultative anaerobic, motile, and gram-negative bacteria (Salehi et al., 2005).

Cite This Article

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The bacterium *Salmonella* causes salmonellosis. The typical symptoms include fever that flares up suddenly, nausea, vomiting, diarrhea, and abdominal pain (WHO, 2018). About 9% of human cases of salmonellosis are brought on by direct contact with animals, but between 55% and 95% of cases are acquired through food consumption (Majowicz et al., 2010; Mead et al., 1999; Oludairo et al., 2022). Depending on the strain, the infectious dosage of *Salmonella* can range from 1 to 10^{10} CFU/g (Pires et al., 2021). An outbreak from a single food source demonstrates that 10^{10} cells can result in salmonellosis (Bhunia, 2018; Yousef & Carlstrom, 2003).

The global escalation of antibiotic-resistant foodborne pathogens has raised public apprehension due to their virulence and increased mortality rate in infected patients (Chiu et al., 2002). The use of antibiotics to treat *Salmonella* infections is significantly impacted by the emergence of multidrug resistance in the serotypes of the bacteria. The invasive serotypes of infection are frequently fatal and need to be treated with strong antibiotics. The preferred medications for treating infections caused by MDR *Salmonella* have been quinolones and third-generation cephalosporins (Karon et al., 2007). The severity of bacterial infections in both humans and animals has grown as a result of the introduction of multidrug-resistant *Salmonella* (Afrin et al. 2023). According to epidemiological research, susceptible strains of *Salmonella* do not induce as severe or prolonged a syndrome as MDR strains, suggesting that MDR strains are more virulent than susceptible strains (Travers & Michael, 2002).

The pathogenicity of *Salmonella* is multifactorial involved in adhesion, invasion, intracellular survival, toxin synthesis, and iron acquisition (Skyberg et al., 2006). Distinct *Salmonella* serovars harbor unique virulence networks that determine their specific pathogenic potential (Tenor et al., 2004). Among these *invA* gene regulates the initial stage of *Salmonella's* intracellular pathogenicity cycle, which involves the invasion of intestinal epithelial cells. The proteins required for bacterial cell membrane invasion into host epithelial cells are encoded by the *invA* gene (Darwin & Miller, 1999; Sharma & Das, 2016). The *fimA* gene encodes proteins for the major fimbrial unit (Yin et al., 2022). Additionally, *sipB* gene is crucial to *Salmonella* pathogenicity (Hayward et al., 2002), that cause cell death through the adaptor proteins (Cook et al., 2007). Virulence genes in *Salmonella* strains resistant to several drugs are linked to a serious infection and pose a serious threat to public health (Tamang et al., 2014).

Due to inadequate hygiene and sanitation standards in market areas, there is a significant risk of cross-contamination between poultry products and nearby vegetables, potentially leading to the spread of pathogenic and multi-drug resistant (MDR) *Salmonella* spp. to humans through the consumption of raw vegetables. Despite the public health implications, there is a lack of documentation on this issue, particularly in regions like Sylhet city. Therefore, the objective of this study is to assess the microbial safety of fresh vegetables sold near poultry markets in Sylhet city by isolating and characterizing *Salmonella* spp. determining their antibiotic resistance profile, and investigating the correlation between multidrug-resistant phenotype and core virulence-associated genes.

Materials and Methods

2.1 Study Area and Sampling Design

This study was conducted at the Animal and Fish Biotechnology Laboratory, Department of Animal and Fish Biotechnology, Sylhet Agricultural University, Sylhet, Bangladesh. The cross-sectional study was carried out from November 2023 to April 2024. For this study, nine different markets of Sylhet city were selected as sampling locations. Seven of these were local markets where vegetables were sold a close proximity (less than 5 meters) to poultry store, remaining two were retail departmental shops with better hygienic standards,

where vegetables were separated and located more than 5 meters away from the poultry section. Three types of widely consumed vegetables—Tomato (*Solanum lycopersicum*), Cucumber (*Cucumis sativus*), and Carrot (*Daucus carota*)—were randomly sampled for this study (Figure 1).

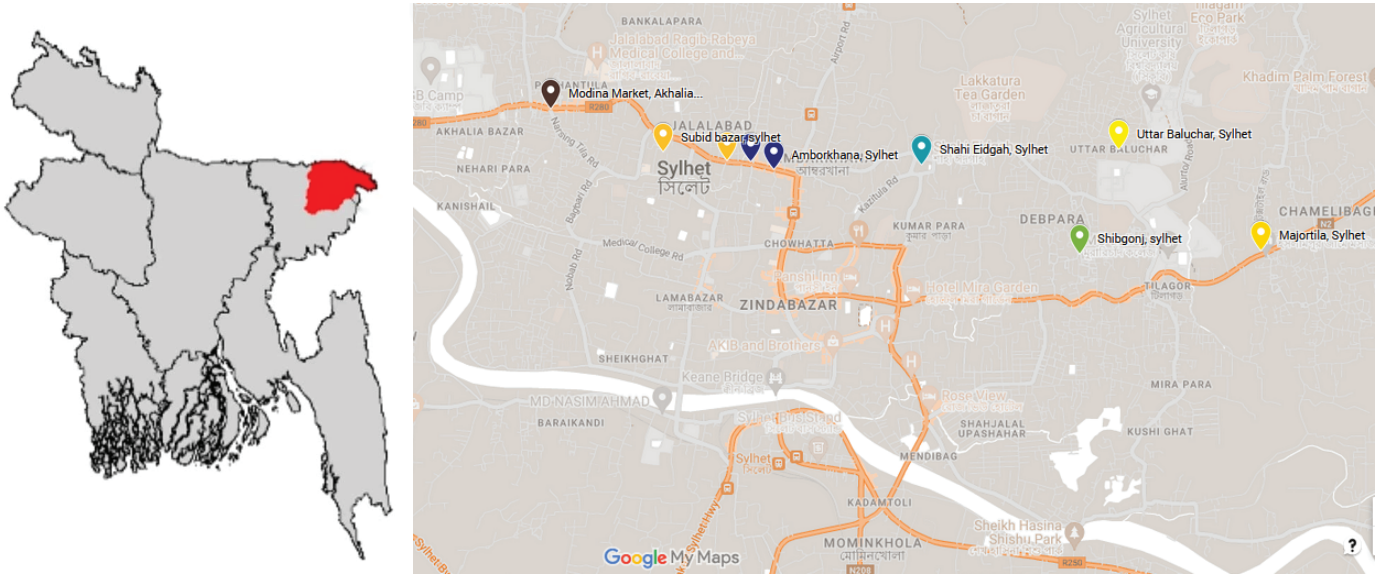


Figure 1. Study Area (Sylhet City)

2.2 Collection of the Sample

A total of 75 fresh vegetable samples were purchased from different markets for this study. After purchasing, every sample was neatly wrapped in a plastic zip lock bag, sealed tightly, and packed individually. Then the samples were carried to the laboratory using a sample collector ice box, where temperature was maintained at 4°C so that the samples didn't get spoiled. Finally, the box was transported to the laboratory as soon as possible and kept between 0–4°C temperature in a refrigerator until it was examined, but not for a period exceeding 36 hours.

2.3 Processing of Sample

After collecting, the samples were processed prior to isolation of *Salmonella*. At first, samples were taken out from refrigerator and were analyzed when the temperature of the samples were at room temperature. Every single vegetable sample was examined twice. The samples were aseptically processed for the isolation of *Salmonella*. At first, the sample was diced into very small, fine pieces using a sterilized knife. Then the sample was homogenized using mortar and pestle.

2.4 Cultivation and Isolation of *Salmonella*

2.4.1 Cultivation of *Salmonella*

Each sample homogenate was inoculated into test tube containing the freshly prepared nutrient broth and marked appropriately. After that, these were aerobically cultured for 24 hours at 37°C in a bacteriological incubator. After incubation, the test tubes were examined for turbidity of the broth, which indicates bacterial growth. Then, a loopful culture of the bacteria was inoculated into a *Salmonella-Shigella* (SS) agar plate from test tubes. The plates were incubated at 37°C overnight. Small, round, black-centered colonies indicated the presence of *Salmonella* (Cheesbrough, 1987; Sarker et al., 2021).

2.4.2 Isolation of *Salmonella*

Salmonella was subcultured in media such as Nutrient Agar (NA), *Salmonella-Shigella* (SS) agar, and MacConkey (MC) agar. Using a sterile inoculating loop, the *Salmonella* inoculum was subcultured in SS agar using the streak plate technique to yield isolated colonies. To develop a culture containing only single colonies, the procedure was repeated as many times as needed, usually at least twice to ensure purity (Cheesbrough, 1987; Sarker et al., 2021). *Salmonella* suspected colonies were further inoculated into MacConkey agar (a differential medium) and Nutrient Agar medium. After overnight incubation, the growth of colourless colonies was observed in MacConkey agar and colonies with grey-white, moist, circular disc with smooth convex surface were seen on nutrient agar medium (Islam et al., 2014).

2.5. Phenotypic and Biochemical Characterization of Isolates

Presumptive *Salmonella* colonies were first examined through Gram staining and morphological characteristics were observed. To further confirm the identity, several biochemical tests were performed, including catalase, indole, methyl red (MR), Voges–Proskauer (VP), urease, and citrate utilization tests, following standard protocols (Krieg & Holt, 1984).

2.6 Screening of Multidrug-Resistant *Salmonella* via Antibiotic Sensitivity Testing

The *in-vitro* test for antibiotic sensitivity was conducted following Kirby-Bauer disc diffusion assay (Bauer et al., 1966). Clinical and Laboratory Standards Institute guidelines specified that breakpoints were used to interpret resistance and susceptibility. Zones of inhibition were classified as susceptible, intermediate, and resistant categories based on the CLSI guideline. Amoxicillin (30 µg), Ciprofloxacin (5 µg), Colistin (10 µg), Erythromycin (5 µg), Gentamycin (10 µg), Nalidixic acid (30 µg), Oxytetracyclin (30 µg) were used. The prevalence of multidrug-resistant *Salmonella* was determined after interpreting the susceptibility and resistant profiling of each tested antibiotic. An isolate was considered as an MDR when it showed resistance against at least three groups of antibiotics, according to the CLSI (2021) and EUCAST (2021) guidelines (CLSI, 2021; EUCAST, 2021). The MAR (Multiple Antibiotic Resistance) index was used to determine the antibiotic resistance pattern of every isolate. Indicators greater than 0.2 were regarded as high risk, which means the multidrug-resistant isolates originated from a source of widespread antibiotic use (Krumperman, 1983).

MAR index = a/b

“a” = Number of antibiotics to which the particular isolate was resistant.

“b” = Total number of antibiotics tested.

2.7 Detection of Virulence Genes by Polymerase Chain Reaction

Multidrug-resistant isolates were selected for the detection of virulence genes (*invA*, *fimA*, *sipB*) using polymerase chain reaction (PCR). At first, the DNA was isolated by using “WIZARD® GENOMIC DNA PURIFICATION KIT following the manufacturer’s instructions. After isolation of the DNA, it was quantitatively assessed using the nanodrop 2000c spectrophotometer. PCR was carried out in a thermal cycler using 25µL reaction mixtures. Cycling parameters were 95° C for 3 minutes, followed by 35 cycles of 95° C for 30 seconds, 53°-58° C for 30 seconds and 72° C for 90 seconds with a final extension 72° C for 5 minutes. After amplification, the PCR products were visualized through electrophoresis onto agarose gel, stained with ethidium bromide and visualized under a UV transilluminator (Table 1, Table 2).

Table 1. List of Primers and their Sequences

Gene	Primer name & sequence (5'-3')	Amplicon Size	Reference
<i>invA</i>	invA-F: GTGAAATTATCGCCACGTTCTGGGCAA invA-R: TCATCGCACCGTCAAAGGAACC	284 bp	Rahn et al., (1992)
<i>fimA</i>	fimA-F: TGCCTTTCTCCATCGTCC fimA-R: TGCGGTAGTGCTATTGTCC	134 bp	Yin et al., (2022)
<i>sipB</i>	sipB-F: GGACGCCGCCCGGGAAAACTCTC sipB-R: AACTCCCGTCGCCGCCTTCACAA	875 bp	Skyberg et al., (2006)

Table 2. Concentration of PCR Reaction Mixture

SL No.	Components	Volume Per Reaction
1.	GoTaq™ G2 Hot Master Mix	12.5 µL
2.	DNA Template (concentration 25-65 ng/µL)	1µL
3.	Forward Primer (concentration 10-20 pMol)	1µL
4.	Reverse Primer (concentration 10-20 pMol)	1µL
5.	Nuclease Free Water	9.5 µL
Total		25 µL

2.8 Statistical Analysis

The total number of *Salmonella*-positive samples, frequency of antibiotic resistance, and number of MDR isolates were calculated. Chi-Square test (Microsoft Excel, 2013) was used to assess variation between local markets and departmental shops. Chi-Square test and Phi (ϕ) coefficient were used to assess the correlation between MDR and virulence genes. Analysis and visualization were performed in R software (version 4.2.2). A p-value <0.05 was considered statistically significant.

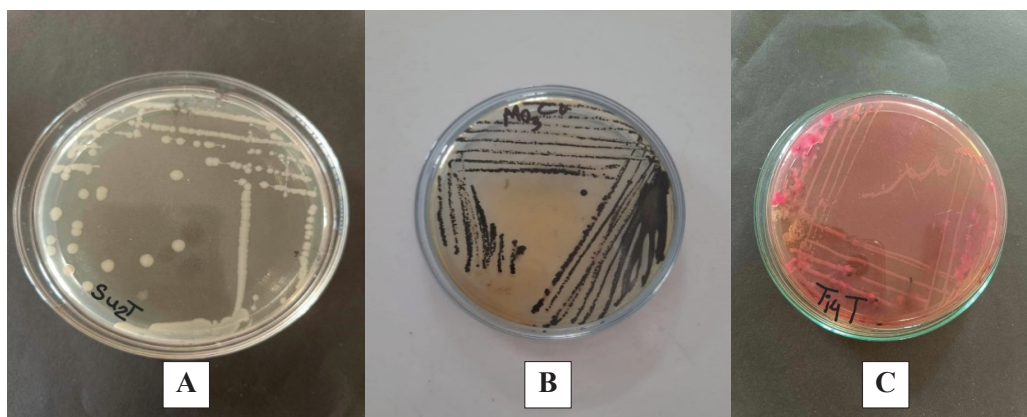
Results

3.1. Cultural Characteristics of *Salmonella* isolates

Presumptive *Salmonella* isolates were initially identified based on its cultural characteristics. On nutritional agar, colonies appeared circular, low convex, smooth, grayish white, and translucent (Table 3, Figure 1). On SS-agar *Salmonella* isolates exhibited characteristic smooth, black-center (Table 3, Figure 1). Furthermore, the isolates produced colourless, or transparent colonies on MacConkey agar confirming the presence of *Salmonella* (Table 3, Figure 2).

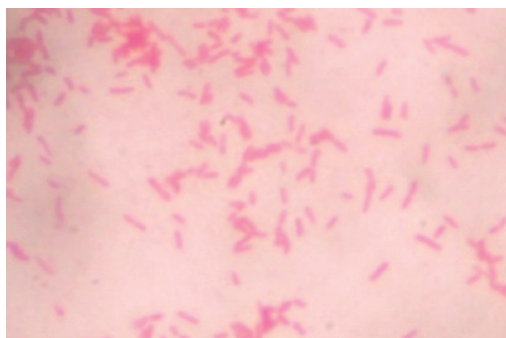
Table 3. Summary of Morphological Characteristics of Salmonella on Different Culture Media

Media	Colony Characteristics	Isolated Bacteria
Nutrient Agar	Circular, smooth, translucent, white colonies	<i>Salmonella</i> spp.
<i>Salmonella-Shigella</i> Agar	Black centered smooth colonies	
MacConkey Agar	Colourless smooth colonies	

**Figure 2.** Cultural Appearance of *Salmonella* spp. on different culture media. (A: Nutrient Agar; B: *Salmonella-Shigella* Agar; C: MacConkey Agar).

3.2. Morphological Characterization by Gram's Staining

Microscopic examination of the pure isolates following Gram staining revealed distinct Gram-negative properties of *Salmonella*. They were characterized as pink, small rod-shaped bacilli appearing predominantly as single cells or in short chains (Figure 3).

**Figure 3.** Gram-Negative Properties of *Salmonella* under Microscope.

3.3. Result of Biochemical Tests for the Identification of Salmonella Spp.

Presumptive *Salmonella* isolates underwent a panel of biochemical tests. The isolates demonstrated distinct biochemical characteristics of the genus *Salmonella*, including positive reactions for catalase, methyl red, and citrate utilization, alongside negative reactions for indole, Voges–Proskauer, and urease production (Table 4, Figure 4).

Table 4. Biochemical Characteristics of the Isolates

Name of the test	Observation	Result	Interpretation
Catalase test	Bubbles were formed	+	<i>Salmonella</i> spp.
Indole test	Absence of red colour	-	
MR test	A distinct red colour on top	+	
VP test	A lack of pink red color	-	
Urease test	The agar slant, but remained light orange	-	
Citrate utilization test	Dark green to vibrant blue	+	

*A positive reaction is denoted by (+) sign, while negative reaction is denoted by (-) sign.

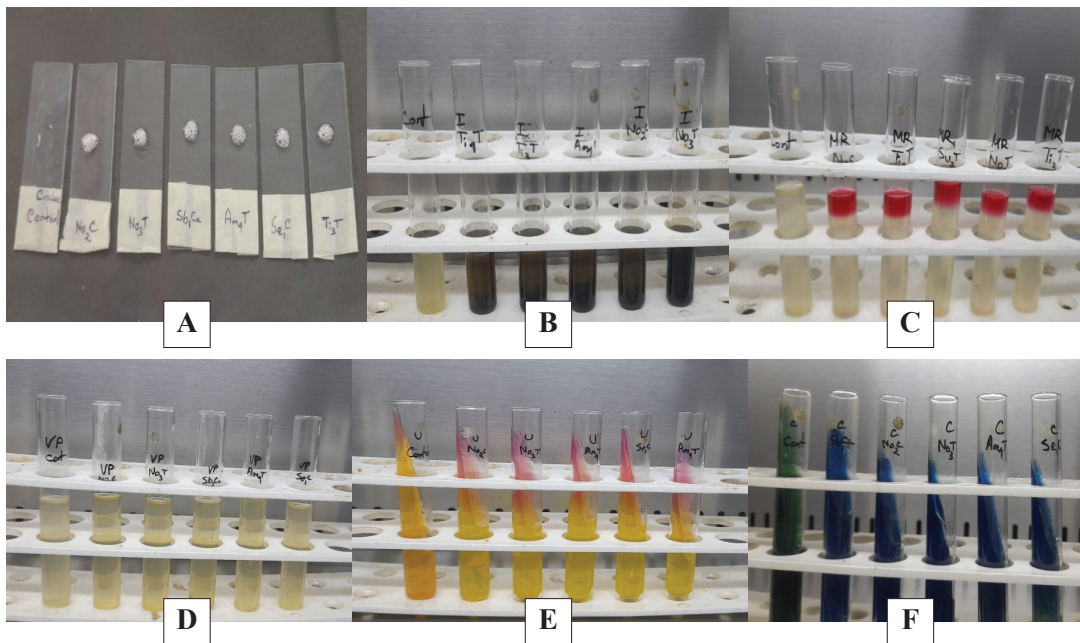


Figure 4. Identification of *Salmonella* Based on Biochemical Test (A) Catalase test (+), (B) Indole test (-), (C) MR test (+), (D) VP test (-), (E) Urease test (-), (F) Citrate utilization test (+).

3.4. Prevalence and Distribution of across Retail Markets

Out of 75 fresh vegetable samples tested, 43 (57.33%) were positive for *Salmonella* spp. *Salmonella* spp. was found in samples from all seven local markets with prevalence of 75.44%. Among the local markets, Subid Bazar and Shahi Eidgah Bazar, exhibited the highest *Salmonell* contamination rates (100%), while Shibgonj bazar showed the lowest prevalence (50%) among the positive locations (Table 5). Conversely, no *Salmonella* spp. contamination was detected in the 18 samples obtained from two retail departmental shops (Swapno and Masala Bazar) (Table 5).

Table 5. Prevalence of *Salmonella* spp. Samples in Different Locations

Type of the Market	Market Name	Time of Collection	Number of Samples	<i>Salmonella</i> Positive	Frequency	P Value
Local market	Noya Bazar	November, 2023	8	5	62.5%	<0.001
	Shibgonj Bazar	December, 2023	6	3	50%	
	Amborkhana Bazar	December, 2023	7	4	57.142%	
	Modina Market	January, 2024	8	6	75%	
	Subid Bazar	March, 2024	9	9	100%	
	Shahi Eidgah Bazar	April, 2024	9	9	100%	
	Major Tila Bazar	April, 2024	10	7	70%	
Departmental shops	Swapno	January, 2024	9	0	0%	<0.001
	Masala Bazar	April, 2024	9	0	0%	

*P value <0.001 indicates highly statistically significant

3.5. Distribution of *Salmonella* in Different Vegetables

Among the three vegetables sampled, the highest prevalence of *Salmonella* spp. was recovered in Cucumbers (64.285%), followed closely by Tomatoes (62.962%). Carrots exhibited the lowest contamination rate (40%) (Figure 5).

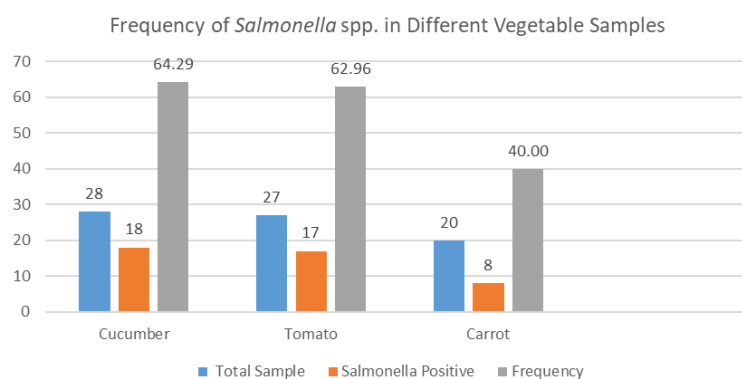


Figure 5. Relative Frequency of *Salmonella* in Different Vegetables.

3.6. Antimicrobial Susceptibility and Resistance Profiling

Antimicrobial susceptibility assay revealed that all the isolates (100%) were resistant to amoxicillin and erythromycin. In contrast, all the isolates showed susceptibility to gentamycin. Notably, 48.84% of the isolates displayed intermediate resistance against colistin (Figure 6, Figure 7).

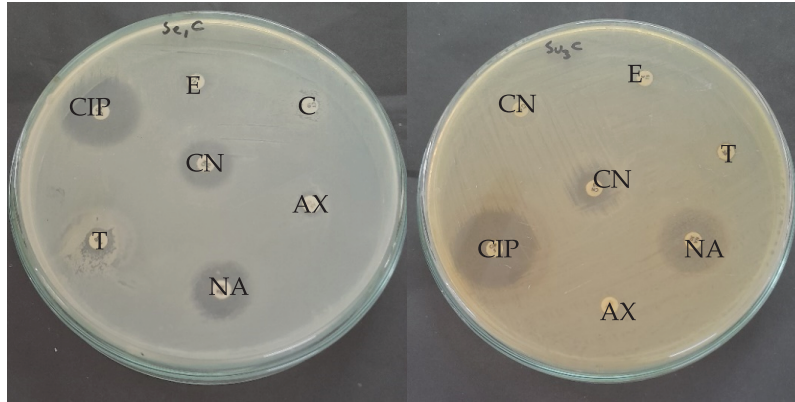


Figure 6. Representative Antibiotic Susceptibility Test Results Using the Disc Diffusion Method.

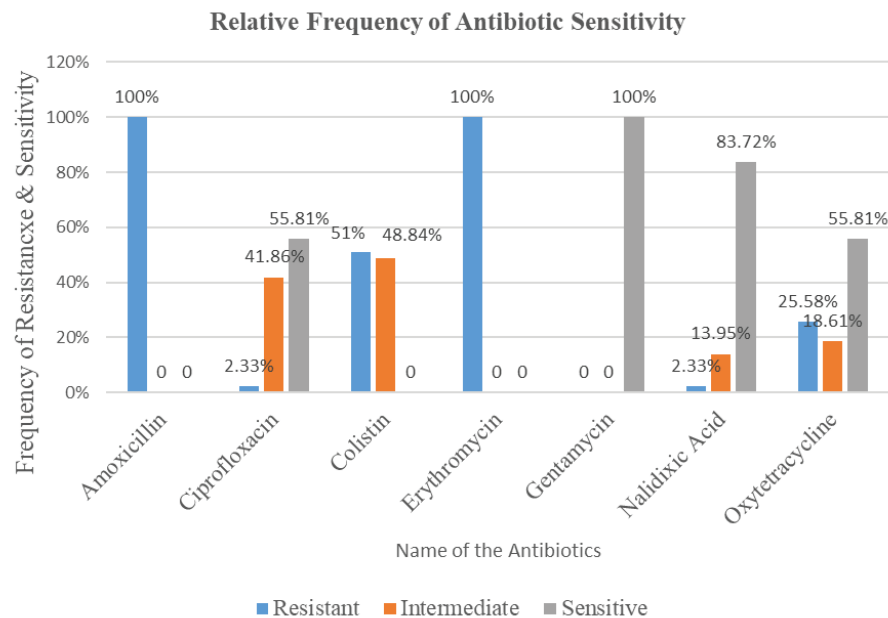


Figure 7. Antibiotic Sensitivity and Resistance Pattern of *Salmonella* Isolates.

3.7. Prevalence of Multidrug-Resistant (MDR) *Salmonella* Phenotypes

The isolates exhibiting resistance to at least three groups of antibiotics were classified as multidrug-resistant (MDR). Based on the criteria, 22 out of 43 (51.2%) were confirmed as MDR *Salmonella*, and the frequency was determined as 51.16%. The highest rate of MDR isolates were obtained from Tomatoes (58.82%), followed by Cucumbers (50%) and Carrots (37.5%). The MDR isolates were resistant against a minimum of three and up to five groups of antibiotics. Notably, a single isolate obtained from a Tomato sample at Modina Market showed the highest resistance against five groups of antibiotics (Amoxicillin, Ciprofloxacin, Colistin, Erythromycin and Oxytetracycline) (Table 6, Table 7, Figure 8).

Table 6. Multiple Antibiotic Resistance (MAR) Index of *Salmonella* Isolates

Location	No. of Isolates	Resistance to Groups of Antibiotics (a)	Total Tested Groups of Antibiotics (b)	MAR Index (a/b)
Noya Bazar	4	2	7	0.28
	1	3	7	0.42
	1	4	7	0.57
Shibgonj Bazar	2	2	7	0.28
Amborkhana Bazar	1	4	7	0.57
	1	2	7	0.28
	1	3	7	0.42
Modina Market	2	4	7	0.57
	4	2	7	0.28
	1	4	7	0.57
Subid Bazar	1	5	7	0.71
	4	2	7	0.28
	1	3	7	0.42
Major Tila Bazar	4	4	7	0.57
	1	2	7	0.28
	6	3	7	0.42
Shahi Eidgah Bazar	6	2	7	0.28
	1	3	7	0.42
	2	4	7	0.57

Table 7. Distribution of Multiple Antibiotic-Resistant Profile of *Salmonella* Isolates by Sample Types

No. of Isolates	Class of Antibiotics	Resistant Phenotype	Number of Isolates	Frequency of Isolates	MAR Index
Tomato (n=17)	2	AX ^R , E ^R	7	41.18%	0.28
	3	AX ^R , CT ^R , E ^R	5	29.41%	0.42
	4	AX ^R , CT ^R , E ^R , T ^R	4	23.53%	0.57
	5	AX ^R , CT ^R , E ^R , T ^R , CIP ^R	1	5.88%	0.71
Cucumber (n=18)	2	AX ^R , E ^R	9	50%	0.28
	3	AX ^R , CT ^R , E ^R	4	22.22%	0.42
	4	AX ^R , CT ^R , E ^R , T ^R Or AX ^R , CT ^R , E ^R , NA ^R	5	27.78%	0.57
Carrot (n=8)	2	AX ^R , E ^R	5	62.5%	0.28
	3	AX ^R , CT ^R , E ^R	1	12.5%	0.42
	4	AX ^R , CT ^R , E ^R , T ^R	2	25%	0.57

AX, CT, E, T, CIP, NA denotes Amoxicillin, Colistin, Erythromycin, Oxytetracyclin, Ciprofloxacin and Nalidixic acid respectively and n belongs to sample number

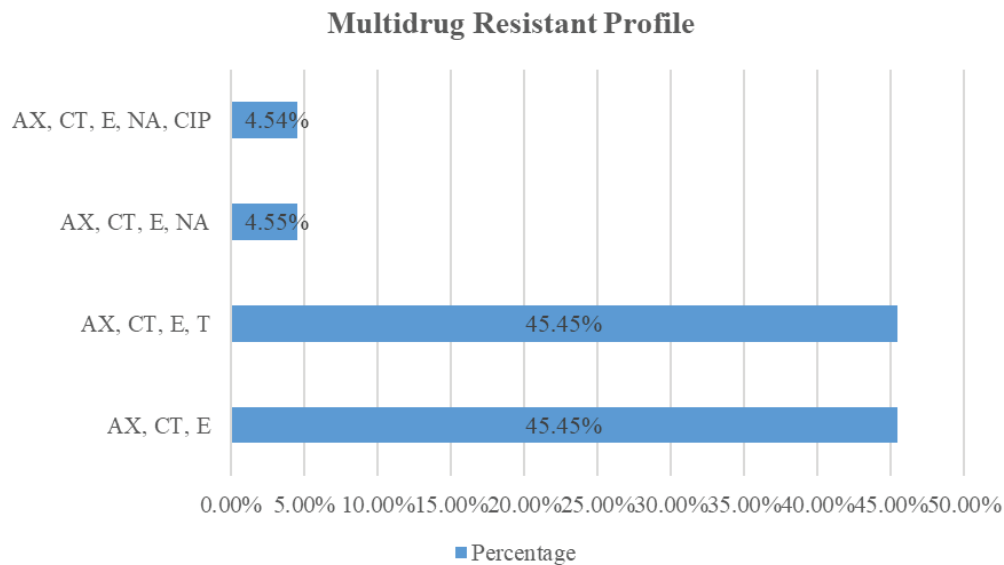


Figure 8. Prevalence of Multidrug-Resistant *Salmonella* spp. According to Drug Groups.

3.8. Molecular Detection of Virulence Genes

To detect the presence of virulence-associated marker genes, PCR primers targeting the *Salmonella*-specific *invA* gene successfully amplified 284 BP fragments in all 43 isolates. The *sipB* gene (875 bp) was amplified in 28 *Salmonella* isolates including 19 MDR isolates (86.4%) and 9 non-MDR (42.9%) isolates. The *fimA* was detected in 12 isolates, consisting of 8 MDR isolates (36.4%) and 4 non-MDR isolates (19.0%) (Figure 9).

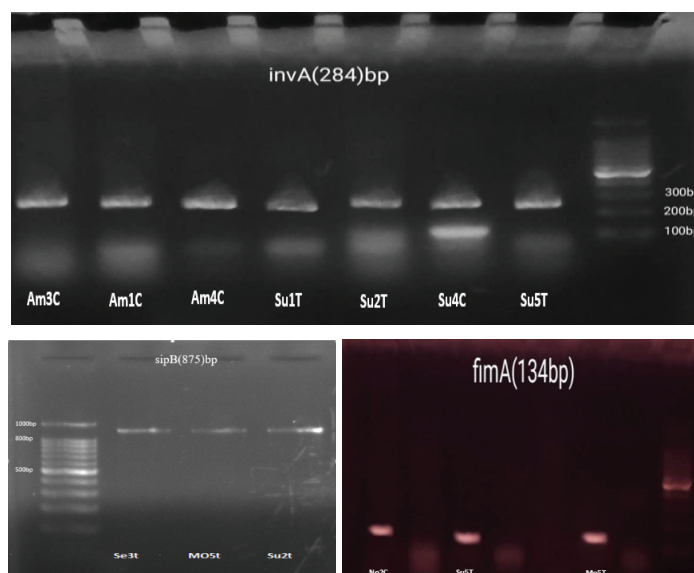


Figure 9. Agarose gel electrophoresis of PCR-amplified virulence genes (*invA*, *sipB*, *fimA*) in selected *Salmonella* isolates.

3.9. Statistical Association between Multidrug Resistance and Virulence Markers

As the *invA* gene was uniformly distributed across all isolates (100%), it was excluded from comparative statistical correlation analysis. The statistical relationship between MDR phenotype and the remaining virulence markers (*sipB* and *fimA*) was evaluated using the Chi-square test and Phi (ϕ) coefficient (Table 8).

Table 8. Correlation between MDR Phenotype and Virulence Genes in *Salmonella* Isolates

Virulence Gene	Test Used	X ² Value	df	p Value	Significance	Interpretation
<i>sipB</i>	Chi-Square Test	8.94	1	0.003	P<0.01	Significant correlation with MDR
<i>fimA</i>		1.61	1	0.20	ns	No significant correlation with MDR

A highly significant association was established between the MDR phenotype and *sipB*. In contrast, *fimA* showed a statistically nonsignificant association with MDR phenotype. According to the Phi (ϕ) coefficient, the *sipB* gene showed a moderate positive correlation with MDR phenotype ($\phi = 0.456$), supporting Chi-square result. In contrast, *fimA* showed only a weak association with MDR phenotype ($\phi = 0.19$), which is consistent with its non-significant p-value (Figure 10, Figure 11).

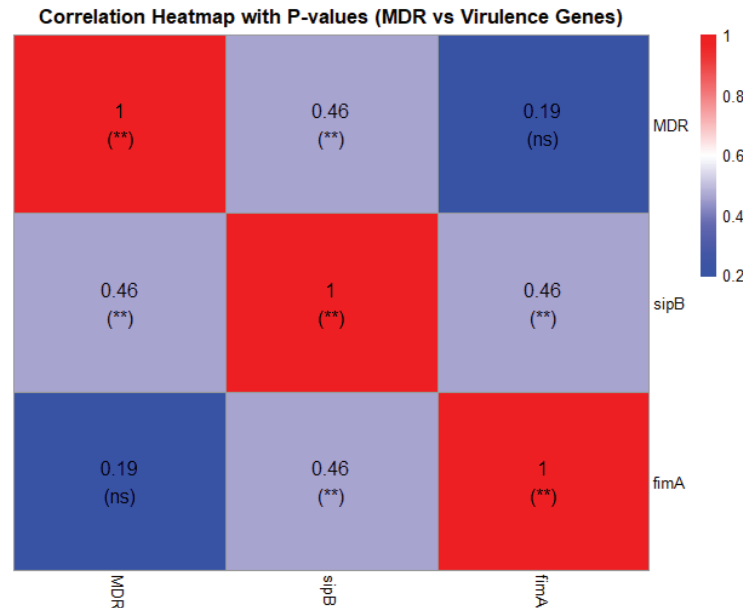


Figure 10. Heatmap Showing the Association between MDR Phenotypes and Virulence Genes.

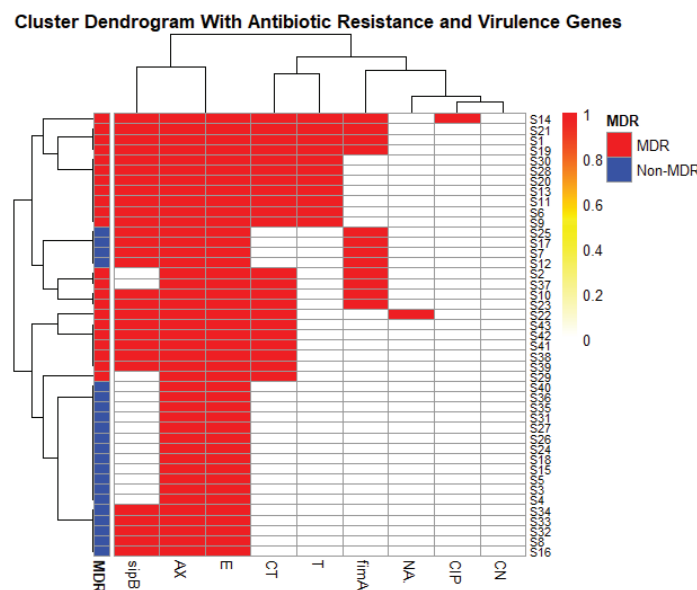


Figure 11. Hierarchical Clustering Dendrogram of *Salmonella* Isolates based on Antibiotic Resistance Profiles and Virulence Genes

Discussion

This study demonstrated a substantial overall *Salmonella* prevalence of 57.33% across fresh vegetable samples collected in Sylhet city. The highest contamination rates were observed in Cucumbers (64.285%), followed by Tomatoes and Carrots with a prevalence of 62.962% and 40%. Crucially, all the positive isolations were obtained from local markets with a frequency of 75.44%, while *Salmonella* contamination was not found in the samples taken from departmental shops. These differences suggest that good hygiene, proper handling, and suitable storage conditions play an important role in reducing bacterial contamination. This study also speculated that the prevalence of *Salmonella* also depends on the temperature or other environmental conditions. Higher prevalence (89.28%) of *Salmonella* was observed during March and April, while a lower prevalence (62.07%) of *Salmonella* was found from November to January. These observations align with previous study that the summer months featured a higher incidence of *Salmonella* infections, as higher temperatures favor the growth and persistence of *Salmonella* in the food supply (Liu et al., 2013). A previous study has also shown the differences in the prevalence of *Salmonella* in vegetables of local markets and departmental shops of Dhaka city (Mamun et al., 2021). Internationally, 28% prevalence of *Salmonella* was reported in Tomatoes, Cucumbers, Carrots, capsicums in Malaysia (Nillian et al., 2011) while 13.9% and 8% *Salmonella* prevalence was documented in the retail vegetables was in Nigeria and Pakistan (Abakpa et al., 2015; Razzaq et al., 2014). The relative high prevalence observed in our study confirms that the prevalence of *Salmonella* in vegetables can vary from one-region to another, possibly due to difference in hygiene, handling practices, and cross contamination.

Antimicrobial susceptibility testing showed a worrying pattern of antibiotic resistance. All of the *Salmonella* isolates (100%) were resistant to amoxicillin and erythromycin. This finding is consistent with Najwa et al. (2015) who also reported that showed 100% resistance of *Salmonella* against erythromycin high susceptibility against nalidixic acid and gentamycin. In contrast, Abatcha et al. (2018) found no resistance to ciprofloxacin in *Salmonella* isolated from vegetables, whereas we detected some ciprofloxacin-resistant isolates. However, our findings slightly contrast with the findings from Vietnam, found which reported moderate to high resistance to Quinolone group (Nhung et al., 2018).

The Multiple Antibiotic Resistance (MAR) indices provided critical insights into regional biosecurity. In this study, all of the *Salmonella* isolates exhibited MAR index 0.28 or higher, indicating that each isolate originated from environments with high antibiotic exposure. Antibiotic-resistant bacteria can be tracked using the MAR index, which is a plausible, solid, and affordable method (Sandhu et al., 2016). In this present study, the MAR index ranges from 0.28 to 0.71 with one isolate exhibiting the highest MAR index of 0.71. Among all isolates, 48.837% had a MAR index of 0.28 index and 23.25% showed a MRA index of 0.42. A previous study reported the MAR index of *Salmonella* serovars ranging from 0.08 to 0.83 (Abatcha et al., 2018), which is comparable with the present study. These reports suggest that vegetables contaminated with multidrug-resistant *Salmonella* may pose a significant public health risk through the spread of antibiotic-resistant bacteria.

All *Salmonella* isolates were further examined for the presence of three virulence genes (*invA*, *fimA*, *sipB*). All 43 isolates (100%) showed bands for the *invA* gene, regardless of vegetable sources indicating the invasive potential of the isolates. Among the MDR *Salmonella* isolates, 86.4% were positive for the *sipB* gene, whereas only 42.9% of non-MDR isolates carried the gene. Chi-square analysis revealed a statistically significant association between MDR status and presence of the *sipB* gene ($\chi^2 = 8.94$, $p < 0.01$), with a Phi coefficient (Φ) of 0.456, indicating a moderate positive correlation. For the *fimA* gene, 36.4% of MDR isolates and 19.0% of non-MDR isolates were positive. The association was not statistically significant ($\chi^2 =$

1.61, $p = 0.20$), with a weak Phi correlation ($\Phi = 0.19$). The detection of *invA* and *sipB* genes in this study is consistent with previous reports. The *invA* gene is recognized as a sensitive screening tool for the genus *Salmonella* and is associated with bacterial invasion of host epithelial cells, making an important virulence determinant responsible for gastroenteritis (Sohi et al., 2023). Similarly, Ed-Dra et al., (2019) detect the 875 bp *sipB* gene in 100% of human and poultry serovars, and 96.6% of domestic animal serovars. Other studies reported that all *Salmonella* isolates carrying the *sipB* gene were resistant to at least one antibiotic. The present are consistent with previous research.

Overall, the findings of this study indicate a significant transmission pathway of *Salmonella* spp. from poultry shops to vegetables sold in nearby markets. The close proximity of vegetable stalls to poultry shops may facilitate cross-contamination through shared handling practices, contaminated surfaces, or water runoff from poultry waste. Consequently, fresh vegetables may serve as vehicles for transmitting antibiotic-resistant and potentially virulent *Salmonella* to consumers, posing a significant public health concern.

Conclusion

The presence of *Salmonella* spp. in fresh vegetables sold near poultry stores in local markets possess serious food safety and hygiene risk. Alarmingly, these isolates are not only multidrug-resistant (MDR), but also harbor virulence genes, making them potentially lethal without effective treatment. To address this public health threat, genomic surveillance, strict market sanitation, and responsible antibiotic use are recommended.

Author Contributions

DA and KMAZ designed outlines and drafted the manuscript. MMC performed the experiments and analyzed the data. MMC, RS, and MBUC wrote the initial draft of the manuscript. KMAZ and DA reviewed the scientific contents described in the manuscript. All authors read and approved the final submitted version of the manuscript.

Conflicts of Interest

There is no conflict of interest among the authors.

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