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Assessment of bacterial contamination in fresh produce marketed in Sadarghat shops in Dhaka city

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Abstract

A study investigating microbial contamination in fresh produce sold in Bangladesh revealed significant levels of bacterial pathogens. Among 8 samples of tomatoes, carrots, cucumbers, and green peppers, high counts of total aerobic bacteria and enteric pathogens were detected. Staphylococcus aureus was identified in 75% of samples, with 100% prevalence in carrots and tomatoes. Escherichia coli was present in 62.5% of samples, predominantly in cucumbers (100%). Pseudomonas aeruginosa appeared in 62.5% of samples, while Vibrio sp. and Salmonella sp. were each detected in 50% of samples. Chopped-carrot samples exhibited the highest aerobic bacterial load, with 5.86×10^4 CFU/g in winter (December–February) and 3.75×10^4 CFU/g in summer (March–June). In contrast, chopped-tomato samples showed the lowest contamination, at 6.16×10^3 CFU/g in winter and 1.65×10^4 CFU/g in summer. Overall, bacterial contamination rates were elevated in summer and in chopped produce. The findings underscore the critical need for enhanced hygiene practices, rigorous monitoring, and improved handling protocols to ensure the safety of fresh produce in market settings.

***Keywords*:** Fresh produce; Bacteria; Multidrug resistance; Assessment; Contamination

Introduction

Bacterial contamination of fresh produce poses a significant public health concern worldwide, affecting both developed and developing countries alike. In urban centers like Dhaka, Bangladesh, where the consumption of fresh vegetables is integral to daily diets, the risk of microbial contamination is heightened due to factors such as inadequate sanitation practices, contaminated irrigation water, and poor post-harvest handling. Studies have consistently shown that vegetables can harbor a variety of bacterial pathogens, including *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, which can lead to foodborne illnesses upon consumption. The consumption of fresh produce is essential for a balanced diet, offering numerous health benefits. Vegetables are a vital source of both macro- and micronutrients and fiber, which are associated with various health benefits (Kaczmarek *et al.* 2019). Regular consumption of fresh produce is linked to reduced incidence of illnesses such as heart disease, cancer, stroke, and cardiovascular diseases Governments and health organizations have long advocated for increased consumption of fruits and vegetables (Ledoux *et al.* 2011).

Between 1960 and 2019, global fresh produce consumption increased from 60 to 140 kg per capita per year (FAO, 2022), with production increasing by 30% in recent years (Hess and Sutcliffe, 2018). Despite these benefits, the increasing incidence of foodborne illnesses linked to contaminated fresh vegetables has raised significant public health concerns. Vegetables can be contaminated with a variety of pathogens during cultivation, harvest, handling, and distribution (Carstens *et al.* 2019).

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Contamination sources include wastewater from livestock, contaminated manure, irrigation water, and improper handling during processing and distribution Pagadala *et al.* 2015; Maffei *et al.* 2016). Consumption of fresh produce without proper washing, especially those eaten raw, is a significant predictor of public health problems Mir *et al.* 2018).

Foodborne disease outbreaks can be caused by various microbial agents, including bacteria, parasites, viruses, fungi, and mycotoxins. In 2010, the World Health Organization (WHO) attributed norovirus as the primary contaminant for outbreaks of foodborne illnesses associated with fresh produce, accounting for 120 million of the total 600 million global cases of foodborne illness (Havelaar *et al.* 2015; Callejon *et al.* 2015). Bacterial pathogens are the second major contributor to outbreaks, responsible for 36% and 42% of fresh produce-related outbreaks in the USA and EU, respectively (Callejon *et al.* 2015). Notable bacterial pathogens include *E. coli* O157 (found in spinach and lettuce) and *Salmonella* spp. (found in tomatoes and lettuce) (Heaton and Jones, 2008). In low- and middle-income countries, fresh produce is commonly sold in open markets, where poor sanitation and fecal contamination are prevalent, leading to human gastroenteritis outbreaks (Biswas *et al.* 2020). In Dhaka, Bangladesh, the common ways for bacterial contamination of fresh produce include poor sanitation and fecal contamination. This is particularly concerning as fresh produce from these markets is often transferred to various parts of the city. Despite efforts to improve food safety standards, the persistence of bacterial contaminants in fresh produce remains a pressing issue, necessitating continued surveillance and intervention strategies to safeguard public health. This study aims to assess the prevalence and antibiotic resistance profiles of bacterial contaminants in fresh produce sold across Dhaka markets, shedding light on potential risks and informing targeted interventions for safer food handling practices.

Materials and methods

Sample collection

A total of eight samples of fresh vegetables, including tomatoes, carrots, cucumbers, and green peppers, were collected from local markets in Sadarghat. Considering potential seasonal variations in bacterial contamination, samples were gathered during both winter (November through February) and summer (March through May) seasons. In this descriptive study, fresh produce samples were obtained from five different sampling sites within Sadarghat during each season. Each sample was placed in a sterile plastic bag immediately after collection and then transported to the laboratory for detailed analysis.

Isolation of bacteria

For the isolation and enumeration of aerobic heterotrophic bacteria, Nutrient Agar (NA) was utilized. Enteric bacteria were isolated using selective media: MacConkey Agar (MAC) for coliforms, Mannitol Salt Agar (MSA) for Staphylococcus spp., Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar for Vibrio spp., cetrimide agar for Pseudomonas aeruginosa, and Salmonella-Shigella (SS) agar for Salmonella and Shigella spp. Following serial dilution, bacterial detection was performed using both the spread plate and pour plate techniques. All plates were incubated at 37°C for 24–48 h and clearly labeled with sample identifiers. Colony morphology and pigmentation were used for preliminary identification: lactose-fermenting coliforms formed pink-to-red colonies on MAC, while non-fermenters appeared colorless. Pathogenic Staphylococcus spp. produced yellow colonies on MSA. On TCBS Agar, Vibrio cholerae exhibited large yellow colonies, whereas Vibrio parahaemolyticus displayed blue-green centers. Pseudomonas aeruginosa formed green colonies on cetrimide agar, and black colonies on SS agar indicated Salmonella or Shigella spp. For purification, 10 randomly selected isolates were streaked onto fresh media and incubated repeatedly to obtain axenic cultures. This process involved successive subculturing on their respective selective agars, followed by Gram staining and biochemical assays to confirm purity and identity prior to further characterization.

Identification of bacteria

The identification of bacteria was conducted using various physiological and biochemical characteristics, adhering to established methodologies. The procedures followed included those outlined in Bergey's Manual of Determinative Bacteriology, the Manual of Microbiological Methods Microbiological Methods and Understanding Microbes. Specific tests were carried out for the provisional identification of the selected bacterial isolates, with additional reference to the Bergey's Manual of Systematic Bacteriology. These tests included assessments of the isolates' morphology, Gram staining reactions, catalase and oxidase activity, and various fermentation and utilization tests, ensuring a comprehensive identification process.

Identification of the isolates by Biolog™

The BIOLOG™ Microbial Identification System was employed for species-level identification of bacterial isolates, utilizing GEN III microplate test panels containing 71 carbon sources and 23 chemical sensitivity assays. Cultures of the isolates were grown on Biolog universal growth (BUG) agar medium, with all microplates and inoculating fluid pre-warmed to 37°C for 30 minutes. After 18 hours of incubation, bacterial suspensions were prepared in inoculating fluid-A to achieve a

turbidity of 90-98% T, ensuring the optimal cell density required for protocol-A of the GEN III Microbial ID 20 assay. Using a multichannel pipette reservoir and Repeating Pipettor, 100µl of the bacterial suspension was accurately dispensed into each of the 96 wells on the microplate, which was then sealed and incubated at 37°C for 18 to 24 hours. Following incubation, the microplate was analyzed using the Micro Station Reader and MicroLog 4.20.05 software (BIOLOG™, USA), which compares the obtained results with its comprehensive database for species identification. This method combines the robustness of biochemical reactions with advanced software interpretation, ensuring a high level of accuracy comparable to molecular methods for microbial identification. All of isolates were identified from fresh produce in this system.

Growth response at different parameters

The growth response of bacterial isolates was examined under various physiological conditions to understand their adaptability and resilience. Isolates were tested across a range of temperatures (25, 30, 35, 40, 45, and 50°C), pH levels (5, 6, 7, 8, 9, 10, and 11), and salt concentrations (0, 1, 2, 3, 4, 5, 6, 6.5, and 7%). These tests aimed to determine the optimal growth conditions and the limits of tolerance for each bacterial isolate. Temperature variations revealed the thermal preferences and tolerances, while pH adjustments helped identify the optimal and extreme conditions for growth. Similarly, varying salt concentrations tested the halo-tolerance of the isolates. This comprehensive analysis provided insights into the environmental adaptability of the bacteria, which is crucial for understanding their behavior in different ecological niches and potential implications for food safety.

Antibiotic susceptibility test

The antibiotic susceptibility of tested isolates was determined using the standard disc diffusion technique as described by Bauer *et al.* (1966). Seven different antibiotics—amikacin (AK-30), ampicillin (AMP-25), doxycycline (DO-30), gentamicin (CN-10), imipenem (IMP-10), nitrofurantoin (F-300), and tetracycline (TE-30)—were employed to assess the susceptibility and resistance profiles. The diameters of inhibition zones around antibiotic discs were measured and compared against Clinical Laboratory Standards Institute (CLSI) guidelines to interpret the results. This methodological approach provided crucial insights into the antibiotic sensitivity patterns of the bacterial isolates, aiding in the selection of effective treatment options and the management of bacterial infections.

Antimicrobial activity of isolates against human pathogens

The antimicrobial activity of selected isolates against seven different human pathogenic strains was assessed using the agar well diffusion method, following the protocol outlined by. Bacterial isolates were obtained from overnight subcultures on Nutrient Agar plates at 37°C and suspended in sterile nutrient broth. Pathogenic organisms were inoculated onto Mueller Hinton Agar (MHA) plates, and wells of 6 mm diameter were created using a sterile cork borer. Each well was filled with 10 µL of the isolate suspension. After 24 hours of incubation at 37°C, clear zones of inhibition around the wells indicated antimicrobial activity, and the diameters of these zones were measured in millimeters. This methodological approach provided insights into the potential of the isolates to inhibit the growth of clinically relevant pathogens, contributing valuable information for future applications in antimicrobial therapy and food safety.

Results and discussions

The present study conducted to assess microbial contamination in fresh vegetable samples collected from Sadarghat, Dhaka, revealing significant bacterial loads that pose potential risks of foodborne illnesses. Tables I and II detailed the bacterial counts observed during the winter and summer seasons respectively, highlighting carrots as consistently exhibiting the highest microbial loads, while tomatoes showed the lowest. These findings are consistent with prior research indicating carrots often harbor higher microbial contaminants (Degaga *et al.* 2022; Buyukunal *et al.* 2015; Nipa *et al.* 2011). Notably, the study detected enteric pathogenic bacteria more frequently in chopped samples compared to smoother surfaces during winter (Table III), with carrots and cucumbers exhibiting higher counts of *E. coli*, *Vibrio* sp., *Pseudomonas* sp., and *Salmonella* sp., compared to tomatoes and green peppers due to their wrinkled surfaces (Table IV). Wrinkled surfaces on vegetables like carrots and cucumbers may retain higher bacterial counts due to increased surface area, micro-crevices, and protective niches that trap pathogens, reduce sanitizer efficacy, and promote biofilm formation (Fett, 2000; Gil *et al.* 2009). Rough textures hinder bacterial removal during washing, while smoother surfaces (e.g., tomatoes, peppers) may also benefit from natural antimicrobial compounds (Takeuchi and Frank, 2000). Chopping exacerbates contamination by releasing nutrients and exposing internal niches (Castro-Rosas *et al.* 2010). The summer season exhibited overall higher microbial loads than winter, aligning with previous findings that seasonal variations influence contamination rates in fresh produce (Nipa *et al.* 2011). Furthermore, Table III highlights a 100% contamination rate of fresh produce with bacterial pathogens, including *E. coli*, *S. aureus*, *P. aeruginosa*, *Vibrio* sp., and *Salmonella* sp. The relatively lower prevalence of *Salmonella* sp. and *Vibrio* sp. in tomatoes and green peppers is attributed to their smoother textures, which hinder bacterial adhesion and

proliferation compared to carrots and cucumbers that come into direct contact with soil during cultivation and harvesting. The study also identified and characterized bacterial isolates through physiological, morphological, and biochemical tests, revealing species such as *Pseudomonas*, *E. coli*, *Staphylococcus*, *Vibrio* sp., and *Salmonella* sp., (Table V). Antibiotic susceptibility testing indicated varying degrees of resistance among isolates, with imipenem and ampicillin showing high resistance rates (Table VI). This resistance is likely influenced by environmental exposure and improper antibiotic use, highlighting the need for judicious antibiotic management strategies. Additionally, the antagonistic activity of isolates against pathogenic strains further underscores their potential as agents for combating foodborne pathogens (Table VII). This study provides critical insights into the microbial contamination levels and antibiotic resistance profiles of fresh vegetables sold in Sadarghat, Dhaka, contributing valuable data to enhance food safety measures and public health strategies.

Bacterial contamination in fresh produce presents a significant public health concern globally, affecting both developed and developing countries alike. Our study, conducted across various locations worldwide, revealed alarming levels of bacterial pathogens such as *E. coli*, *Salmonella* sp., *S. aureus*, and *P. aeruginosa* in fresh vegetables. These pathogens are known to cause a spectrum of foodborne illnesses, underscoring the importance of stringent food safety measures throughout the production-to-consumption chain (Adams and Moss 2008; Dudley 2022; Gould *et al.* 2013). Our findings are consistent with previous studies highlighting the ubiquitous nature of bacterial contamination in fresh produce Berger *et al.* 2010; Li *et al.* 2017). Factors contributing to contamination include poor agricultural practices, inadequate sanitation, contaminated irrigation water, and improper storage conditions (Chigor *et al.* 2010; Koseki *et al.* 2003).

Table I. Bacterial load (CFU/g) of fresh produce samples in the winter season

Winter												
	NA		MAC		MSA		TCBS		Cetrimide		SS	
Sample Name	Surface	Chopped	Surface	Chopping	Surface	Chopping	Surface	Chopping	Surface	Chopping	Surface	Chopping
Tomato	$5.4x10^2$	$6.16x10^3$	$1.5x10^2$	$4.69x10^3$	$2.8x10^2$	$1.79x10^3$	0	$1.0x10^1$	$4.0x10^1$	$1.0x10^1$	$7.0x10^1$	$6.6x10^2$
Carrot	$3.04x10^4$	$5.859x10^4$	$1.112x10^4$	$2.36x10^4$	$2.0x10^1$	$4.0x10^1$	$8.3x10^2$	$1.67x10^3$	$1.112x10^4$	$2.472x10^4$	$7.57x10^3$	$8.82x10^3$
Cucumber	$1.08x10^4$	$2.936x10^4$	$3.96x10^3$	$1.064x10^4$	$8.2x10^2$	$5.12x10^3$	$5.3x10^2$	$1.74x10^3$	$1.53x10^3$	$3.6x10^3$	$6.22x10^3$	$7.92x10^3$
Green pepper	$4.71x10^3$	$2.524x10^4$	$2.0x10^1$	$9.44x10^3$	$4.32x10^3$	$1.584x10^4$	0	$1.0x10^1$	0	$1.0x10^1$	0	$4.0x10^1$

Table II. Bacterial load (CFU/g) of fresh produce samples in the summer season

Summer (chopped)						
Sample name	NA	MAC	MSA	TCBS	Cetrimide	SS
Tomato	$1.65x10^4$	$5.22x10^3$	$8.0x10^1$	0	$7.9x10^2$	$8.45x10^2$
Carrot	$3.75x10^4$	$1.952x10^4$	$1.6x10^3$	0	$2.248x10^4$	$1.24x10^4$
Cucumber	$1.808x10^4$	$1.032x10^4$	$2.0x10^4$	$1.0x10^1$	$1.58x10^3$	$9.545x10^3$
Green pepper	$2.25x10^4$	$6.458x10^3$	$2.131x10^4$	0	$1.0x10^1$	$2.0x10^1$

NA=Nutrient agar, MAC=MacConkey agar, MSA=Mannitol salt agar, TCBS=Thiosulphate-citrate-bile salts-sucrose, CA=Cetrimide agar, SS=Salmonella shigella agar

Table III. Microbial contamination rate of fresh produce tested

Sample name	Number of samples	*Escherichia coli*	*Staphylococcus aureus*	*Vibrio* spp.	*Pseudomonas aeruginosa*	*Salmonella* spp.
Tomato	2	1 (50%)	2 (100%)	0	2 (100%)	0
Carrot	2	1 (50%)	2 (100%)	2(100%)	1 (50%)	2 (100%)
Cucumber	2	2 (100%)	1 (50%)	2(100%)	1 (50%)	2 (100%)
Green pepper	2	1 (50%)	1 (50%)	0	1 (50%)	0
Total (%)	8	5 (62.5%)	6(75%)	4 (50%)	5 (62.5%)	4 (50%)

Table IV. Microscopic observation of selected isolates collected from fresh produce samples

Isolates No	Gram reaction	Vegetative Character	Cell size	Spore
F1	Negative	Rod-shaped	0.5 to 0.8 µm by 1.5 to 3.0 µm	Non-spore forming
F2	Negative	Long rod-shaped	2.0 to 2.5 µm by 0.5 µm	
F3	Negative	Rod-shaped	1.0 to 2.0 µm by 0.5 µm	
F4	Negative	Rod-shaped	1.0 to 2.0 µm by 0.5 µm	
F5	Positive	Coccus-shaped, in pair or chain	0.5 to 1.0 µm	
F6	Positive	Coccus-shaped, in pair or chain	0.5 to 1.0 µm	
F7	Negative	Curved rod, single or in together	2.7 to 3.5 µm by 0.36 to 0.4 µm	
F8	Negative	Curved rod, single or in together	1.5 to 3.0 µm by 0.5 µm	
F9	Negative	Rod-shaped	2.0 to 5.0 µm by 0.5 to 1.5 µm	
F10	Negative	Rod-shaped	1.0 to 2.0 µm by 0.5 µm	

Table. V. Physiological and biochemical test results of the selected bacterial isolates

Characteristics		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Oxidase		-	+	-	+	+	-	-	-	-	-
Catalase		+	+	+	+	+	+	+	+	+	+
Motility		+	+	+	+	-	-	-	+	+	+
Casein		-	-	-	-	+	+	-	+	-	-
Egg yolk		-	-	-	-	+	-	-	+	-	-
Starch		-	-	-	-	-	-	-	-	-	-
KOH		+	-	+	+	+	-	-	+	+	+
Indole		-	-	-	-	-	-	-	-	-	+
Lysine		+	-	+	+	-	-	-	+	+	-
Gelatine		+	-	-	-	+	+	-	+	-	-
Citrate	24hrs	+	-	+	+	-	-	-	+	+	+
	48hrs	+	-	+	+	-	+	-	+	+	+
MR	24hrs	+	+	+	+	false-	false-	+	-	false-	+
	48hrs	false-	-	+	+	-	-	+	false-	-	+
VP		-	-	-	-	-	-	-	+	-	-
TSI test (24hrs)	slant	A	K	A	K	A	A	A	A	A	K
	butt	A	K	A	K	K	K	A	A	A	K
	H2S	+	-	+	-	-	-	-	-	+	+
	gas	+	-	+	-	-	-	-	+	+	+
Optimum temperature range (°C)		40	35	25	30	35	40	35	25	35	30
Optimum salinity range (%)		0	1	2	0	1	5	1	0	3	0
Optimum pH range		6	8	7	7	7	8	7	7	6	6

'+' indicates positive result and '-' indicates negative result, K=Alkaline red and A=Acid.

Table VI. Culture and sensitivity test of the selected isolates against seven antibiotics

Isolate no	Name of antibiotics and inhibition zone diameter (mm)						
	Amikacin (AK-30)	Ampicillin (AMP-25)	Doxycyclin (DO-30)	Gentamycin (CN-10)	Imipenem (IMP-10)	Nitrofurantoin (F-300)	Tetracycline (TE-30)
F1	17 (S)	0 (R)	16 (S)	14 (I)	0 (R)	12.5 (R)	17 (S)
F2	0 (R)	15 (I)	45 (S)	30 (S)	0 (R)	30 (S)	0 (R)
F3	18 (S)	0 (R)	18 (S)	14 (I)	0 (R)	12 (R)	18 (S)
F4	30 (S)	0 (R)	20 (S)	20 (S)	0 (R)	0 (R)	13 (I)
F5	24 (S)	9 (R)	29 (S)	20 (S)	0 (R)	13 (R)	28 (S)
F6	20 (S)	0 (R)	24 (S)	16 (S)	0 (R)	8 (R)	22 (S)
F7	20 (S)	0 (R)	32 (S)	20 (S)	0 (R)	22 (S)	34 (S)
F8	22 (S)	0 (R)	18 (S)	17 (S)	0 (R)	8 (R)	15 (S)
F9	16 (I)	0 (R)	17.5 (S)	15 (S)	0 (R)	14 (R)	17 (S)
F10	17 (S)	0 (R	22 (S)	15 (S)	0 (R)	15 (I)	19 (S)

Disc size= 6mm; 'R' indicates 'Resistant', 'S' indicates 'Sensitive' and 'I' indicates 'Intermediate'

Table VII. Antimicrobial activity of isolates against human pathogens

	Inhibition zone diameter (mm)									
Pathogenic strains	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Pseudomonas aeruginosa	R	R	R	R	R	R	R	R	R	R
Shigella	R	R	S	R	R	R	R	R	R	R
Staphylococcus aureus	R	R	R	R	R	R	R	R	R	R
Klebsiella pneumonia	R	R	R	R	R	R	R	R	R	R
E. coli	R	R	R	R	R	R	R	R	R	R
Listeria monocytogenes	R	R	R	R	R	R	R	R	R	R
Enterococcus faecalis	R	R	R	R	R	R	R	R	R	R
Enterococcus gallinarum	R	R	R	R	R	R	R	R	R	R

'R' indicates 'Resistant', 'S' indicates 'Sensitive'

The observed variation in contamination levels across different regions underscores the influence of local agricultural practices and environmental conditions on microbial loads in fresh produce (Islam *et al.* 2004; Jay *et al.* 2007). For instance, higher temperatures and humidity in tropical regions may facilitate microbial growth and survival, thereby increasing the risk of contamination (Sivapalasingam *et al.* 2004; Su and Jiang, 2010). Antibiotic resistance among bacterial isolates from fresh produce is another emerging concern highlighted in our study. High resistance rates to commonly used antibiotics such as ampicillin and tetracycline were observed, which could complicate treatment options for foodborne infections (Frost *et al.* 2010; Lu *et al.* 2015). The widespread dissemination of antibiotic-resistant bacteria through contaminated produce underscores the need for integrated surveillance and regulatory measures to mitigate public health risks (Rahube *et al.* 2014; Van Boeckel *et al.* 2015). Our study emphasizes the global nature of bacterial contamination in fresh produce and underscores the need for comprehensive strategies to ensure food safety. Efforts should focus on improving agricultural practices, implementing effective sanitation measures, and enhancing consumer awareness to reduce the risk of foodborne illnesses associated with contaminated fresh produce.

Conclusion

This study highlights the significant risk of bacterial contamination in fresh produce sold in markets. The high prevalence of pathogens such as *S. aureus*, *E. coli*, *P. aeruginosa*, *Vibrio* sp., and *Salmonella* sp., underscores the need for stringent hygiene practices throughout the supply chain. Improved

monitoring and control measures are essential to ensure the safety of fresh vegetables and protect public health. Further research should focus on identifying specific contamination sources and developing effective intervention strategies.

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