Microbial contamination of ice used in street beverages in Dhaka

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Declaration

It is hereby declared that

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

Student's Full Name & Signature:

Student Full Name	**Student Full Name**
Student ID	Student ID

Approval

The thesis titled "*Microbial contamination of ice used in street beverages in Dhaka*" Submitted by Mohammad Arif (21126011) & Sumya Tabassum (21126005) of Spring 2025 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor's in Microbiology on 23-06-2025.

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Departmental Head: (Chair)	________________________ Md. Firoze H. Haque, PhD Associate Professor and Chairperson, Department of Mathematics and Natural Sciences Brac University

Ethics Statement

This research was conducted under proper supervision and is the original work of the author. No industrial or branded juice or ice samples were disclosed during the experiments. The thesis does not contain any previously published or third-party material without appropriate citation and accurate referencing. Ice samples were collected from local vendors in Merul Badda and Gulshan. All procedures were carried out following proper safety protocols and in accordance with the rules and regulations of the Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University.

Abstract

Street-vended beverages are widely consumed in Dhaka, especially during hot and humid weather. While previous studies have focused on microbial contamination in juices, limited attention has been given to the ice used during preparation despite its potential to transmit enteric pathogens when handled or produced under poor sanitary conditions. This study aimed to investigate microbial contamination in both ice and juice samples from two busy areas of Dhaka Merul Badda and Gulshan-1 and to evaluate associated public health risks. A total of 18 samples of juice and ice (from orange and watermelon juices) were collected. Microbiological analysis included serial dilutions, culture on selective media (MacConkey and KPC agar), Gram staining, polymerase chain reaction (PCR), and antimicrobial susceptibility testing (AST). Out of 18 samples, 6 (33.3%) tested positive for bacterial contamination. Among these, 4 samples (22.2%) were positive for *Escherichia coli*, all from watermelon juice and related ice in Gulshan-1**.** Two samples (11.1%) were positive for *Klebsiella pneumoniae*, one from orange juice ice in Merul Badda and another from watermelon juice ice in Gulshan-1. The highest bacterial loads were observed in Gulshan-1, with *E. coli* reaching 6.8×10^4 CFU/mL in juice and 8.2×10^4 CFU/mL in ice. PCR was conducted to amplify the DNA of the isolated bacteria, and gel electrophoresis revealed distinct bands—at 585 bp for *E. coli* and 130 bp for *K. pneumoniae*—supporting their identification. The study aims at examining the Antibiotic Resistance Profile of *Escherichia coli* (n=4) and *Klebsiella pneumoniae* (n= 2) battery isolated through Antimicrobial Susceptibility Testing (AST) in samples under clinical investigation. The findings showed that there was a huge rate of multidrug resistance and that the two species are 100% resistant to most of the antibiotics that were used in the test. The *E. coli* exhibited partial resistance towards ciprofloxacin (25%) and nitrofurantoin (50%) and the *K. pneumoniae* displayed resistance of 50% to nitrofurantoin only. These results indicate the increasing problem of the antimicrobial resistance and the serious necessity of effective antibiotic stewardship, constant surveillance, and elaboration of alternative treatment regimens to address resistant bacterial infections.

Keywords: Street drinks, microbial contamination, ice, *Klebsiella pneumoniae*, *Escherichia coli*, public health, Dhaka

Dedication

I would also like to dedicate this thesis to my family whose support, encouragement, and cherish have been my biggest strength during this journey.

Acknowledgment

To begin with, I would like to extend my sincere thanks to Almighty Allah, providing me with the strength and endurance to accomplish my research thesis.

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List of Acronyms

AST	Antibiotic Susceptibility Testing
AMR	Antimicrobial Resistance
BFSA	Bangladesh Food Safety Authority
BSTI	Bangladesh Standards and Testing Institution
CFU	Colony Forming Unit
DA	Clindamycin
E. coli	*Escherichia coli*
K. pneumoniae	*Klebsiella pneumoniae*
PCR	Polymerase Chain Reaction
UV	Ultraviolet
AK	Amikacin (Antibiotic Disc Code)
AMR	Antimicrobial Resistance
AST	Antibiotic Susceptibility Testing
BFSA	Bangladesh Food Safety Authority
bp	Base Pair
CAZ	Ceftazidime
CIP	Ciprofloxacin
CPM	Cefepime
CRO	Ceftriaxone
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
ECO-F / ECO-R	Forward/Reverse Primer for *E. coli*

EDTA	Ethylenediaminetetraacetic Acid
F	Nitrofurantoin (Antibiotic Disc Code)
KPC	*Klebsiella pneumoniae* Carbapenemase
KP Pf-F / KP Pr1-R	Forward/Reverse Primers for *K. pneumoniae*
LE	Levofloxacin
MAC	MacConkey Agar
MEM	Meropenem
mm	Millimeter
mL	Milliliter
NOR	Norfloxacin
PCR	Polymerase Chain Reaction
TAE	Tris-Acetate-EDTA
TGC	Tigecycline
UTI	Urinary Tract Infection
UV	Ultraviolet
WHO	World Health Organization
°C	Degree Celsius

Chapter 1

Introduction

1.1 Background of the Study

The beverages that are sold by street vendors are very popular in Bangladesh especially in the urban centers such as Dhaka and people enjoy them because of their good taste, low prices as well as their availability. The beverages are frequently consumed on ice and they are very common especially in hot and damp seasons. Nevertheless, along with their wide popularity, the questions of their safety are becoming increasingly raised, in particular with respect to the ice employed in their preparation. The study arose out of personal instances of gastrointestinal illnesses such as vomiting, diarrhea, and stomach pain following the drinking of street-vended drinks in Dhaka. These cases caused the suspicion that the ice employed in these kinds of drinks could be contaminated with disease-causing microorganisms. On further investigation it was found that most of the local juice sellers work in dirty environments and might be using ice that was made with contaminated water or ice that was not sanitized.

In places with a high population, such as Dhaka, where pollution and the lack of infrastructure already are a health concern, the informal manufacturing and distribution of ice introduce a new dimension of the issue. Ice can be made with untreated or surface water, and then treated by many people without hygiene or protection measures. The small-scale producers might not observe the safety rules, and the vendors have no awareness or training regarding the healthy handling procedures. Contamination of ice by microorganisms, especially enteric pathogens like *Escherichia coli* and *Klebsiella pneumoniae* can cause severe health complications like diarrhea, food poisoning, urinary tract infections (UTIs) and even respiratory diseases. Food and water safety is a major concern to public health, as these diseases are particularly treacherous in societies with reduced access to the healthcare system. Regardless of these health consequences, there is little research on ice employed in street-vended drinks in Dhaka in regards to its microbiological quality. This research paper will seek to address that gap by identifying the microbial contamination of ice in the common street drinks and assessing the health risks that are associated with it hence this will form part of the efforts that will be aimed at ensuring that the standards of food safety in Bangladesh are improved.

1.2 Rationale and Problem Statement

High population density and prevalent use of street-available beverages, particularly in hot seasons, followed by microbial contamination of ice used to prepare such drinks, is one of the most feasible and severe threats to public health, in the case of Dhaka. Ice may be prepared, in unsanitary conditions, with untreated water, and often constitutes a significant source of enteric pathogens. Although it has the capability of bringing severe complications to the health status of different people, including but not limited to food poisoning, urinary tract infections, respiratory diseases, or an overarching failure of the systems in an organism and/or a specific at-risk population, the problem has not attracted much attention among researchers or state officials. The problem is further perpetuated because of a lack of appropriate regulation and control which in turn is disproportionately afflicting populations with lower incomes as well as children and populations with restricted health care access. Such risks are presented in this study in order to create awareness about a serious health hazard that has escaped serious attention.

1.3 Objectives of the Study

The principle aim of study was to conduct a study about presence of the enteric bacteria called *Escherichia coli* and *Klebsiella pneumoniae* in ice that was used in drinking vended drinks in Dhaka. The purpose of the study is to be informed about the extent of microbial contamination, and the way the contamination can be impacting on the populations. This study will be informing food safety processes, among other things, by explaining which risks microbially ice consumption could cause. In addition, it will also eradicate incidences of water borne disease outbreaks in the urban populations.

- To determine the level of microbial contamination in ice in orange juice (of Merul Badda) and watermelon juice (of Gulshan-1).
- To find out whether these pathogens are present through the use of molecular protocols Polymerase chain reaction (PCR) and Gel electrophoresis.
- To perform antibiotic susceptibility test (AST) of the isolated bacterial organisms to determine their profile of resistance.
- To educate the masses and the relevant authorities of the risks related to contaminated ice of the street drinks and to improve the state of hygiene and regulation in the sector.

1.5 Significance of the Study

The study is important in boosting the education among the people, food safety, and microbiological surveillance in the urban setting of Dhaka. Vended drinks are cheap and available on the streets, which are usually potentially dangerous due to the use of ice that cannot be controlled as a hidden source of pathogen spreading leading to diarrhea, food poisoning, UTI, and pneumonia. The presence of *Escherichia coli* and *Klebsiella pneumoniae* in ice samples raises necessity to be able to improve enforcements of hygienic conditions during the manufacture and processing of ice. Moreover, the article draws attention to the increase in the presence of antimicrobial resistance (AMR), which was detected by sensitivity testing of the antibiotics since resistant strains were identified that would complicate treatment. The conclusions will be used to guide policy makers, health organizations and the researcher in the fight against AMR and water borne diseases. Finally, the findings will inform future policy, citizen education programs and training of vendors, as well as a basis to carry out a study into more information on environmental microbiology and health risks associated with informal food sectors in cities.

1.6 Scope and Limitations

This thesis examines ice contamination due to microbial contaminations in orange and water melon juices sold by street-vendors in the Dhaka's area of Merul Badda and Gulshan 1. It dwells upon the detection of enteric bacteria, especially, *Escherichia coli* and *Klebsiella pneumoniae*, both by the means of traditional microbiology and by the means of molecular techniques such as PCR and gel electrophoresis. Antibiotic susceptibility test was also carried out to test the resistance behaviour of the isolated bacteria. The results will contribute towards awareness creation of health hazards due to impure ice and enhancement of better hygiene habits among vendors.

Chapter 2

Literature Review

2.2 Role of Ice in Beverage Safety

Ice used in street-vended beverages plays a significant but often overlooked role in microbial contamination. Although consumers view ice as harmless and refreshing, research shows it can carry more pathogens than the drinks themselves (Nawawee, Abu Bakar, & Zulfakar, 2019). Typically, this ice is produced in unhygienic conditions using untreated or poorly treated water in makeshift setups like home-based or street vendor stalls, exposing it to contamination from dirty water, equipment, and surfaces (Settanni et al., 2017). Handling and storage practices worsen contamination risks, as ice is often exposed to contaminated containers, dirty scoops, and vendor hands, with ice machines and storage bins rarely cleaned regularly (Settanni et al., 2017). Some bacteria, including Escherichia coli, Enterococcus, and Pseudomonas spp., can survive freezing and remain viable in melted ice, posing health risks (Dickens, DuPont, & Johnson, 1985). An international survey detected pathogens such as E. coli, Enterococci, Pseudomonas spp., Staphylococcus aureus, Yersinia spp., Vibrio cholerae, and fungi in ice samples from various countries (Caggiano et al., 2020). One study found 6.3% of ice samples from beverage production lines contained E. coli, while 0.7% harbored dangerous pathogens like Vibrio cholerae or Salmonella typhimurium, highlighting ice as a key contamination vector in street drinks (Nababan et al., 2017).

2.3 Microbial Contaminants Commonly Found in Ice

This research invariably demonstrates that, apart as it may purport to be, ice can indeed be the breeding ground of numerous pathogenic microorganisms that can wreak havoc to human health in general. Studies in different environments, including home-made ice production and commercial ice production environments, have ascertained the idea that more often than not, ice serves as a carrier of bacteria, viruses, and fungi (Triggiano et al., 2024). For instance, one study found coliform bacteria in 9% of ice used directly in beverages and *Escherichia coli* in 1%, with even higher contamination in ice used for food storage. International surveys have detected diverse strains of *E. coli* in ice samples, including diarrheagenic pathotypes in packaged ice sold in Mexico City, highlighting ice as a direct source of gastrointestinal pathogens (Pusparini et al., 2018). In Europe and North America, microbial contamination of ice from restaurants and bars has also been documented; Italian ice cubes contained *Salmonella, Yersinia, and fungi*, with outbreaks traced to contamination via bar-to-glass pathways (Caggiano et al., 2025). Pathogen persistence is due to freezing only inhibiting but not killing microbes, contaminated source water often being untreated or inadequately treated, and poor handling practices such as scooping without gloves and storing ice in contaminated containers (Kassa, Harrington, Baroudi, & Silverman, 2017).

2.4 Water Quality and Ice Production in Developing Cities

In many developing cities like Dhaka, access to safely treated drinking water is limited, with about 2.2 billion people relying on unimproved, often contaminated sources (World Health Organization: WHO, 2023). When such water is used for ice production—especially in informal or small-scale settings—it poses significant health risks. Street vendors frequently use pathogen-contaminated water and have inadequate hand hygiene, making microbial contamination of ice almost unavoidable (Shah & Shrimali, 2017). Global research shows that the microbial quality of ice depends heavily on water source and production hygiene. Poorly sanitized equipment or untreated water used to make ice or produce poorly sanitized drinking water may contain pathogens such as *E. coli*, *Salmonella* and *Klebsiella*. Meanwhile, this ice machine and storage bin may not be thoroughly cleaned, which causes the treated water also to be contaminated. To resolve this, water quality should be enhanced, equipments should be regularly sanitized, vendors should be trained and guidance that are practical in low-resources places. The risks are aggravated in developing countries by poor infrastructure and lack of enforcement and adequate regulations, thus necessitating such interventions to stop the cycle of contamination (Koumassa et al., 2025).

2.6 Regulatory Standards for Food Safety in Bangladesh

In Bangladesh, the main authority in charge of the food safety is Bangladesh Food Safety Authority (BFSA) introduced under Food Safety Act of 2013. One of the responsibilities imposed on BFSA is the creation and implementation of food safety laws, mainstreaming surveillance compliance, and interaction with other agencies to safeguard the health of the people. Despite its comprehensive mandate, the implementation of standards—especially in informal sectors like street vending—faces challenges due to limited resources and coordination among agencies, (Digicomply, n.d.). Alongside BFSA, the Bangladesh Standards and Testing Institution (BSTI) plays a vital role as the national standards body. BSTI develops both mandatory and voluntary standards for food products, drawing heavily from international frameworks such as Codex Alimentarius and ISO norms. Although BSTI has established over 600 food-related standards (with about 76 compulsory) and operates specialized laboratories for testing—including those for water and beverages—many street-vended products remain unregulated due to gaps in enforcement and resource constraints, (Food Safety Innovation Lab, 2021).

Chapter 3

Materials and Methods

3.1 Study Design and Sampling Strategy

The study design was experimental which aimed at examining the microbial contamination in ice utilized in the street-vended drinks. The entire laboratory work was performed at the BRAC University Microbiology Laboratory with no field testing being done. Field samples were taken, delivered under controlled conditions and processed in the university lab. The convenience sampling plan was followed, whereby two important urban areas of Dhaka region were chosen, Merul Badda and Gulshan-1. The zones were selected based on a high concentration of the population as well as street vendors and closeness to the university which eased the transportation and sample handling processes. Eighteen samples were collected from them one was orange juice sample with ice and one watermelon juice sample with ice of Merul Badda and one watermelon juice with ice of Gulshan-1. These samples were bought in disposable cups with the street vendors and put in clean, sealed plastic bags to reduce contamination during transportation. The samples were tried to be kept out of direct sunlight and heating. Lab safety guidelines were observed during collection and transportation to guarantee sample integrity and eliminate cross-contamination. On reaching the lab, the samples were refrigerated at 4^{o} C in a refrigerator. The serial dilutions and plating onto selective media were done upon short arrival, however, other lab procedures, Culture plating, Gram staining, molecular diagnostics (PCR and agarose gel electrophoresis and antibiotic susceptibility testing (AST), were done the next days following the laboratory schedule and the needs of the protocols. This procedure enabled the systematic and proper exploration of the microbial content of the collected samples.

3.3 Sample Collection Procedures

From each location, a single vendor was selected to represent the street-vended beverages commonly consumed in that area. At Merul Badda, an orange juice sample along with its corresponding ice was collected. At Gulshan-1, a watermelon juice sample and the ice used in it were obtained. Each sample was clearly marked with its location and time of collection to maintain accurate tracking. The handling process was carried out with strict attention to hygiene and contamination prevention. Laboratory-issued gloves were worn throughout the collection process, and all samples were placed into a thermal, heat-insulated carrying box provided by the university microbiology lab to preserve their condition during transport.

3.4 Microbiological Analysis Techniques

3.4.1 Serial Dilution and Spread plate

Serial dilution method and the spread plate were used to determine the microbial contamination in samples of juice and ice. Samples of juice and ice were obtained aseptically by using samples of various juice sellers in the area and taken to the laboratory where they were kept cold. Serial ten-fold dilutions (10^1 to 10^3) were performed on each of the samples based on the predicted load of microorganism present. To the every dilution, 0.1 mL was spread over the selective agar plates, all plates were incubated on an incubator at 37^o C degrees centigrade overnight to enable the growth of colonies. To identify the *Escherichia coli*, MacConkey agarselective to the gram-negative enteric bacteria, was used. A selective medium which was used to isolate *Klebsiella pneumoniae* especially the strains that produce carbapenemase was KPC agar. Colony forming units were determined by multiplying the colonies counts by the inverse of the dilution factor and then dividing by the volume used. Juice and respective ice were treated separately by dilutions. The samples of water melon juice and orange juice together with ice were examined parallelly.

3.4.2 Streaking and Gram Staining

Following colony development from the spread plates, representative colonies were picked and streaked on KPC media for further isolation. Colonies selected from both MacConkey and KPC media were subjected to Gram staining to determine their morphological characteristics. The staining revealed Gram-negative bacilli (rod-shaped) for both *Escherichia coli* and *Klebsiella pneumoniae*. To visualization microscopic examination was used under a 100x oil immersion lens, where the pink observation could be confirmed by Gram-negative and Gram-positive nature of the organisms.

Gram Staining

A thin bacterial smear was prepared on a clean glass slide and allowed to air dry. The smear was then heat-fixed by passing the slide gently through a flame several times to adhere the bacteria to the surface. The fixed smear was first flooded with crystal violet stain, the primary stain, and left for one minute. After rinsing gently with distilled water, Gram's iodine solution was applied for another minute to act as a mordant, forming a complex with the crystal violet inside the bacterial cells. The slide was then decolorized by applying 95% ethanol dropwise for 10 to 20 seconds until the runoff became clear, followed immediately by rinsing with distilled water to stop the decolorization process. Finally, a counterstain of safranin was applied for 30 to 60 seconds to stain any decolorized cells. After a final rinse and gentle blotting to dry, the slide was examined under a light microscope using oil immersion at 100x magnification. Gram-positive bacteria appeared purple due to retention of the crystal violet-iodine complex, while Gram-negative bacteria appeared pink or red from the safranin stain.

3.4.3. DNA Extraction

Using a sterile loop, one colony of *E. coli* and *Klebsiella pneumonae* was isolated in a streaked KPC agar plate. A sterile Eppendorf tube (150 µL TE buffer) was used to pipette the colony and mix or shake the mixture. The suspension was well vortexed or pipetted to make sure that the bacterial cells are fully suspended. The tubes were next incubated in the heat bath at 100^{o} C in a 15-minute interval to lysis the bacterial cells and to have the genomic DNA freed. Following addition of heating, room temperature or short ice incubation was performed. The cell debris was then pelletized at 13000 rpm in 15 minutes period. The extracted DNA that was suspended in a clear supernatant was transferred to a fresh sterile micro-centrifuge tube without disrupting the pellet. The DNA that was extracted was either used as a template to perform the PCR or refrigerated at -20 C to be utilized in future purposes.

3.4.3 PCR and Agarose Gel Electrophoresis

Polymerase Chain Reaction (PCR) was conducted for molecular confirmation of *Escherichia coli* and *Klebsiella pneumoniae* presence using specific primers: ECO primers F and R used for *E. coli* and KP primers F and R for *K. pneumoniae*.

Supplementary Table 1: Primers and PCR Conditions used in this Study

Agarose Gel Electrophoresis

Name of bacteria	Primer Designation	Primer Sequence	Product size	PCR conditions
Klebsiella pneumoniae	KP Pf-F: KP Pr1-R:	5′-ATTTGAAGAGGTTGCAAACGAT-3′ 5′-TTCACTCTGAAGTTTTCTTGTGTTC-3′	130 bp	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.
Escherichia coli	ECO- F ECO-R Nuc -R	5′-GACCTCGGTTTAGTTCACAGA-3′ 5′-CACACGCTGACGCTGACCA-3′ 5'-AGC CAA GCC TTG ACG AAC TAA AGC-3'	585 bp	Initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 45s, annealing at 45°C for 45s and extension for 1 min followed by a final extension at 72°C for 5 min.

A 1% agarose gel was prepared by dissolving 1 g of agarose powder in 98 mL of distilled water and 2 mL of 1X TAE working solution. The mixture was heated until the agarose completely dissolved and then cooled to about 50–60°C. Ethidium bromide (4 µL) was added to the cooled agarose solution for DNA visualization. The agarose solution was poured into a gel casting tray with combs to form wells and allowed to solidify at room temperature for 30–45 minutes. The gel was then placed in the electrophoresis tank and covered with 1X TAE buffer. DNA samples and a DNA ladder were loaded into the wells, and electrophoresis was run at 100 volts for 45 minutes. After completion, DNA bands were visualized under a UV Tran illuminator and documented.

3.4.4 Antibiotic Susceptibility Testing (AST)

Antibiotic susceptibility of the isolated strains was evaluated using the Kirby-Bauer disk diffusion method. A panel of antibiotics was used for both *Escherichia coli* and *Klebsiella pneumoniae*, with results recorded based on the diameter of inhibition zones.

Table 2: The following antibiotics were applied:

Serial no	Antibiotic	Group	Effective against	Disc code	Disc potency (µg)	Interpretative Criteria		
						Sensitive mm or more	**Intermediate mm**	**Resistant mm or less**
1	Clindamycin	Lincosamide	Gram-positive cocci (Staph, Strep)	DA	2	≥ 21	15–20	≤ 14
2	Nitrofurantoin	Nitrofuran	Enterobacteriaceae, Staph, Enterococcus	F	300	≥ 17	15–16	≤ 14
3	Meropenem	Carbapenem	Gram-positive and Gram-negative	MEM	10	23	20–22	19
4	Cefedime	Cephalosporin	Gram-positive and Gram-negative	FEP	30	25	19–24	18
5	Norfloxacin	Fluoroquinolone	Gram-negative rods (mainly urinary)	NOR	10	≥ 17 [1]	13–16	≤ 12
6	Ceftazidime	3rd-gen Cephalosporin	Pseudomonas, Gram-negative rods	CAZ	30	≥ 18	15–17	≤ 14
7	Ceftriaxone	3rd-gen Cephalosporin	Gram-negative & positive (broad)	CRO	30	≥ 21	14–20	≤ 13
8	Amikacin	Aminoglycoside	Gram-positive and Gram-negative	AK	30	17	15–16	14
9	Ciprofloxacin	Fluoroquinolone	Gram-positive and Gram-negative	CIP	5	21	16–20	15
10	Tigecycline	Glycylcyline	Gram-positive and Gram-negative	TGC	15	18	15-17	15
11	Levofloxacin	Fluoroquinolone	Broad-spectrum (Gram-positive & negative)	LE	5	≥ 17	14–16	≤ 13

Chapter 4

Results

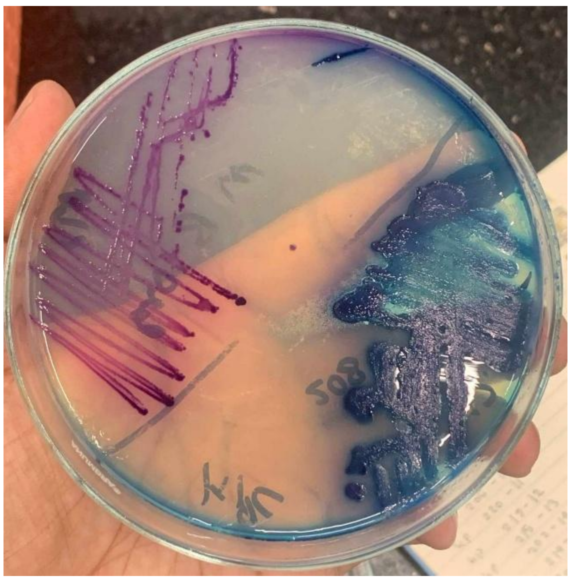
4.1 Microbial Load in Ice from Orange and Watermelon Juices

An ice sample associated with orange juice, diluted at 10^{-1}, exhibited a *Klebsiella pneumoniae* count of 1.86×10^3 CFU/mL on KPC agar, indicating a substantial level of contamination. Another ice sample, linked to watermelon juice and diluted at 10^{-3}, showed 8.3×10^4 CFU/mL of *K. pneumoniae*, which, although lower in dilution, still signifies the presence of potentially pathogenic bacteria in consumable ice. In addition, multiple ice samples related to watermelon juice were found to contain *Escherichia coli*. At a 10^{-1} dilution, one ice sample yielded 1.93×10^3 CFU/mL, while another at 10^{-3} dilution demonstrated 8.2×10^4 CFU/mL, both on MacConkey agar. The colonies exhibited characteristic pink pigmentation, indicating lactose-fermenting *E. coli*. Similarly, the microbial load in the watermelon juice itself showed high *E. coli* contamination, with 2.46×10^3 CFU/mL at a 10^{-1} dilution and 6.8×10^4 CFU/mL at 10^{-3} dilution.

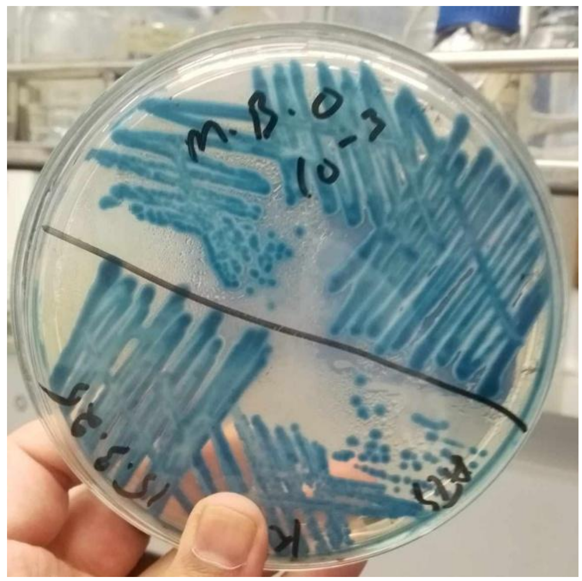
Table 3: Bacterial Load (CFU/mL) in Ice and Juice Samples

Sample Type	Organism Detected	Calculated CFU/mL
Ice (Orange Juice)	*Klebsiella pneumoniae*	1.86×10^3
Ice (Watermelon Juice)	*Klebsiella pneumoniae*	8.3×10^4
Ice (Watermelon Juice)	*Escherichia coli*	1.93×10^3
Ice (Watermelon Juice)	*Escherichia coli*	8.2×10^4
Juice (Watermelon)	*Escherichia coli*	2.46×10^3
Juice (Watermelon)	*Escherichia coli*	6.8×10^4

Colony Morphology and Selective Media Growth.

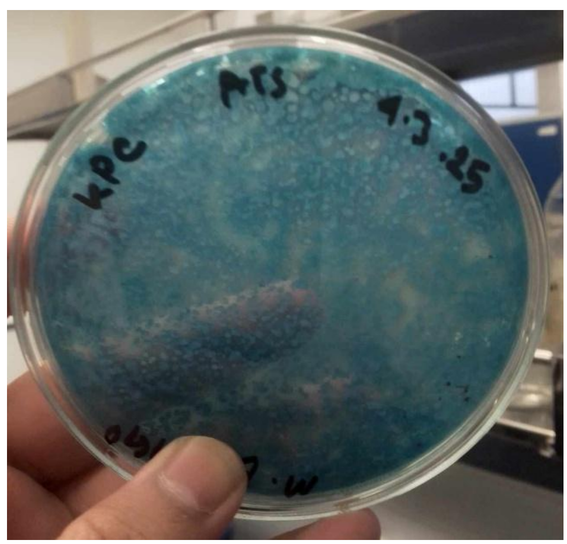


A



B

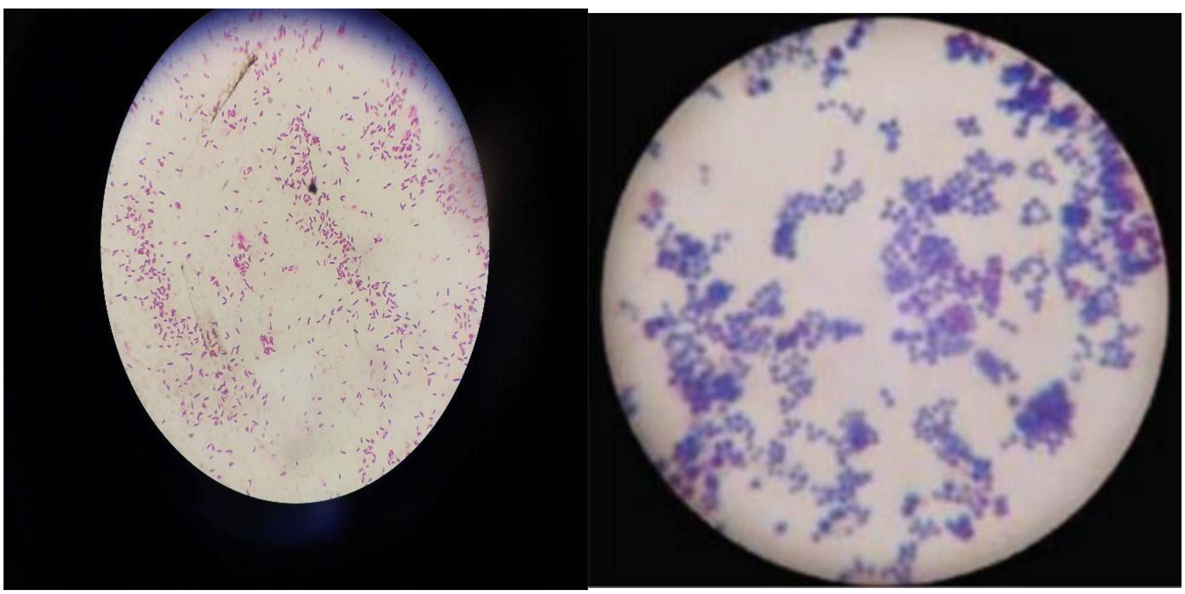
C



D

Figure 1. Image A, shows the both isolated (*K. pneumoniae* and *E. coli*) streaked on a plate on KPC media. Image B, shows the completely isolated colonies of *K. pneumoniae* on KPC media. Image C, shows the spread plate of *E. coli* on MAC media and lastly image D, shows the spread plate of *K. pneumoniae* on KPC media.

Gram Staining:

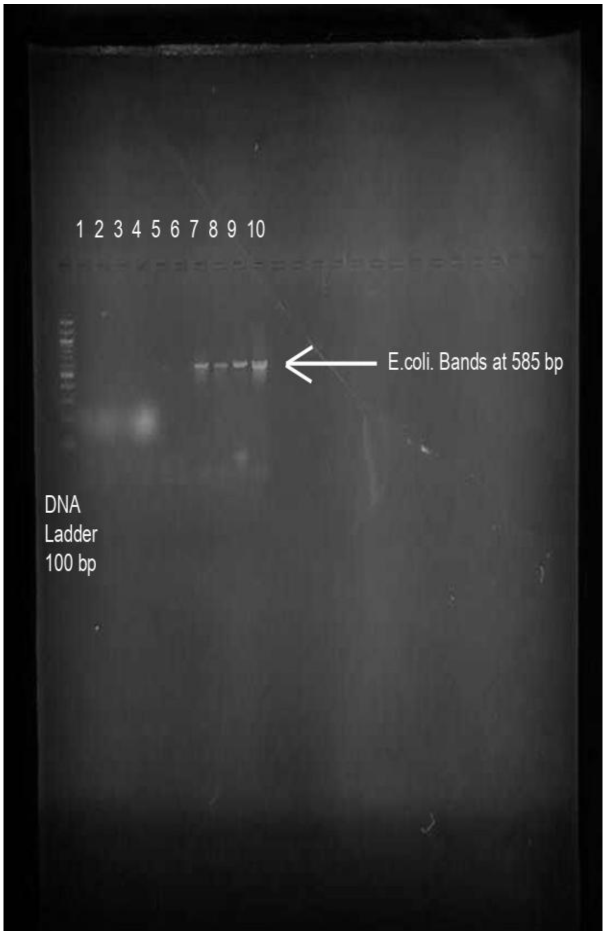


A B

Figure 2. A. the Gram staining of *E.coli* seen as gram negative with pinkish color under the 100x microscopic view. On other side, B image shows the Gram staining of *K. pneumoniae* with purple color under the 100x microscopic view.

4.2 Identification of microbes ***(K. pneumoniae and E. coli)***

E. coli and *K. pneumoniae* molecular confirmation by Agarose Gel Electrophoresis

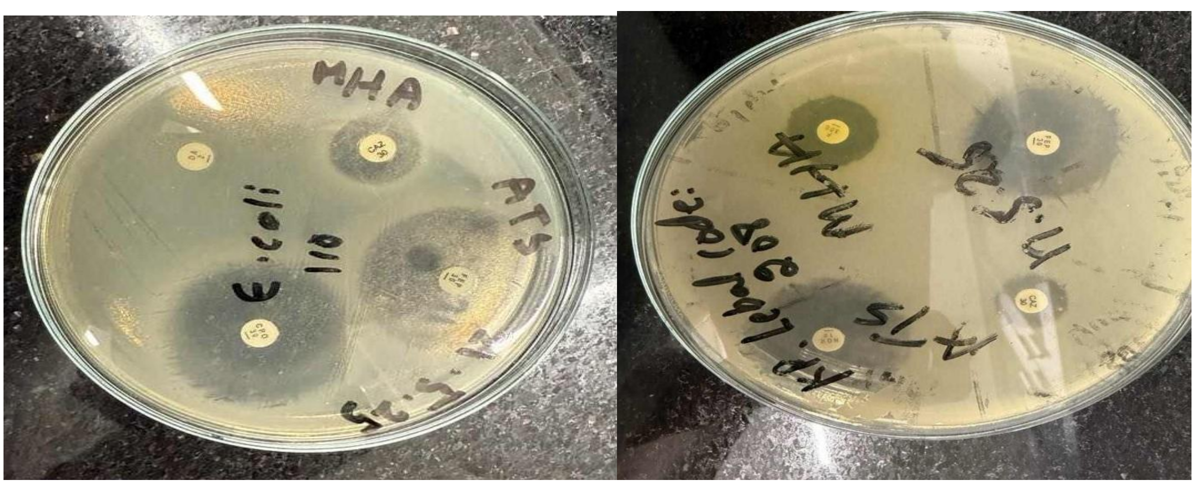


A B

Figure 3: Image A, shows the *E. coli* bands at 585 base pairs and other side image B, the bands 130 base pairs can be seen for *K. pneumoniae***.**

4.3 Antibiotic Resistance Patterns of ***E.coli*** and *K. pneumoniae*

The AST result show the profile of antibiotic resistance of *Escherichia coli (E. coli)* and *Klebsiella pneumoniae (K. pneumoniae)* using a small sample number that consists of four isolates of *E. coli* (n=4) and two of *K. pneumoniae* (n=2) isolates. These are figure 5, which show the percentage of made by each bacterial species towards several of the most common antibiotics. The resistance profile is of a worrying nature in the case of *E. coli*. The bacteria showed resistance to 9 antibiotics out of the eleven antibiotics that were used with a resistance rate of 100% in clindamycin, levofloxacin, ceftriaxone, cefepime, morepen, amikacin, norfloxacin, tigecycline and ceftazidime. It is an indication that indeed these isolates have a major multidrug resistance issue that was absolutely resistant to these types of antibiotics. Nonetheless, a partial resistance whereby 25 percent and 50 percent of the isolates were resistant to ciprofloxacin and nitrofurantoin respectively was noted. These findings mean that ciprofloxacin and nitrofurantoin can still be effective against E. coli albeit at limited levels since the drug is becoming resistant. On the *K. pneumoniae*, the resistance pattern is equally worrying. The bacteria showed absolute resistance towards seven antibiotics out of eight i.e. clindamycin, levofloxacin, ceftriaxone, cefepime, norfloxacin, tigecycline, and ceftazidime. The resistance rate was lower only with nitrofurantoin (50%), that is, one of the two isolates remained susceptible. This indicates how severe the resistance is among the *K. pneumoniae* strains and an indication that few treatment options exist.



A B

Figure 4: A. AST for *Escherichia coli* B. AST for *Klebsiella pneumoniae*

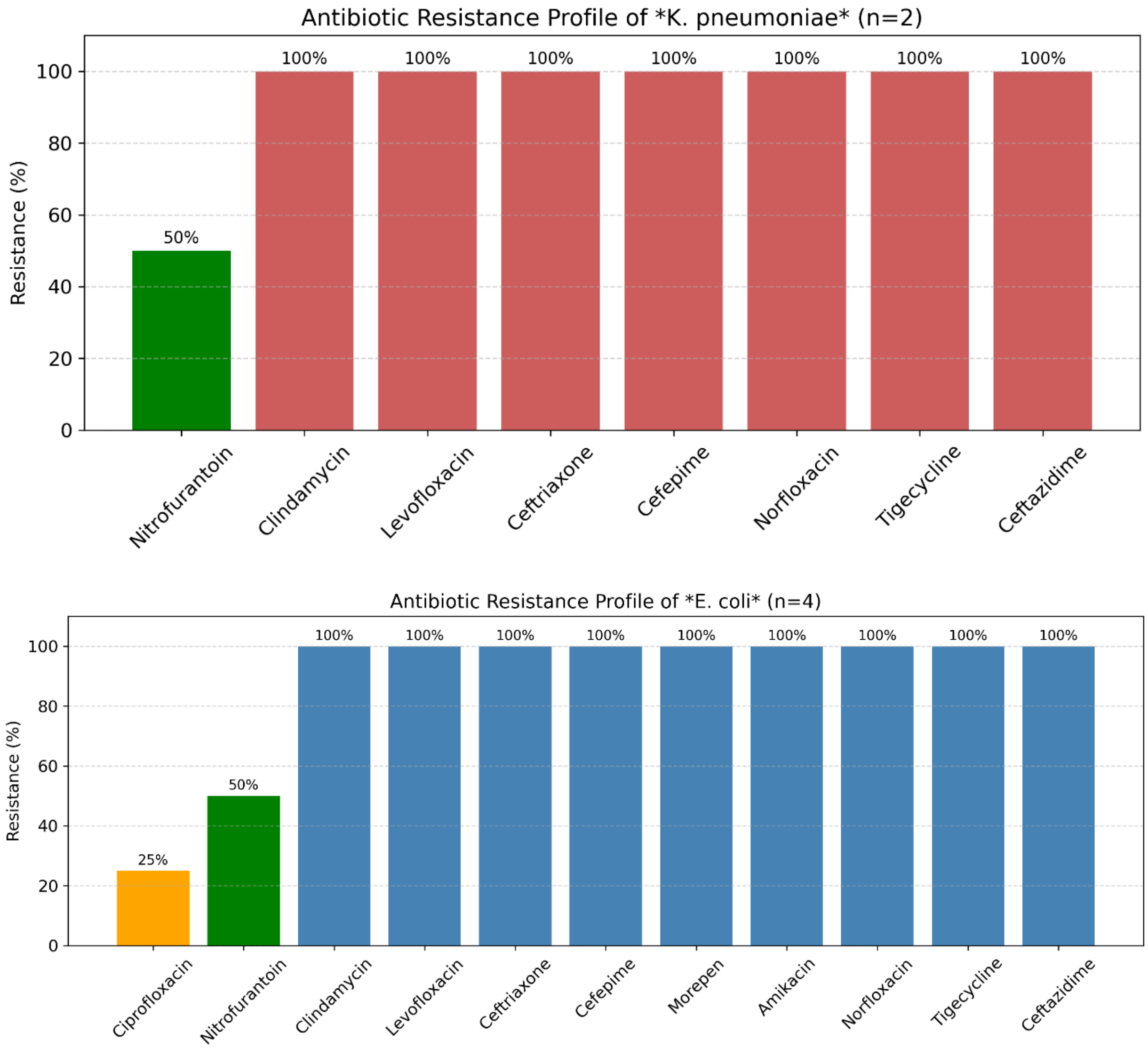


Figure 5: *Escherichia coli* (n=4) and *Klebsiella pneumoniae* (n=2) are incredibly high in resistance of photo-indicated antibiotics as indicated in their antibiotic resistance profiles, with *Escherichia coli* 100% resistant to the majority of the antibiotics tested, and *Klebsiella pneumoniae* 100% resistant to more than half of the antibiotics tested. *E. coli* was equally partly resistant to ciprofloxacin (25%) and nitrofurantoin (50%) and *K. pneumoniae* only 50% to nitrofurantoin hence being limited to therapy.

Chapter 5

Discussion

5.1 Interpretation of Microbiological Findings

This study revealed significant microbial contamination in ice and juice samples collected from street-vended fruit juices in Dhaka, particularly those associated with orange and watermelon juices. Two opportunistic and potentially pathogenic bacteria—*Klebsiella pneumoniae* and *Escherichia coli*—were consistently isolated, raising serious public health concerns due to poor hygiene, unsafe water usage, and inadequate storage conditions. In Merul Badda, an ice sample from orange juice showed *K. pneumoniae* growth of 1.86×10^3 CFU/mL at a 10^{-1} dilution on KPC agar, indicating a substantial bacterial load. Sample of ice connected to watermelon juice cocktail had displayed an even higher contamination level in Gulshan-1, as it persisted to be 8.3×10^4 CFU/mL containing 10^3 dilution. These results prove that the presence of microbes is still high even at a higher dilution, which indicates the utilization of the dirty water in the production of ice. The prevalence of E. coli was also high especially in Gulshan-1 samples. Watermelon juice samples exhibited contamination levels of 2.46×10^3 CFU/mL at a 10^{-1} dilution and 6.8×10^4 CFU/mL at 10^{-3}. The corresponding ice samples yielded 1.93×10^3 CFU/mL at 10^{-1} and 8.2×10^4 CFU/mL at 10^{-3} on MacConkey agar. Lactose fermentation (an important E. coli feature) was established by the pink pigment colonies. The watermelon juice was incomparably more microbenic than the orange juice, perhaps, due to the high sugar content, which possibly, favors the bacterial growth as also the reason being poor handling and exposure to the environment.

The PCR amplification further confirmed the bacterial identification whereby the bands were identified at about 130 bp in the case of *K. pneumoniae* and 585 bp in the case of *E. coli*. Furthermore, gram staining also confirmed the type of identification in that both isolates were identified as being gram-negative, rod-shaped bacteria. Having such pathogens in unprocessed food such as raw juice is of great risk to the population and more so to those people whose immune systems may be weak.

The graphs present the antibiotic resistance patterns of the Escherichia coli (n=4) and Klebsiella pneumoniae (n=2) indicating the pattern of multidrug resistance (MDR) in the isolated strains being in critical position. The resistance levels of the two strains of bacteria were very high when tested under common antibiotics presenting a great concern on the problems of normal antimicrobial-based treatment.

The resistance was 100% of isolated strains against nine of the eleven antibiotics in the case of *E. coli* and the antibiotics where resistance was observed included clindamycin, levofloxacin, ceftriaxone, cefepime, morepen, amikacin, norfloxacin, tigecycline and ceftazidime. The common type of resistance groups sheds light on the capability of the organism to resist a wide range of types of antibiotics, which creates a major challenge of treatment. Ciprofloxacin (25%) and nitrofurantoin (50%), partial resistance was observed indicating that although still there are agents that are yet to be resistant, emerging resistance to even these agents was clearly seen. These trends of resistance can express excessive or incorrect use of these antibiotics at hospitals. In the same vein, *K. pneumoniae* showed hundred percent resistant to seven out of eight tested antibiotics thus showing its ability to have an extensive mechanism of antimicrobial resistance. Partial resistance (50%) was recorded in nitrofurantoin with one of the isolates still being susceptible. The general resistance profile fits with the worldwide description of *K. pneumoniae* as a high-risk MDR pathogen. These results highlight the necessity to develop the intensification of antimicrobial stewardship and surveillance programs. The low susceptibility rate among the two species of nitrofurantoin could provide a therapeutic alternative, although its utility places a significant potential of the drawbacks as it has a small sample size and a certain indication of resistance even at the preliminary indications. Finally, these findings underline the increasing concern of the antimicrobial resistance (AMR) and the necessity to implement strict infection control measures, selection of antibiotics, and continuous research on the development of new types of antimicrobial agents.

5.2 Comparison with Existing Studies

Few past studies in South Asia and other parts of the world have documented the microbial contamination in street-vended foods and beverages especially juices, milk-based drinks, and ice made with water. In a study carried out in India (Tambekar et al., 2009), the results showed a great extent of bacterial contamination in the street vended juices, with E. coli representing the most isolated organism. On the same note, (Noor, 2016) carried out a study in Dhaka and found more than 60 percent of the tested ice samples, served by street vendors, positive to the presence of fecal coliforms, indicating low water quality and sanitary conditions during ice making and storage.

The current study aligns with these findings and further adds molecular confirmation of pathogen identity using PCR—a methodological advancement compared to earlier studies relying solely on cultural and biochemical techniques. Moreover, while studies such as those by (Rahman et al., 2013) in urban Bangladesh confirmed microbial presence in raw street foods, fewer have specifically focused on the ice used in fruit juices, which is often assumed to be clean and safe.

This study contributes to this gap by not only identifying pathogens but also providing AST profiles. The antimicrobial resistance observed in *E. coli* and *K. pneumoniae* reflects global trends in AMR as documented by WHO (2022), where *E. coli* in particular is frequently associated with multidrug resistance in clinical and community settings. This implies that street food contamination is not just a matter of gastrointestinal infection but could also be contributing to a broader, silent crisis in antibiotic resistance.

5.3 Likely Sources of Contamination

Multiple sources of contamination can be inferred based on field observations and microbiological results. The most likely sources include:

1. Contaminated Water for Ice Preparation: The ice used in these fruit juices is often manufactured using untreated or poorly treated municipal water, stored in non-hygienic environments, and transported in open containers without proper covering. This leaves sufficient chances of microbial contamination.
2. Hygiene and Handling Practices of Vendors: The vendors do not wear gloves, use open or dirty utensils, and work in dusty or sewage nearby areas especially in Merul Badda. Poor handwashing or utensil washing can result in contamination of the juice preparation process with organisms of fecal origin such as E. coli.
3. Environmental Factors: The presence of open drains and poor waste disposal systems alongside heavy human traffic in urban neighborhoods such as Merul Badda pose the risk of airborne or contact based contamination. The environment of Merul Badda has a greater risk of contamination compared to Gulshan-1 which is a comparatively cleaner and controlled commercial area.

4. Storage Conditions: Ice is often kept in large plastic tubs with little cooling or insulation, they partially melt and support the growth of microbes on the surface. This could be the reason why the bacteria count is bigger in watermelon juice ice, the melting process might have catalyzed the distribution of microbial.

5.4 Implications for Public Health in Dhaka

This research has important implications on the public health of the urban dwellers in Dhaka and in particular individuals who depend on the street vendors to get their daily refreshment on basis of affordability and proximity. Enteric and opportunistic infections continue to expose the populace due to the existence of pathogens such as *E. coli* and *K. pneumoniae* in consumables such as juice ice. Additionally, the realization of antimicrobial resistance among these isolates further potentiates the danger. Routinely consumed resistant strains could colonize the human gut microbiota and cause problems with treatment when people become ill, even due to unrelated infections. This aligns with One Health concerns—highlighting how environmental, human, and microbial health are interconnected.

5.5 Limitations of the Study

While the study offers important insights, several limitations should be acknowledged:

1. Limited Sample Size and Scope: Only three samples (two ice, one juice) from two urban locations were analyzed. This restricts generalizability and statistical comparisons across broader regions or seasons.
2. No Biochemical Confirmation: The study relied on cultural, morphological, and molecular identification, omitting confirmatory biochemical tests that could further validate species-level identification.
3. No Replication of AST: Antimicrobial susceptibility testing was not repeated for verification. Given its importance, repetition could have ensured greater accuracy.
4. Lack of Intermediate Resistance Data: The study classified isolates as resistant or sensitive, with no intermediate category analysis, possibly oversimplifying the resistance profiles.

5. No Environmental or Water Quality Testing: Testing the water used to make the ice or cleaning tools was beyond the study's scope. Such testing could have pinpointed the primary sources of microbial load.
6. No In-depth Statistical Analysis: Descriptive statistics were used, but the study lacked advanced statistical treatment (e.g., t-tests, chi-square) to support significance of findings.

Chapter 6

Conclusion

And

Recommendations

6.1 Key Findings Summary

This study assessed the extent of microbial contamination in ice and juice samples from street-vended orange and watermelon juices, collected from two urban areas of Dhaka—Merul Badda and Gulshan-1. The investigation confirmed the presence of two opportunistic and potentially pathogenic bacteria, Klebsiella pneumoniae and Escherichia coli, through colony enumeration, selective media growth, Gram staining, and species-specific PCR amplification and bands were seen in Gel electrophoresis.

In Merul Badda, an ice sample associated with orange juice revealed *K. pneumoniae* contamination of 1.86×10^3 CFU/mL at a 10^{-1} dilution on KPC agar. In Gulshan-1, an ice sample linked to watermelon juice showed even higher *K. pneumoniae* levels at 8.3×10^4 CFU/mL at a 10^{-3} dilution. These findings indicate substantial contamination of consumable ice, particularly in beverages not subjected to further processing. *E. coli* contamination was more extensive in Gulshan-1. Watermelon juice samples yielded 2.46×10^3 CFU/mL at a 10^{-1} dilution and 6.8×10^4 CFU/mL at 10^{-3}, while corresponding ice samples showed 1.93×10^3 CFU/mL at 10^{-1} and 8.2×10^4 CFU/mL at 10^{-3} on MacConkey agar. The distinct pink colonies confirmed lactose-fermenting *E. coli*. In comparison, orange juice samples showed lower microbial loads. This suggests that watermelon juice and its associated ice provide a more favorable environment for microbial survival, likely due to higher sugar content and poor hygiene practices. Although Merul Badda presented the highest single count of *K. pneumoniae*, Gulshan-1 demonstrated consistently higher *E. coli* contamination in both juice and ice. Despite Gulshan-1's reputation for cleaner urban conditions, the findings indicate inadequate ice handling, unsafe water usage, and possible cross-contamination during juice preparation. The use of a triangulated identification approach—colony morphology on selective media, Gram staining, and PCR—ensured accurate detection. PCR results confirmed *K. pneumoniae* and *E. coli* with distinct bands at approximately 130 bp and 585 bp, respectively. The results of the current investigation highlight a worrying trend of multidrug resistance in both Escherichia coli and Klebsiella pneumoniae isolates where both organisms have a high resistance to the majority of all routinely used antibiotics. The results proved that there was a very limited opportunity to treat infection with only the nitrofurantoin and ciprofloxacin being somewhat effective. The pattern of resistance clearly demonstrates the necessity to increase antimicrobial stewardship, sensible antibiotic treatment, and strict infection prevention procedures to curtail disease-resistant pathogens. Moreover, constant monitoring and studies at the molecular level are needed to know resistance mechanisms and allow corresponding therapeutic approaches to be established. The fact that even last-resort antibiotics face some resistance indicates the utmost importance of consistent work in research, policy-making, and the sphere of disease prevention as the problem of antimicrobial resistance recently appears to be significant.

6.2 Recommendations for Vendors and Public Health Authorities

In light of the results obtained in this study, a number of specific recommendations can be put forward with a view to curbing microbial contamination and the public health risks that are linked to this. The use of safe water to prepare ice should be a priority to street vendors; unlike using the municipal tap water or any other untreated source of water. The safe water should be either treated or filtered. Furthermore, the hygienic handling of the food needs to be enhanced; the vendors are supposed to wear gloves, regularly sanitize their utensils, and not expose ice to open-air places where it is likely to get contaminated. Ice storage is also of prime importance- ice must be stored in insulated, closed containers, to reduce melting and Microbial growth. As part of facilitating these changes, local health departments are encouraged to prepare and conduct elementary food safety training packages particularly designed to street vendors.

6.3 Suggestions for Future Research

In order to elaborated on the results of this study and fill in the existing gaps, future researches ought to contemplate expanded sampling, as well as improved methodology. The generalizability of the results would be strengthened by a bigger and more varied number of samples, taken in various places and at different times of the year, to identify any spatial or seasonal patterns of microbial contamination. Furthermore, to enhance reliability of the results, antimicrobial susceptibility testing (AST) ought to be replicated by duplicate or triplicate testing, and resistance analysis should be done in the future to provide more detailed picture by incorporating the intermediate resistance category. The researchers ought to perform environmental source tracking to identify the major causes of microbial contamination, especially the quality of water used to make ice and the hygienic condition of tools and containers. Also, an inferential statistical analysis, as ANOVA or chi-square test, would enable a more effective validation of results and would help to comprehend significant differences among locations or conditions. Comprehensively, the presented study forms an important basis of a future investigation and emphasizes the necessity of conducting an immediate improvement of the street food safety and microbial monitoring situation in urban Bangladesh.

Chapter 7

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