Comparative Microbiological and Antibiotic Resistance Analysis of Street-Vended Fruit Juice and Ice

By

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

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing the degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Ethics Statement

The laboratory procedures were all conducted in accordance with biosafety and standard microbiological protocols in the institution. No human subjects or animals were used in the research because the research included microbiological analysis of food and environmental samples (fruit juice and ice). Therefore, ethical approval was not required. All street vendors were informed that the samples would only be utilized in academic research and gave verbal consent during the collection of samples. In order to conduct a comprehensive geographic and chemical study of the microbial risk, the particular points of sampling and type of juices are revealed in this report. But in order to safeguard the privacy and to preserve the livelihoods of the individual vendors, the names and the precise addresses of the stalls were anonymized and substituted by uniquely assigned codes during the data analysis.

Abstract

Street-vended fruit juices are widely consumed in urban Bangladesh, yet their microbiological safety remains a concern. The study evaluated the quality and antimicrobial resistance of bacterial pathogens in 20 samples of street-vended fruit juices and ice in Dhaka, Bangladesh. Sixty-six isolates, including *Escherichia coli* (n=11), *Klebsiella pneumoniae* (n=20) and *Vibrio* spp. (n=35), were confirmed. Out of the 20 samples, 50% of the samples had total aerobic counts exceeding the recommended safety limits. *Klebsiella* spp. and *Escherichia coli* exceedance of microbiological limits was reported in 55% of samples each, whilst *Vibrio* spp. contamination was over regulatory levels in each sample (100%), indicating a significant issue to the community. Kirby-Bauer Disk Diffusion, which is a phenotypic susceptibility testing, showed a wide level of resistance to a large proportion of Amoxicillin (60% in ice; 41.3% in juice). It is interesting to note that isolates derived by juices were more resistant to the carbapenem Imipenem (32.6%) than those derived by ice (10%). On the other hand, Levofloxacin and Norfloxacin had 100 percent efficacy in all the samples. Such results point to the high public health hazard urging tightening of the hygiene policies and the education of the vendors to reduce the foodborne AMR pathogens.

Keywords: Street-vended beverages; Fruit juice; Ice; Antimicrobial resistance; Food safety; Bangladesh

Dedicated to those supporters and people who motivated us, and towards the hope of a better and healthier world.

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List of Acronyms

TAC	Total aerobic Count
FDA	Food and Drug Administration
AMR	Antimicrobial Resistance
MDR	Multidrug Resistance
CLSI	Clinical and Laboratory Standards Institute
CFU	Colony Forming Unit
PCR	Polymerase Chain Reaction
UV	Ultraviolet
EDTA	Ethylenediaminetetraacetic Acid
E. coli	*Escherichia coli*
K. pneumoniae	*Klebsiella pneumoniae*
EMB	Eosin Methylene Blue
SSA	Salmonella–Shigella Agar
XLD	Xylose Lysine Deoxycholate Agar
TCBS	Thiosulfate–Citrate–Bile Salts–Sucrose Agar
µL	Microliter
TAE	Tris Acetate EDTA

Chapter 1

Introduction

1.1 Overview

In highly populated urban centers like Dhaka the population consumes beverages, especially fruit juices and ice to cool them, which are sold in the streets because they are cheap and readily available, especially among the poor groups. Although these beverages are important in terms of nutrition and economy, they pose significant threats to the general health of the people because of poor hygienic preparation and handling procedures. The contamination is commonly caused by untreated or contaminated water, the insufficient sanitation at the vending areas, and the lack of standard food safety precautions by the vendors (Afroz et al., 2024). A number of food safety investigations have confirmed high occurrence of enteric pathogens in beverages sold in the streets, where most of the commonest culprits found to be fecal-carriers and hygienically unsound include *Escherichia coli*, *Klebsiella pneumoniae*, and *Vibrio* spp. (Uddin et al., 2017). These are microorganisms that are related to gastrointestinal diseases such as diarrhea and cholera. Although the ice that is employed as a cooling system seems to be under-studied, still, it is a crucial source of microbial contamination despite the fact that the majority of research is conducted on fruit juices. The ice is made out of non-potable water and manipulated in unhygienic conditions, which allow the spread of pathogenic bacteria into drinks (Nordmark et al., 2022).

The emerging issue of increasing cases of AMR also contributes to the problem of the public health issue. The existence of MDR bacteria, such as Carbapenemase-producing Enterobacteriaceae (CPE), in the street-food supply chain has been reported in the recent literature (Sinha et al., 2025). Such organisms can be harboring resistance determinants like the Klebsiella pneumoniae carbapenemase (KPC) gene that make them resistant to carbapenems- the antibiotics that are usually used as the last line of treatment. Their spread with the help of the most popular items offered at the stands and sold in the streets is a significant burden to clinical therapy and points to the necessity of constant observation and control.

Chapter 2

Literature Review

2.1 Microbiological Quality of Street-Vended Beverages

In fast growing cities such as Dhaka, street-side beverages act as a two-sided sword- it is a source of inexpensive hydration, but also the major source of environmental contaminants such as fecal matter, dust and untreated water. According to World Health Organization (WHO, 2022), foodborne diarrheal diseases are a leading health crisis on the global agenda, which is often due to abject sanitation in informal food circles. Local tests conducted in Dhaka have revealed that these fresh juices are infested with bacteria. Intriguingly, *Salmonella* and *Shigella* cannot always be found, but *Vibrio* spp. is almost omnipresent in such samples (Jahan et al., 2022). This is reflected in data of Chittagong, which identified street juices as hot spots of *Klebsiella pneumoniae*, *Vibrio cholerae*, and *Staphylococcus aureus*, most of which are already resistant to first-line agents such as amoxicillin (Paul et al., 2018).

Such a heavy load of microbes is often carried with them due to a three-step contamination: improper hygiene of the vendor, the use of tap water to dilute it, and the mere lack of clean running water to wash the utensils (Khan et al., 2015). It is also important to note that the high acidity (pH less than 4.0) of certain juices such as lime or orange perhaps is the sole factor that does not allow an even greater number of pathogens such as *Salmonella* (Yin, Gyles, & Gong, 2012).

2.2 Pathogen Profiles

The detection of Enterobacteriaceae (specifically *E. coli* and *K. pneumoniae*) in a drink is a "red flag" for fecal-oral contamination. *K. pneumoniae* is also very problematic with cold drinks. It does not merely remain in the water, rather, it forms rough and sticky biofilms on the inside of the ice-making machines and storage bins, which is virtually impossible to clean with regular detergents (Li & Ni, 2023). In the meantime, Vibrio species can survive in such chilled and wet conditions and thus, they continue to be a constant, climate-tolerating menace to any individual taking street drinks (Abram & Uff, 2023). The level of *E. coli* in South Asian Street food is usually above the level of safety standards provided by international regulatory bodies, which is a primary indication that the drink is unsafe to drink (Ahmed et al., 2024).

2.3 The Emergence of Antimicrobial Resistance (AMR)

The emergence of the so-called superbugs among the street-food suppliers is a silent but lethal crisis. In South Asian cities, investigators have detected *E. coli* and *Vibrio* isolates in fruit drinks that are utterly resistant to Ampicillin or Cefotaxime (Sharma et al., 2020). Worse still, there is the development of Carbapenem-Resistant Enterobacteriaceae (CRE). As the name suggests, these bacteria possess the KPC (Klebsiella pneumoniae carbapenemase) gene, which is basically an enzyme that basically digests carbapenems - the antibiotics which physicians resort to as a final option. Their presence in a typical street drink is an indication that the food chain has become a significant road through which hospital-grade resistance makes its way into the general population (Huang et al., 2023).

2.4 The Hidden Risk: Ice as a Major Vector

Although there is everyone who is concerned about the juice, the ice that is introduced to the juice is usually the true culprit. The bacterial density of cooling ice is often high compared to that of the juice (Nichols, Monsey, & de Louvois, 2000). This ice is typically manufactured in factory blocks of Dhaka with poor-quality water, scraped on the unsanitary floors, and broken in unhygienic machines. The research on ice-based products conducted internationally demonstrates that once the basic sanitation is overlooked, ice turns out to be the leading vehicle of *E. coli* and *K. pneumoniae* delivery (Papadopoulou et al., 2023).

2.5 Literature Gaps

Although the risks of the street-vended refreshments are evident, there is a marked absence of comparative data that considers ice and juice as independent variables, although interacting. Majority of the researches concentrate only on the end product and do not identify the source of the MDR pathogens and whether these are in the juice or the ice that has been added. This study will address this gap that is so essential by conducting a parallel analysis of the two types of samples so that the data can be used to inform the public health policy through data.

2.6 Objectives

Comparison of the microbiological quality and antibiotic resistance pattern of the bacteria isolated in the fruit juice and ice sold on the streets is the main purpose of this research. In particular, the study will seek to:

1. Isolate and identify the target bacterial pathogens, i.e., *E. coli*, *K. pneumoniae*, and *Vibrio* spp., of street-vended fruit juice and ice in such juices.
2. Measured and compared microbial load and prevalence of the identified pathogens in the tissue fruit juice and ice samples.
3. Determine the susceptibility profiles of the isolated bacteria to the antibiotics by the Kirby-Bauer disk diffusion test.
4. Identify the markers of antimicrobial resistance, such as carbapenemase production (e.g., KPC) by phenotypic means and polymerase chain reaction (PCR).
5. Compare the results in terms of contamination and resistance of fruit juice and ice to know which one poses a more significant risk to the population in general.

Chapter 3

Materials and Methods

3.1 Sample collection and transporting

A total of 20 samples were collected from February to July, of which 11 were juice samples and 9 were ice samples. The samples of fruit juice and ice provided by the vendors on the streets were gathered at various urban areas across Dhaka city such as Gulshan, Mohakhali, Merul Badda, Banasree, Uttara and Mohammadpur. The study also included different fruit juices of freshly made drinks (orange, watermelon, papaya, lemon, mango and pineapple) and the ice that was put in the drinks to ensure representative sampling. Detailed information on the samples is provided in Table 1.

The samples of fruit juice and ice were taken in disposable cups at the street vendors and packed in tightly closed plastic bags. The samples were all taken to the laboratory in ice box that was insulated and all processed within 2-4 hours of collection to reduce alteration of microbial load and avoid contamination. These samples were melted at room temperature before being analyzed in the university laboratory.

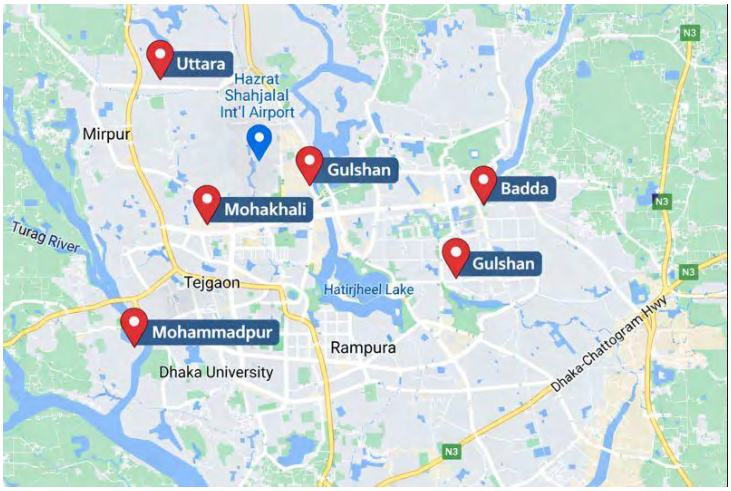

Figure 1: Map of Sampling Sites in Dhaka, Bangladesh

Table 1: Identification and Geographical Distribution of Street-Vended Fruit Juice and Ice Samples

Sample Type	Sample Area	Given Name
Orange Juice-1	Gulshan	GLOJ-1
Orange ice-1	Gulshan	GLOI-1
Watermelon juice-1	Mohakhali	MWJ-1
Watermelon ice-1	Mohakhali	MWI-1
Papaya Juice-1	Merul Badda	MBPJ-1
Papaya ice-1	Merul Badda	MBPI-1
Lemon juice-2	Banasree	BLJ-2
Lemon ice-2	Banasree	BLI-2
Orange juice-2	Merul Badda	MBOJ-2
Mango Juice-2	Merul Badda	MBMJ-2
Lemon Juice-3	Merul Badda	MBLJ-3
Lemon ice-3	Merul Badda	MBLI-3
Lemon Juice -3	Uttara	ULJ-3
Lemon Ice-3	Uttara	ULI-3
Watermelon Juice-3	Gulshan	GWMJ-3
Watermelon ice-3	Gulshan	GWMI-3
Pineapple juice-4	Uttara	UPAJ-4
Pineapple ice-4	Uttara	UPAI-4
Lemon juice-4	Mohammadpur	MMLJ-4
Lemon ice-4	Mohammadpur	MMLI-4

3.2 Sample processing and bacterial isolation

All the fruit juices and associated melted ice samples were well homogenized before analysis. Juice and ice samples were diluted tenfold separately using sterile normal saline. 0.1 ml aliquots of the dilution were aseptically spread on selective and differential agar media using standard spread plate and streak plate methods. To isolate certain bacterial pathogens, HiCrome KPC agar and Eosin Methylene Blue (EMB) agar were used to isolate *E. coli* and *K. pneumoniae* respectively. All the inoculated plates were incubated at 37° C, 18-24 hours. The colonies with a typical morphology were counted after incubation, and the number of bacteria

was reported as colony-forming units per milliliters (CFU/ml) to estimate the TAC and pathogen loads using this formula (Khan et al., 2015).

$$\text{CFU/ml} = \frac{Number\ of\ colonies \times Dilution\ factor}{Volume\ plated\ (mL)}$$

3.2.1 Enrichment Using Alkaline Peptone Water

For selective enrichment of *Vibrio* spp. and *Salmonella* spp., 5 ml of each fruit juice and melted ice sample was inoculated into 45 ml Alkaline Peptone Water (APW). The enrichment broths were incubated at 37°C for 5 hours under aerobic conditions in a shaker incubator. The alkaline pH (about 8.5) and high peptone concentration of APW promote the preferential growth of Vibrio species while suppressing competing microorganisms. After enrichment, the samples were diluted and 100 µl of each sample from dilution were plated on TCBS agar and Salmonella-Shigella (SS) agar and for the isolation of *Vibrio* spp. and *Salmonella* spp. respectively. All the plates were incubated at 37°C for 48 hours (Uddin et al., 2017).

3.3 Bacterial Culture

3.3.1 Isolation of bacteria

The original and enriched samples were spread on selective media, which allows the growth of desired bacteria and not others to recover the target organisms. Enrichment enhanced the chances of identifying organisms that were of low concentration. The colonies that exhibited unique morphological characteristics were assumed to be the possible isolates. The picked colonies were then selected based on the media employed and their respective characteristics in the colonies were summarized in Table 2 and finally colonies were picked and streaked onto the selective media for further screening and carry out the additional analysis.

Table 2: Colony Characteristics of Bacterial Isolates on Selective Media

Target Pathogen	Gram Reaction	Media	Colony Appearance
E. coli	Gram-negative	HiCrome KPC Agar	Purple to pink colonies
		EMB	Metallic Green Sheen
K. pneumoniae	Gram-negative	HiCrome KPC Agar	Blue or light blue colonies
Vibrio spp.	Gram-negative	TCBS agar	Yellow colonies
Salmonella spp.	Gram-negative	SS Agar	Colorless colonies with a black center
		XLD agar	Red colonies with black centers

3.4 DNA Extraction

3.4.1 Preparation of Bacterial Templates

Rapid thermal lysis protocol was used to get a viable DNA template to be used in downstream molecular analysis of *E. coli*, *K. pneumoniae*, *Salmonella* spp. and *Vibrio* spp. This was done by picking one, well-isolated colony in each respective selective media, i.e. KPC chromogenic agar with the Enterobacteriaceae, XLD with *Salmonella* spp. and TCBS agar with Vibrio isolates. A pure colony was then transferred with a sterile inoculation loop to 1.5 ml micro-centrifuge tube with 150 µL of TE buffer. The use of TE buffer was also intentional, as it offers a stable state where nucleic acids can be maintained and it can counteract the degradation of the latter during the following heat-shock period (Packeiser et al., 2013).

3.4.2 Thermal Disruption via Dry Heat Block

High-speed vortexing was done until the bacterial cells were fully mixed and the suspension became uniform. The tubes were put into a 15-minute dry heat block with the capacity of 100 C in order to trigger the release of the genomic DNA. This heat treatment denatures cellular proteins and disrupts cell membranes, releasing genomic DNA into the buffer (Dashti et al., 2009).

3.4.3 Centrifugal Clarification and Supernatant Recovery

Just after the heating session, the tubes were moved to an ice rack to spend a brief stabilization period. A centrifugation of 15 minutes at 13,000 rpm was done in order to separate the liberated DNA and the precipitated cellular debris and proteins. The rapid spin formed a dense solid pellet at the base of the tube. The supernatant was now clear and the released DNA was carefully aspirated using a micropipette. Those were careful not to mix up the underlying pellet in order to avoid the addition of PCR inhibitors to the final template. This Tablet DNA sample was long-term stored at -20° C or immediately used as a template in Polymerase Chain Reaction (PCR) amplification (Dashti et al., 2009).

3.5 PCR Amplification and Gel Electrophoresis

Molecular identification of *E. coli, K. pneumoniae, Salmonella* spp. and *Vibrio* spp. was done by the use of species-specific primers in Polymerase Chain Reaction (PCR). A total of 13 µL of template DNA and forward and reverse primers, PCR master mix, and nuclease-free water were used in PCR reaction.

Table 3 outlines the primers sequences and conditions of cycling in this paper. 1.5 % agarose gel was prepared by adding 2 ml 50X working TAE buffer to the distilled water and followed by heating until completely melted. After cooling down the mixture, 4 µL ethidium bromide was added. Then the mixture was poured into the casting tray with comb to form the wells. After solidification, the gel was placed in the electrophoresis tank in submerged 1X running TAE buffe. DNA samples along with a DNA ladder were carefully loaded into the wells, and electrophoresis was carried out at 100 V for 50 minutes. The gel was seen under UV light with the help of a gel documentation system.

Table 3: Oligonucleotide Primer Sequences and Optimized Thermal Cycling Conditions for the Molecular Identification of Target Isolates

Targeted Organism	Primer	Primer sequence	PCR conditions	Amplicon Size (bp)	Reference
E. coli	ECO- F	5′-GACCTCGGTTTAGTTCACAGA-3′	Initial denaturation at 95°C for 10 min; 35 cycles of denaturation at 95°C for 30s, annealing at 58°C for 30s and extension for 1 min, 72°C for 1 min followed by a 7 min final extension at 72°C	585	(Odetoyin et al., 2022)
	ECO- R	5′-CACACGCTGACGCTGACCA-3′			
K. pneumoniae	KP Pf-F	5′-ATTTGAAGAGGTTGCAAACGAT-3′	The cycling conditions were 10 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 20 s at 57 °C and 20 s at 72 °C, followed by a 10 min hold at 72 °C	130	Liu et al. (2008)
	KP Pr1-R	5′-TTCACTCTGAAGTTTTCTTGTGTTC-3′			
Vibrio Spp.	V.G-F:	5'-GTCARATTGAAAARCARTTYGGTAAAGG-3'	The cycling conditions were 5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 60 °C and 30 s at 72 °C, followed by a 10 min hold at 72 °C	689	(Fukuda, Tsunashima, & Usui, 2023)
	V.G-R	5'-ACYTTRATRCGNGTTTCRTTRCC-3'			

3.6 Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion test on Mueller Hinton agar was used as the methods of antibiotic susceptibility testing of confirmed isolates using Clinical and Laboratory Standards Institute (CLSI) guidelines. The suspension of bacteria was brought to 0.5 McFarland standard and spread onto agar plates evenly. The plates that were inoculated were incubated with antibiotic disks that represented the various classes. Plates were incubated at 37º C with a time of 18-24 hours and the size of inhibition zone in millimeters.

Table 4: Interpretive Standards for Antibiotic Susceptibility Testing (AST) and Zone Diameter Breakpoints

No.	Antibiotic Class	Antibiotic Name	Disk Code (µg)	Range		
				Resistance (mm) or less	Intermediate (mm)	Susceptible (mm) or more
1	Aminoglycoside	Amikacin	AK 30	14	15-16	17
2	Penicillin	Amoxyclav	AMC 30	11	12-14	15
3		Amoxicillin	AML 10	13	14-17	20
4	Cephalosporin	Ceftazidime (3rd)	CAZ 30	14	15-17	18
5		Ceftriaxone (3rd)	CRO 30	13	14-20	21
6		Cefepime (4th)	FEP 30	18	19-24	25
7	Fluoroquinolone	Ciprofloxacin	CIP 5	15	16-20	21
8	Tetracycline	Doxycycline	DO 30	15	16-19	20
9	Carbapenem	Imipenem	IPM 10	19	20-22	23
10	Aminoglycoside	Kanamycin	K 30	13	14-17	18
11	Fluoroquinolone	Levofloxacin	LEV 5	13	14-16	17
12	Carbapenem	Meropenem	MEM 10	19	20-22	23
13	Fluoroquinolone	Nalidixic acid	NA 30	13	14-18	19
14		Norfloxacin	NOR 10	12	13-16	17
15	Nitrofuran	Nitrofurantoin	F 300	14	15-16	17
16	Penicillin	Piperacillin	PRL 100	17	18-20	21
17	Sulfonamides	Trimethoprim/ Sulfamethozole	SXT 25	13	14-18	19
18	Tetracycline	Tetracycline	TE 30	14	15-18	19

Chapter 4

Results

4.1 Incubation on selective media

Bacterial growth was observed on all samples of fruit juices and ice on selective media, and this means that there is contamination by microorganisms. On Eosin Methylene Blue (EMB) agar, presumptive isolates of *E. coli* formed typical metallic green sheen colonies. The isolates of *K. pneumoniae* produced big, mucous, blue colonies on the HiCrome KPC agar. On TCBS agar, presumptive *Vibrio* spp. gave yellow colonies, which means that it ferments sucrose. On Salmonella-Shigella (SS) agar and XLD agar, no typical *Salmonella* colonies were detected in any of the samples. The morphologies of observed colonies were coincident with the standard descriptions of the colonies in the literature on microbiological research of street-vended beverages (Khan et al., 2015).

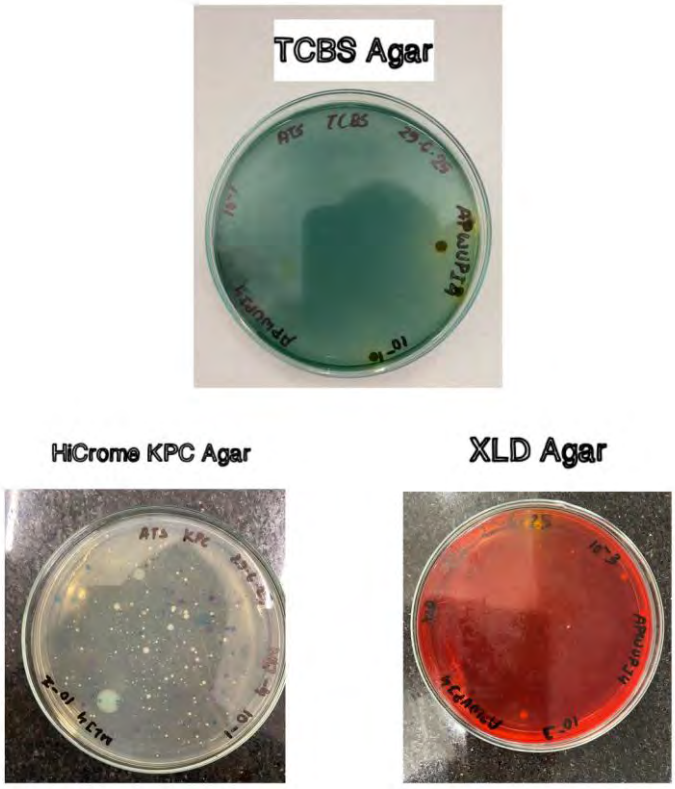

Figure 2: Morphological Observation of Bacterial Isolates on KPC, TCBS, and XLD Agar via the Spread Plate Method

4.2 Total aerobic plate count of the samples

Table 5 gives a summary of the TAC and pathogen-specific bacterial loads of fruit juice and ice samples. In general, the import of fruit juice samples showed significantly more bacteria loads as compared to their ice counterparts. The fruit juice samples in the TAC had a range of 2.3 x 10^3 to 5.4 x 10^6 CFU/ml and the ice samples had comparatively lower counts of 1.5 x 10^3 -2.2 x 10^4 CFU/ml. The juice samples, watermelon juice (GWMJ-3) and lemon juice (BLJ-2), showed the best TAC values. Fruit juice samples had a count of 1.2 10^4 to 3.6 10^4 CFU/ml of *E. coli* whereas ice samples had lower counts (0 to 2.0 10). Klebsiella spp. were found in all samples of juices with a range of concentrations of 3.8 × 10^4 to 7.9 × 10^4 CFU/ml, and in ice samples with an average of 2.1 × 10^1 to 3.9 × 10^2 CFU/ml. Both juice and ice samples always harbored *Vibrio* spp., but the amount of *Vibrio* spp. was higher in juice samples (2.9×10^3 to 7.2×10^3 CFU/ml) than in ice samples (8.0x10^2 to 2.4x10^3 CFU/ml). It can be explained by the increased bacterial content in fruit juices as the result of such factors as improper handling, the presence of contaminated water in which the juices are diluted, being exposed to environmental pollution, which has been reported in other studies (Khan et al., 2015).

Table 5: Recommended microbiological limits for fruit juices and ice according to international food safety guidelines

Parameter	Accepted Limit (m)	Maximum Limit (M)	Guideline Reference
TAC	<10^4 CFU/ml	10^5 CFU/ml	(GSO, 2000; ICMSF, 2002)
E. coli	Not detectable (0)	<10^2 CFU/ml	(GSO, 2000; CFS, 2014)
Vibrio spp.	Not detectable (0)	Not detectable (0)	(WHO, 2011; BFSA, 2023)
Klebsiella spp.	Not detectable (0)	<10^2 CFU/ml	ICMSF (2002)
Salmonella spp.	Not detectable (0)	Not detectable (0)	FDA (2021)

The recommended limit of the total aerobic count in ready-to-drink beverages according to the ICMSF and WHO guidelines is 104 CFU/mL and must be free of *E. coli*, *K. pneumoniae*, and *Vibrio* spp. The majority of samples in the present study were above these permissible limits.

Table 6: Bacterial Load of Street-Vended Fruit Juice and Ice Samples. Values shown in bold indicate samples exceeding the maximum permissible limit.

Sample Name	TAC (CFU/ml)	*E. coli* (CFU/ml)	*K. pneumoniae* (CFU/ml)	*Vibrio* spp. (CFU/ml)
GLOJ-1	2.3×10^{3}	**1.2×10^{4}**	**4.5×10^{4}**	**3.1×10^{3}**
GLCI-1	**1.1×10^{4}**	2.0×10^{2}	3.5×10^{2}	**1.2×10^{3}**
MWJ-1	**4.2×10^{6}**	**2.8×10^{4}**	**6.1×10^{4}**	**5.5×10^{3}**
MWI-1	1.5×10^{3}	0	2.2×10^{1}	**8.0×10^{2}**
MBPJ-1	**3.7×10^{6}**	**1.9×10^{4}**	**5.2×10^{4}**	**4.2×10^{3}**
MBPI-1	2.2×10^{4}	1.3×10^{2}	3.0×10^{2}	**2.1×10^{3}**
BLJ-2	**5.1×10^{6}**	**3.3×10^{4}**	**7.4×10^{4}**	**6.8×10^{3}**
BLI-2	9.0×10^{3}	4.0×10^{1}	8.5×10^{1}	**1.1×10^{3}**
MBLJ-2	**3.9×10^{6}**	**2.1×10^{4}**	**4.8×10^{4}**	**3.9×10^{3}**
MMJ-2	**4.5×10^{6}**	**2.5×10^{4}**	**5.9×10^{4}**	**5.1×10^{3}**
MBLJ-3	**2.8×10^{6}**	**1.5×10^{4}**	**3.8×10^{4}**	**2.9×10^{3}**
MBLI-3	1.8×10^{4}	1.1×10^{2}	2.1×10^{2}	**1.9×10^{3}**
ULJ-3	**3.1×10^{6}**	**1.7×10^{4}**	**4.2×10^{4}**	**3.5×10^{3}**
ULI-3	1.3×10^{4}	9.0×10^{1}	2.4×10^{2}	**1.5×10^{3}**
GWMJ-3	**5.4×10^{6}**	**3.6×10^{4}**	**7.9×10^{4}**	**7.2×10^{3}**
GWMI-3	8.5×10^{3}	3.2×10^{1}	7.0×10^{1}	**9.5×10^{2}**
UPAJ-4	**4.8×10^{6}**	**2.9×10^{4}**	**6.5×10^{4}**	**5.8×10^{3}**
UPAI-4	2.0×10^{4}	1.6×10^{2}	3.9×10^{2}	**2.4×10^{3}**
MMLJ-4	**3.5×10^{6}**	**1.8×10^{4}**	**4.4×10^{4}**	**3.7×10^{3}**
MMLI-4	1.1×10^{4}	8.5×10^{1}	2.1×10^{2}	**1.3×10^{3}**

4.4 Molecular Identification of Bacterial Isolates

Both the samples of fruit juice and ice were found to contain the target bacterial species as confirmed by PCR amplification. The amplification of the *E. coli-specific* gene produced a 585 bp amplicon, *K. pneumoniae*, a 130 bp amplicon and *Vibrio* spp. a 689 bp amplicon, as seen on agarose gel electrophoresis (Figures 4-6).

The results of the selective culture and the phenotypic characterization were supported by the molecular findings that demonstrated the presence of these pathogens in the fruit juices and ice sold in the streets. Bangladesh and other underdeveloped countries have already carried out molecular confirmation of foodborne pathogens.

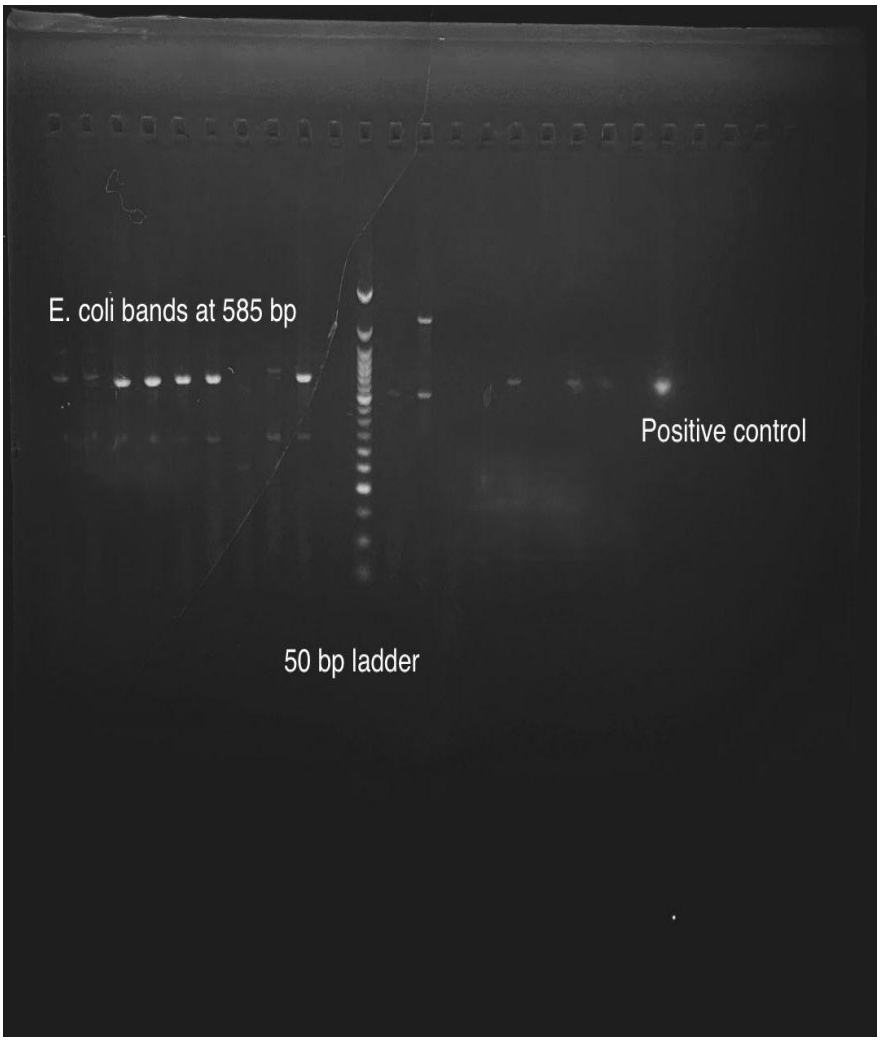

Figure 3: Agarose Gel Electrophoresis of PCR-Amplified Products for the Molecular Confirmation of *E. coli*

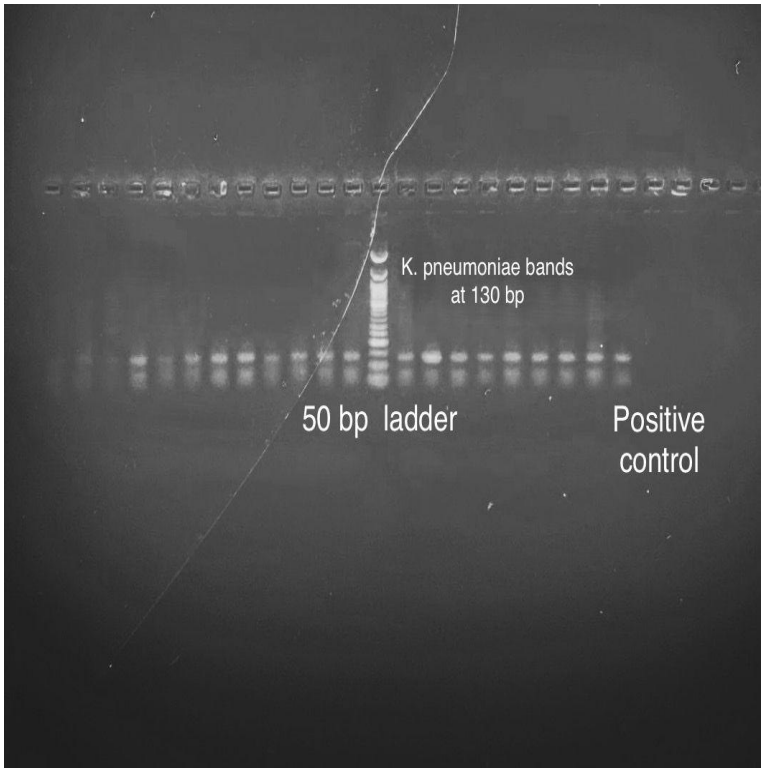

Figure 4: Agarose Gel Electrophoresis of PCR-Amplified Products for the Molecular Confirmation of *K. pneumoniae*

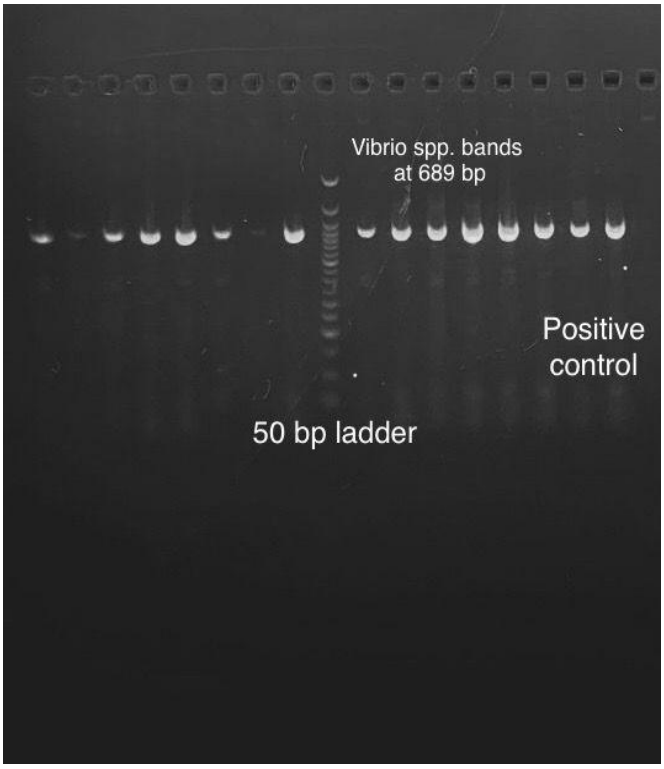

Figure 5: Agarose Gel Electrophoresis of PCR-Amplified Products for the Molecular Confirmation of *Vibrio* spp.

No amplification was observed for *Salmonella* spp., indicating its absence in the analyzed samples.

Vibrio spp. was the most prevalent in the samples of juice (47.5%), then *K. pneumoniae* (35.0%), and finally *E. coli* (17.5%). Equally, *Vibrio* spp. (58.3%) was the most prevalent contaminant of ice samples, with frequencies of *K. pneumoniae* (25.0%) and *E. coli* (16.7%. Both types of samples did not contain *Salmonella* spp.

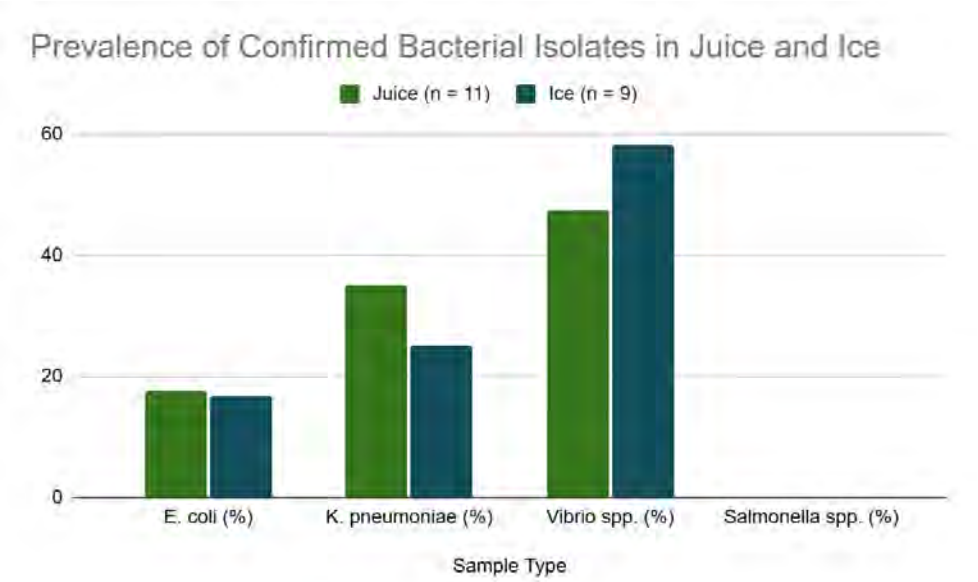

Figure 6: Prevalence and Distribution of Confirmed Bacterial Isolates in Juice and Ice

4.5 Antimicrobial susceptibility profiles

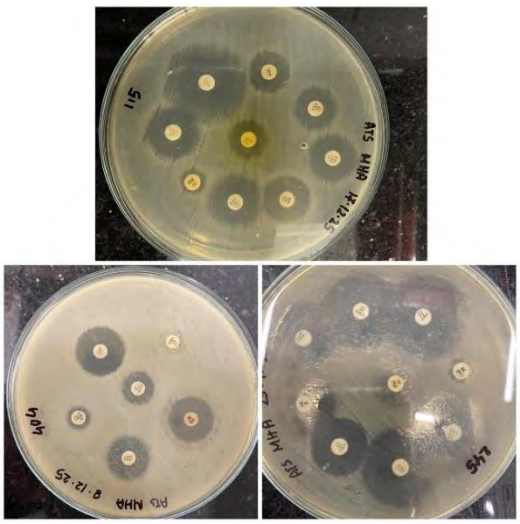

Figure 7: Inhibition zones of antibiotics disks of *E. coli, K. Pneumoniae and Vibrio* spp.

4.5.1 Resistance Profiling of *E. coli* Isolates

Experiment was carried out to identify the phenotypic resistance patterns of the confirmed isolates of *E. coli* through the use of the Kirby-Bauer disk diffusion technique. One of the outcomes of this research was that the prevalence of resistance to carbapenems, or more precisely to Meropenem, was considered very high (82.5%). Conversely, Imipenem resistance was much lower with 17.5 percent and then Cefepime (17.5%), Piperacillin (10%), and finally Nitrofurantoin (8.5%). The difference in the level of Meropenem resistance and Imipenem resistance is an indication of a complicated resistance mechanism across the recovered strains.

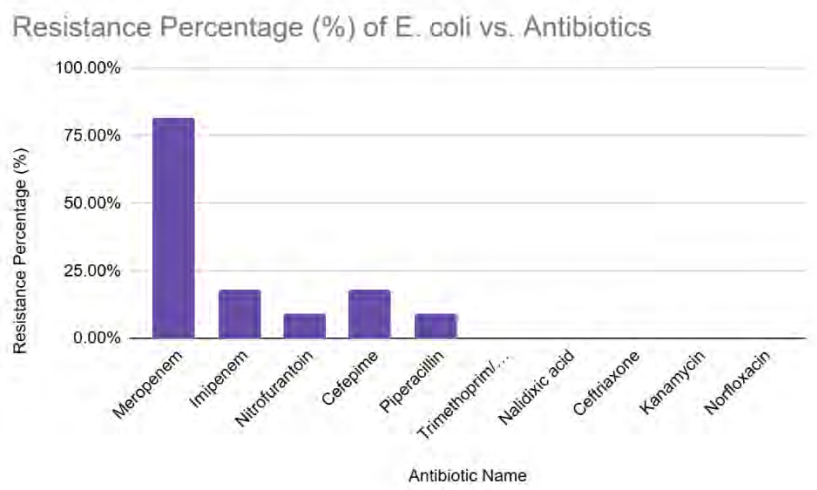

Figure 8: Antibiotic susceptibility testing based on inhibition zone diameters of *E. coli*

4.5.2 Resistance Profiling of *K. pneumoniae*

The isolates of *K. pneumoniae* had their own and alarming MDR profile. Ceftazidime and Imipenem had the highest resistance with the highest level of resistance being 83.33%.

Amoxicillin Clavulanic Acid (38.89%) and Cefepime (38.89%) were noted to have a moderate level of resistance, but the isolates were found to be relatively more susceptible to Kanamycin (5.56%).

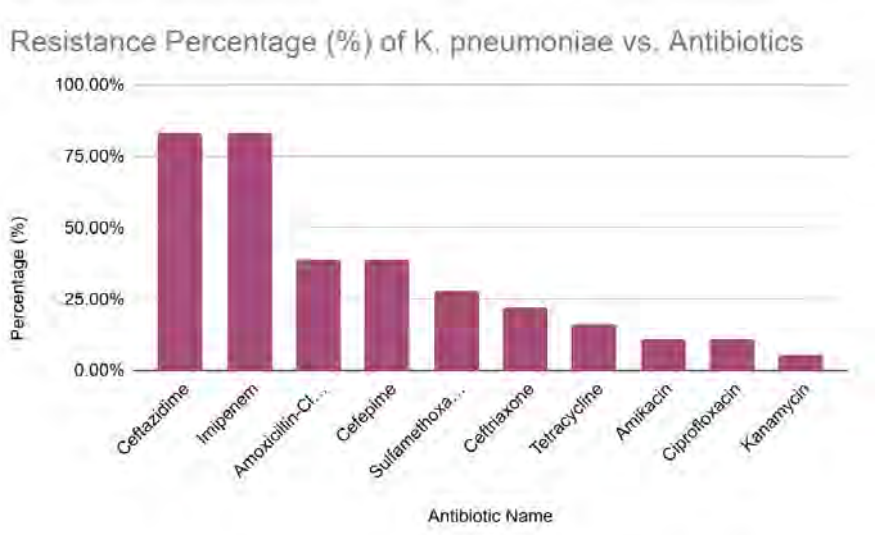

Figure 9: Antibiotic susceptibility testing based on inhibition zone diameters of *K. pneumoniae*

4.5.3 Resistance Profiling of *Vibrio* spp.

The *Vibrio* spp. isolates were almost completely resistant to Amoxicillin as 96.67% of the isolates did not exhibit sensitivity. It was then followed by Doxycycline (27.78) and Nalidixic Acid (25%).

The prevalence of resistance to Ceftriaxone (12.5%) and Sulfamethoxazole-Trimethoprim (9.52%) was not so high meaning that these agents could still have some activity in this infection against aquatic pathogens.

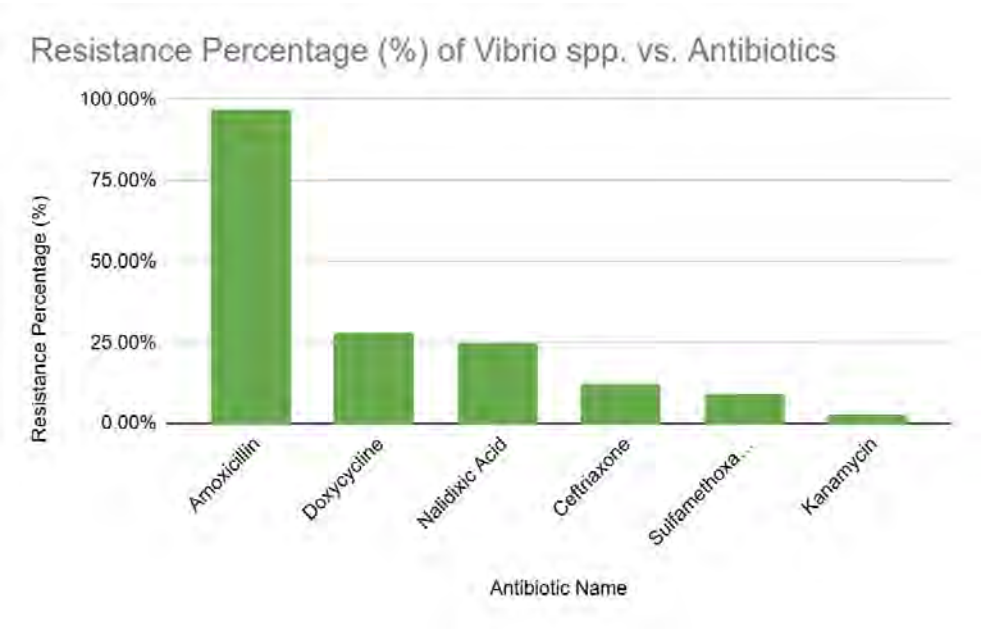

Figure 10: Antibiotic susceptibility testing based on inhibition zone diameters of *Vibrio* spp.

4.5.4 Comparative Antibiogram Summary (Total Isolates)

In the study conducted on all the 66 isolates recovered on the samples of street-vended juice and ice, Amoxicillin was found to be the least effective antibiotic with a general percentage resistance in the study amounted to 51.11. The resistance rate of imipenem in the overall sample population was 25.76% and Ceftazidime was 22.73%. Comparative analysis of the sample source showed that the drug resistance percentages of drugs such as Amoxicillin (approx. 42%), Imipenem (approx. 33%), etc. were generally higher in juice samples than in the ice samples that were similar to each other at 60% resistance to Amoxicillin but had low-percentage resistance to other agents.

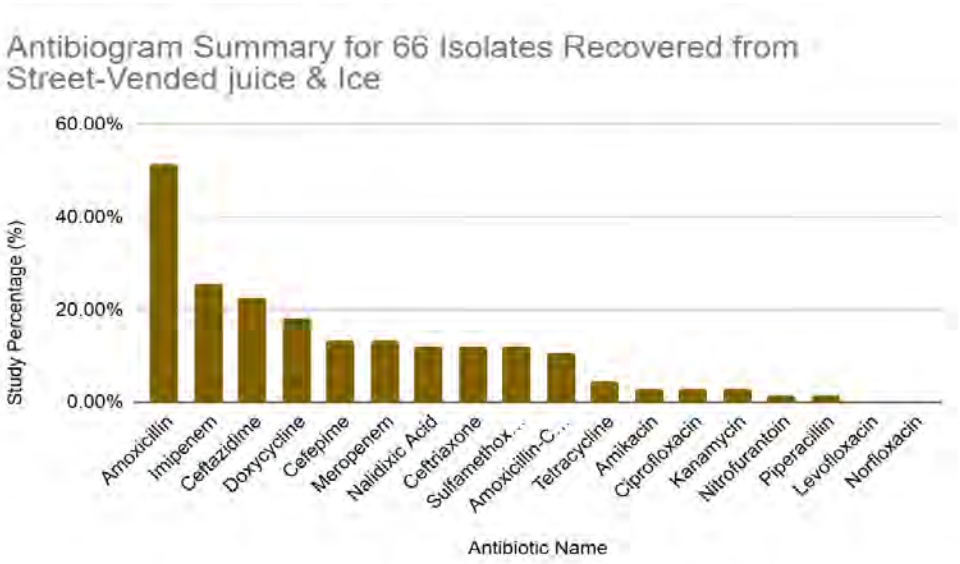

Figure 11: Cumulative Antibiogram Summary for 66 Isolates Recovered from Street-Vended Juice & Ice

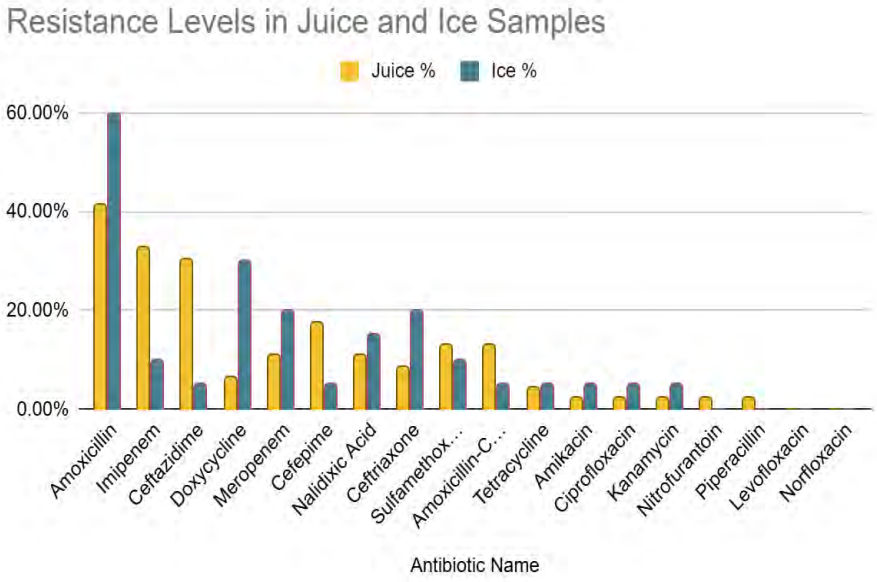

Figure 12: Comparison of Resistance Levels in Isolates Recovered from Juice and Ice Samples

4.5.5 Prevalence of Multidrug Resistance (MDR) among Isolates

Following the initial antimicrobial susceptibility testing, the isolates were screened for MDR. Multidrug resistance was defined as resistance to three or more classes of antimicrobial agents in accordance with international standards.

Out of the total 66 bacterial isolates recovered from street-vended fruit juices and ice samples, 16 isolates (24.24%) were identified as MDR. The distribution of these resistant strains varied significantly across the three species tested (Table 7).

Table 7: Distribution of Multidrug-Resistant (MDR) Isolates

Bacterial species	*Total isolates (n)*	*MDR isolates (n)*	*MDR (%)*
E. coli	*11*	*1*	*9.09*
K. pneumoniae	*20*	*8*	*40*
***Vibrio* spp.**	*35*	*7*	*20*
Total	*66*	*16*	*24.24*

Chapter 5

Discussion

The current research proves that the fruit juice and ice samples of the two locations in Dhaka were microbiologically polluted and contained antibiotic resistant bacteria, which imposes questions about their safety as human food. The isolation of *E. coli*, *K. pneumoniae* and *Vibrio* spp. both types of samples demonstrate the fecal and environmental contamination that is usually related to bad hygiene, contaminated water sources and poor handling in the process of preparation and storage.

The fact that bacterial loads in samples of fruit juices are higher than in those on ice supports the idea that more significantly contributes to the microbial contamination are the process of preparing fruit juices. Possible causes of high total aerobic counts observed in juices could be manual handling of the fruits, poor washing, long stays in open conditions and dilution by untreated water. The same tendencies were reported in the previous research carried out in Bangladesh and other countries, whereby fruit juices contained higher bacterial counts as compared to ice samples, although in general, ice samples contained lower counts (Ahmed et al., 2009). However, the prevalent presence of the pathogenic bacteria in the ice samples indicates its contribution as a significant secondary contamination source, which is consistent with the previous findings that ice served as a street drink is often made of non-potable water and is handled non-hygienically (Mohd Nawawee, 2019).

It is also important to note that *E. coli* was found in the fruit juice and ice samples because this organism is commonly known as a fecal contaminant. Its existence indicates that it uses contaminated water to prepare juices, clean the utensils or even ice. Similar rates of *E. coli* contamination in beverages sold on the streets have been reported in Dhaka and Chittagong where the inaccessibility of safe water was recognized as one of the driving factors (Khan et al., 2015). The reason for such a high prevalence of *K. pneumoniae* in fruit juices could be the fact that it is capable of surviving on food-contact surfaces and form biofilms, which further enhances the chances of persistence and cross-contamination. The past research has shown that *K. pneumoniae* is able to live in ice-making devices and storage facilities, thus polluting beverages despite having been prepared (Centeleghe, 2023). *Vibrio* spp. also confirms the role of contaminated water sources by the fact that these microorganisms are inherently related to water body ecology and can survive in refrigerated or chilled temperatures (Papadopoulou, 2023).

The lack of *Salmonella* spp. in the samples studied in this paper has been observed in similar studies on acidic fruit juices. *Salmonella* is also known to be inhibited by acidic environments, especially in citrus-based juices, which could be the reason why it is undetected despite other enteric bacteria (Rovira et al., 2024). This observation is in line with the fact that intrinsic factors like pH may be used to determine survival of pathogens in beverages sold in the streets. Phenotypic characterization and molecular identification using PCR demonstrated good concordance, which validated the effectiveness of the methods of identification applied. The amplification of species-specific genes of *E. coli, K. pneumoniae,* and *Vibrio* spp. is successful, which helps to support the previous findings on the significance of integration between culture-based and molecular methods to detect accurately foodborne pathogens (Bainotti et al., 2025). In particular, in food safety research, molecular confirmation is important since closely related species might have similar phenotypic properties.

The susceptibility testing to antibiotics showed that the prevalence against the most commonly used antibiotics including amoxicillin, amoxiclav, tetracycline and nalidixic acid was high. It is a similar pattern of resistance documented in foodborne isolates of street foods and beverages in South Asia and where the indiscriminate use of antibiotics and the spread of antimicrobial residues into the environment have led to the growing rates of resistance (Tabassum et al., 2018). The relative increased resistance to carbapenems and aminoglycosides in this case indicates that the antibiotics are still active against the majority of the isolates; the decreased resistance in a few *K. pneumoniae* isolates is a cause of concern. Such incidents involving recently emerged carbapenem resistance in *K. pneumoniae* of environmental and food chain contexts have raised concerns regarding the dissemination of clinically relevant resistance genes beyond healthcare settings (Zaman et al., 2025).

On analysis of the graphical representation, it has been found that the number of bacterial isolates with MDR was approximately 16 out of 66, thereby having an MDR prevalence of 24.24%. On analysis of the isolates, it has been found that *K. pneumo*niae showed the highest MDR of approximately 40%, followed by *Vibrio* spp. (20%), while *E. coli* had the lowest prevalence of MDR (9.09%). This difference in prevalence can be associated with differences in capacities to possess resistance genes. However, it would appear that the presence of resistant bacteria in ready-to-drink street-vend beverages poses a cause for concern, considering that these resistant bacteria can limit resort to antimicrobials, which can contribute to rising antimicrobial resistance in the community.

Overall, the findings of the current study are in line with the existing literature, which confirms the evidence of poor microbiological quality and the presence of antibiotic resistance in street-vended drinks. Nonetheless, the relative evaluation of fruit juice and ice gives further understanding of the contribution of ice as a contributory source of contamination which is usually ignored in food safety evaluation. The findings highlight the necessity to engage in better hygiene behavior among the vendors, access to safe water, frequent microbiological surveillance and community health initiatives to prevent microbial contamination and subsequent transmission of antimicrobial resistance via street-vended food items.

Chapter 6

Conclusion

This research indicated microbiological contamination of fruit juice and ice samples sold by street vendors in various regions of Dhaka as well as the presence of antibiotic-resistant bacteria and a potential hazard to human health. The *E. coli*, *K. pneumoniae* and *Vibrio* spp. of both fruit juice and ice have been isolated and confirmed by molecular means showing fecal and environmental contamination, due to poor hygienic practices, unsafe water sources and poor handling during preparation and storage.

Comparative analysis showed that fruit juice samples always had greater bacterial loads than ice samples implying that there is more contribution of preparation processes in juice preparation to the microbial contamination. Nevertheless, given the fact that the presence of pathogenic bacteria in ice samples was regularly observed, it is essential to note that ice is a valuable secondary source of contamination, and ice must be regarded just like food as an equally important safety concern. The fact that all the samples contained no *Salmonella* spp. could be explained by acidic fruit juices which could have provoked the lack of its survival.

The results of antibiotic susceptibility test showed very high resistance to the most frequently used antibiotics such as amoxicillin, amoxiclav, tetracycline, nalidixic acid and the susceptibility to carbapenems and aminoglycosides were relatively high. The occurrence of multidrug-resistant *E. coli* and *K. pneumoniae* isolates in ready-to-consumer drinks is of great concern since it indicates the possible spread of antimicrobial resistance foods into the community via the food chain.

In general, the results of this research highlight the high level of urgency in the context of hygiene measures of street vendors, the availability of clean water to prepare juices and produce ice, and the regular inspection of street-vended drinks by the regulatory bodies. Awareness of the populace and training of the vendors are crucial to enhance the lack of microbial contamination and restrict the proliferation of antimicrobial resistant microbes. The research also provides a useful source of information regarding the microbiological quality and resistance of fruit juice and ice sold in the streets and emphasizes the need to assess ice as a food safety measure to enhance the health of consumers.

According to the results of this research, street vendors should be advised to be trained on the elementary food hygiene measures such as good hand hygiene, clean utensils as well as appropriate storage facilities. Washing of fruits, making of juice and the use of ice on the use of potable water should be monitored strictly. Microbiological monitoring to the drinks sold in the streets should be conducted on the regular basis, and in the case of ice as a source of contamination, it is to be given special attention by the regulatory authorities. Education on the dangers of contaminated street foods to the health of people can also contribute to the mitigation of contact with pathogenic and antimicrobial-resistant bacteria.

Chapter 7

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