Public Health Risks of Fresh Fruit Juices: Bacterial Isolation, Fecal Contamination Assessment, and Antibiotic Resistance Patterns

By

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

Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences,

BRAC University

April, 2025

Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing a 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 that has been accepted or submitted for any other degree or diploma at a university or other institution.

4. We have acknowledged all the main sources of help.

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Abstract

Fresh fruit juices are widely consumed across Bangladesh due to their nutritional value, taste, and accessibility in urban markets and roadside stalls. This study investigates the microbiological quality and health risks of freshly made fruit juices sold in nine locations across Dhaka city. 27 Juice were collected from vendors to count fecal coliform count (FCC) alongside aerobics plate count (APC). Also, samples are diluted and spread on selective media for isolation and identification. After that PCR-based identification revealed that juice samples are contaminated with *Klebsiella pneumoniae*, *Vibrio* spp., *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus* which pose an entirely significant health risk. Moreover, 96.3% of the samples contained bacteria where *Klebsiella pneumoniae* leading as the dominant microorganism. Approximately 66.7% of the samples exceeded the acceptable limits for FCC, and 44.44%% failed to meet the standard microbiological safety threshold for APC. Among 147 bacterial isolates, *Klebsiella pneumoniae* exhibited high resistance to Ceftazidime. Multidrug resistance was also observed in *Staphylococcus aureus* (78.57%) and *Salmonella* spp. (75%). These findings highlight significant public health risks associated with pathogenic contamination and antibiotic resistance in fresh fruit juices.

Ethics Statement

This study obtained its samples from juice vendors through strict adherence to safety protocols and hygiene standards. Microbial analysis and antibiotic susceptibility testing occurred in the Microbiology Laboratory at BRAC University. Research experiments did not require human beings or animals throughout the study period.

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1. Introduction

Fresh fruit juices remain popular throughout Bangladesh since they offer nutritional benefits alongside their delicious taste together with their accessibility in markets located in urban areas and along roadsides. People worldwide drink fresh fruit juices since they provide essential vitamins and minerals and antioxidants, which support general wellness. Fresh juice possesses high water activity together with neutral pH and rich nutrients to support the growth of microorganisms and contamination occurrences (Aneja et al., 2014). Unlike commercially prepared juices, street vended juices in urban areas of Bangladesh are very rarely pasteurized or treated in some manner to reduce microbes. The combination of fresh juice preparation with unhygienic handling conditions allows pathogens derived from fecal materials to spread and contaminate the product. Worldwide, food borne disease is a critical public health concern and related closely to poor food handling and sanitation practice (Rifat et al., 2022).

It has been estimated that one tenth of people on earth (approximately 600 million) fall victims of consuming contaminated food (World Health Organization: WHO, 2024). Similar to other low-income countries, it is estimated that over 200 diseases are transmitted through contaminated foods (Loukieh et al., 2018). At least 30 million people suffer foodborne diseases of one type or the other in Bangladesh every year (Khairuzzaman et al., 2014). Scientific research reveals that unregulated fruit juice production facilities allow pathogenic bacteria *Escherichia coli*, *Salmonella* spp., *Vibrio* spp., *Staphylococcus aureus*, and *Klebsiella pneumoniae* to survive within these products. Research from different parts of the world has detected pathogenic microorganisms in fresh juice products. The *Salmonella* spp. caused a significant outbreak of orange juice that impacted sixty-two cases of patients from 21 states of the USA (Cook et al., 1998). The entry of pathogens happens through contaminated water from feces or because of unhygienic food handlers and through contamination that occurs when cutting and blending fruits or when storing them. The presence of fecal coliforms in juices serves as an essential sign that shows improper handling may introduce dangerous pathogens from fecal matter.

Escherichia coli, *Salmonella* spp., *Vibrio* spp., *Klebsiella pneumoniae*, and *Staphylococcus aureus* are known to cause foodborne diseases. *E. coli* together with *Salmonella* and *Vibrio* and *Klebsiella*, represent enteric pathogens that primarily affect the human gastrointestinal tract

through food and water contamination. Although the *Staphylococcus* spp. does not fall under the category of enteric bacteria; it remains a major concern for public health because it produces heat-resistant enterotoxins, which trigger food poisoning. Research conducted in Bangladesh identified major bacterial pathogens from fruit juices, consisting of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp.*, Staphylococcus aureus*, *Klebsiella pneumonia,e* and *Enterobacter* species (Uddin et al., 2017).

Excessive microbial growth in juice products above worldwide maximum standards set by the WHO identifies a major public health risk to consumers. During the 1990s United State of America (USA) experienced a disease outbreak from fruit juice consumption that raised public health warnings because the outbreak caused 1,366 illnesses from *Salmonella*, *Escherichia coli* O157:H7 and *Cryptosporidium* agents (Vojdani et al., 2008). Data from the 2017 World Health Organization (WHO) report reveals that foodborne diseases affect 600 million people yearly, resulting in deaths of 420,000 people alongside 125,000 children under 5 years of age (World Health Organization: WHO, 2024). According to the standards from the Bangladesh Food Safety Authority (BFSA), FCC is checked to determine whether it reaches or exceeds the allowed level. The Emirates Authority for Standardization and Metrology (ESMA) sets the standards for APC, which allow them to check if the number of bacteria exceeds what is acceptable.

Klebsiella pneumoniae stands out due to its growing frequency as a resistant opportunistic pathogen among the bacteria found in contaminated food products. Street-sold food products containing *Klebsiella pneumoniae* serve as indicators for potential health risks due to their association with infections affecting the respiratory and urinary, and gastrointestinal systems (Struve & Krogfelt, 2004). The presence of *Vibrio* spp. and *Salmonella* spp. poses significant public health risks because these pathogens can lead to diarrhea and typhoid fever, and cholera infections. Research conducted in Ethiopia showed that all fresh fruit juice samples contained *E. coli* (50.00%) and *Salmonella* spp. (41.67%) together with *Staphylococcus aureus* (67.71%), which may pose hazard to the health of consumers (Wedajo & Kadire, 2019). Diarrheal diseases are the most prevalent kind of foodborne diseases in Bangladesh; enteric fever and hepatitis. Findings of a report by the Dhaka-based Institute of Epidemiology, Disease Control and Research (IEDCR) revealed that acute watery diarrhea is the most reported outcome from the incidence of food poisoning in the country with approximately 0.28 million cases in 2015 (Banna et al., 2021).

Two other common foodborne diseases, enteric fever and hepatitis, affect 30000 people, and 500 people each year respectively (Project, 2022).

Antimicrobial resistance (AMR) is an important menace to public health in the world. In Asia, AMR is on the rise much faster than in any other part of the world (Chereau et al., 2017). Food safety is also a major concern to public health in the country which has been poorly tackled. Another investigation in Dhaka reported that *E. coli*, *Salmonella*, *Klebsiella*, and *Vibrio* spp. isolated from fruit juices exhibited resistance to multiple antibiotics. Ampicillin was notably the least effective antibiotic (Uddin et al., 2017). The improper application of antibiotics during food production along with healthcare practices, has generated Multidrug-resistant bacterial strains. Bacteria in raw juices can transmit resistance genes to the human gut microbiota when people consume these juices through ingestion. The development of drug-resistant bacteria complicates treatment accessibility and enhances outbreak risks.

Research on microbial contamination of fresh fruit juices as a public health risk remains poorly studied in Bangladesh despite its prominence throughout the region. Studies currently available demonstrate serious drawbacks because they perform minimal testing from a limited geographic area using small sample sizes while investigating very few pathogenic organisms. Very few studies combine research design elements, including aerobic plate count (APC) alongside fecal coliform count (FCC), identification of bacteria, and antimicrobial resistance profiling. Notably, there is a lack of recent research that thoroughly investigates the risks associated with multidrug-resistant (MDR) organisms in fresh fruit juices sold throughout Dhaka's densely populated areas

Given these significant gaps, the purpose of this research is to deliver a thorough microbial safety assessment of fresh juice products distributed in Dhaka city locations based on assessment outcomes. The assessment procedure involves counting aerobic bacteria along with testing for fecal contamination by counting fecal coliform bacteria and identifying harmful bacteria and their drug resistance characteristics. The research findings will provide valuable information to public health stakeholders to identify necessary interventions for maintaining fresh juice microbiological quality in local markets.

1.1 Objectives

1.1.1 General Objective

To isolate and identify bacteria from street vended fresh fruit juice samples and evaluate their antimicrobial susceptibility using the disc diffusion (Kirby-Bauer) method.

1.1.2 Specific Objectives

- To determine the APC using Nutrient Agar.
- To assess FCC through the membrane filtration technique on M-FC agar.
- To isolate bacteria using selective culture media.
- To identify the bacterial isolates using gene-specific Polymerase Chain Reaction (PCR).
- To perform antimicrobial susceptibility testing (AST) of the identified isolates using the Kirby-Bauer disc diffusion method.
- To determine the percentage of multidrug-resistant (MDR) isolates among the identified bacteria.

2. Materials and Methods

2.1 Work Plan

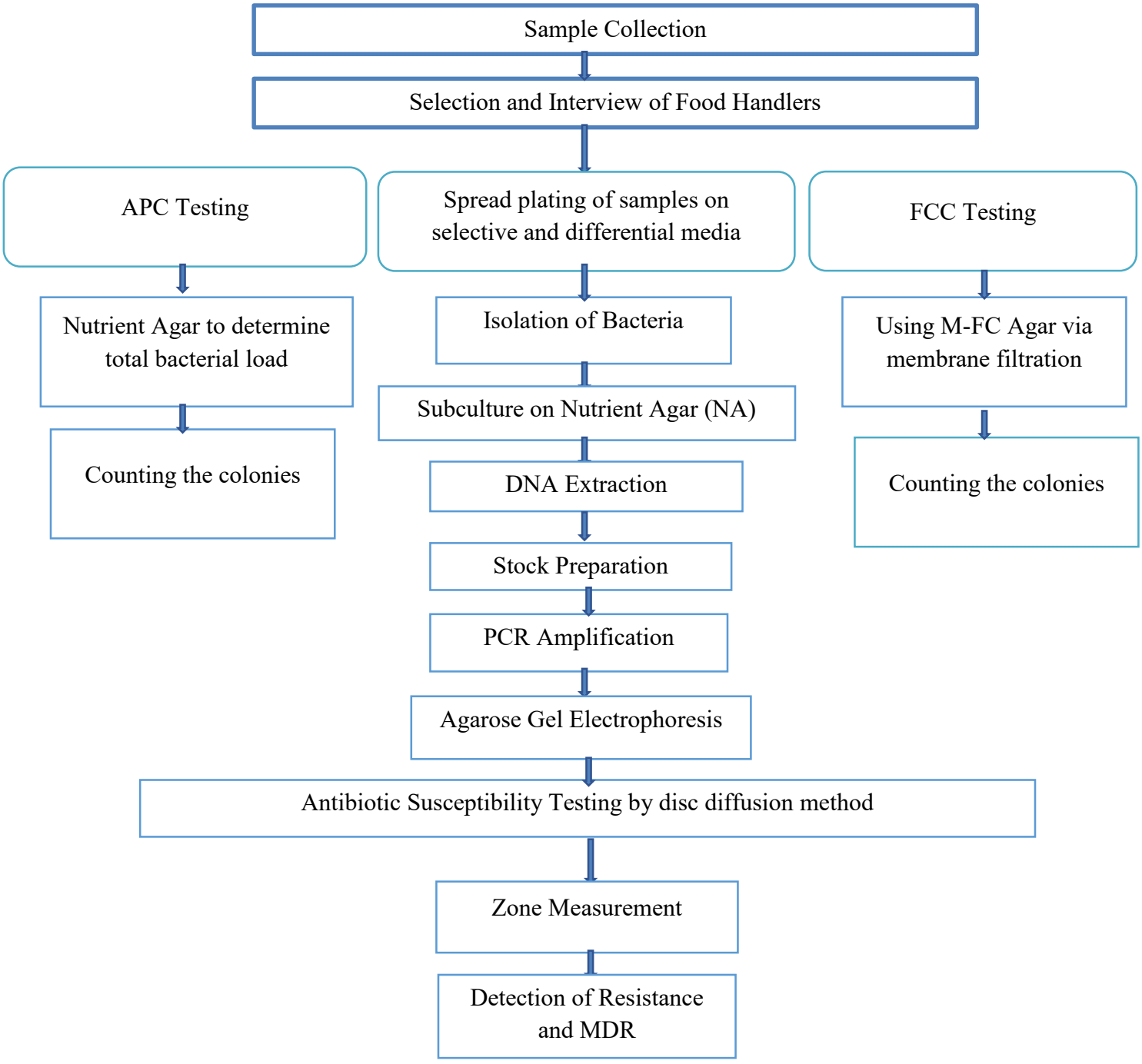

Figure 1: Work Plan of this study

2.2 Description of the study area

A cross-sectional study examined the microbiological quality and potential health risks of freshly made and sold fruit juices found in different Dhaka city in Bangladesh. Nine locations, including Badda, Notun Bazar, Mohakhali, Purbachal, Motijheel, Baily Road, Khilgaon, Uttara, and Mirpur, were used for sample collection to represent diverse residential, commercial, and high-traffic zones. The locations are indicated with a red location indicator (Figure 2).

Figure 2: Areas of Dhaka city from where samples were collected

2.3 Sample collection

All juice samples were collected in clean 250 mL screw-capped bottles and processed within 2 hours. They were kept in an insulated icebox to prevent bacterial growth. All microbiological tests were done at the Microbiology Laboratory of BRAC University.

2.4 Data Collection

The study obtained information about food safety and hygiene practices through semi-structured questionnaires, which included an observational checklist (Table 1). From this, the hygiene condition and other information for contamination can be identified. The checklist indicated the condition of 9 different areas, and hygiene conditions. Here total checklist, N = 9.

Table 1: A semi-structured questionnaire that included an observational checklist

VARIABLES	PARAMETERS	CHECKLIST	FREQUENCY%
Washing Hands	With water only	7	77.78
	With water and soap	2	22.22
Wearing aprons while serving juice	Yes	2	22.22
	No	7	77.78
Waste receiving receptacles	Sacks, Bin without cover	5	55.56
	Bin with cover	4	44.44
Hand-washing equipment	Available	3	33.33
	Not available	6	66.67
Methods of preservation of fruit	On Shelf	6	66.67
	In a bucket	3	33.33
	On the floor	0	0.00
Place to keep the juice after preparation	In Jag	0	0.00
	In the squeezing machine	4	44.44
	In a refrigerator	5	55.56
The frequency of cleaning the material used to keep the juice	After every use	2	22.22
	Once a day	7	77.78
Received any food hygiene or safety training	Yes	0	0.00
	No	9	100.00
	Bottled water	0	0.00

Water used for making juice	Filtered water	5	55.56
	Local water jar	4	44.44
	Tap water	0	0.00
	Tube well water	0	0.00
Reuse leftover juice the next day	Sometimes	2	22.22
	Never	7	77.78

2.5 Sample size and collecting a sample

A total of 27 fresh fruit juice samples were collected from various locations in Dhaka city. Three types of juices were taken from each vendor. In total, nine common fruit types were sampled: Papaya, Orange, Olive, Lemon, Mango, Sugarcane, Mixed Fruit, Pineapple, and Watermelon. All samples were carried in a sterile ice box to the laboratory to maintain their microbial quality and prevent spoilage.

2.6 Study variables

The dependent variable in this study is the bacteriological quality of locally prepared fruit juices by analyzing APC and FCC results and identifying bacteria *E. coli, Salmonella* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Vibrio* spp. together with antibiotic resistance testing for MDR detection.

The independent variables include several factors related to hygiene and safety. These are socio-demographic characteristics of the food handlers, such as whether they received training in food hygiene and safety, juice house hygiene conditions, including the presence of latrine facilities, waste disposal systems, and personal hygiene and safety practices.

2.7 Sample Processing

All juice samples underwent processing within a contamination-free setting through the use of a laminar airflow cabinet. Sterilized surgical knives were used to carefully open each sample to handle the juice during processing. Ten milliliters of juice from each sample were transferred into sterile test tubes. Laboratory personnel used sterile distilled water to make the dilutions for bacteria viability counting.

2.8 Aerobic Plate Count

Procedure for APC required the proper dilution of samples before spreading on nutrient agar surfaces. A dilution process starting with 10^{-1} followed by 10^{-4} using sterile NaCl. These plates were then incubated at 37°C for 24 hours to allow the growth of aerobic bacteria. Bacteria colonies were counted after incubation to determine the number of colony-forming units per mL of juice.

APC (CFU/mL) = Number of Colonies×Dilution Factor/ Volume of Sample Plated (mL)

2.9 Fecal coliform count

The membrane filtration method served as the main technique for FCC detection. The preparation of the diluted sample involved mixing 400 µL of juice solution with 99.6 mL of sterile distilled water to achieve a total volume of 100 mL. An automated vacuum filtration system used a 0.45 µm sterile membrane filter to transfer the solution. The filter was placed on M-FC agar plates, which are designed to detect fecal coliform bacteria. The M-FC agar showed blue-colored colonies after its incubation at 44.5 degrees Celsius, indicating the presence of fecal coliforms.

FCC (CFU/100 mL) = Number of Colonies×100/ Volume of Original Juice Used (in mL)

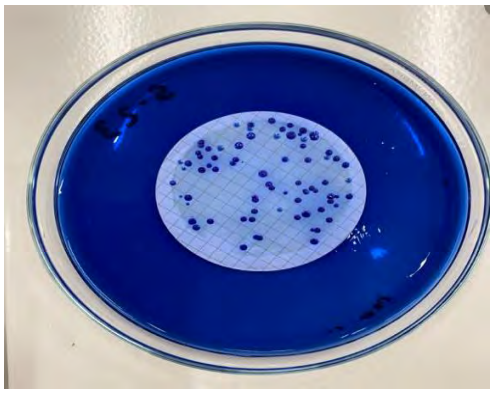

Figure 3: Fecal coliform bacteria in M-FC Agar

2.10 Bacterial isolation and identification

1. The isolation of *Klebsiella pneumoniae* required HiCrome KPC Agar as the culture medium because the medium produces distinct chromogenic colony outcomes.
2. Visual identification of *Escherichia coli* bacteria occurred through HiCrome UTI Agar since this medium produced visible color changes.
3. The mannitol fermentation of *Staphylococcus aureus* led to yellow-colored zones around colonies grown on Mannitol Salt Agar.
4. The detection of *Salmonella* spp. works through Salmonella-Shigella (SS) Agar that produces visible H_2S-producers. Also, XLD Agar was used to isolate Salmonella, which produced red colonies with black centers.
5. The isolation and differentiation of *Vibrio* spp. took place on Thiosulfate Citrate Bile Salt Sucrose (TCBS) Agar medium because it specifically selects for *Vibrio* species and allows colony color recognition.

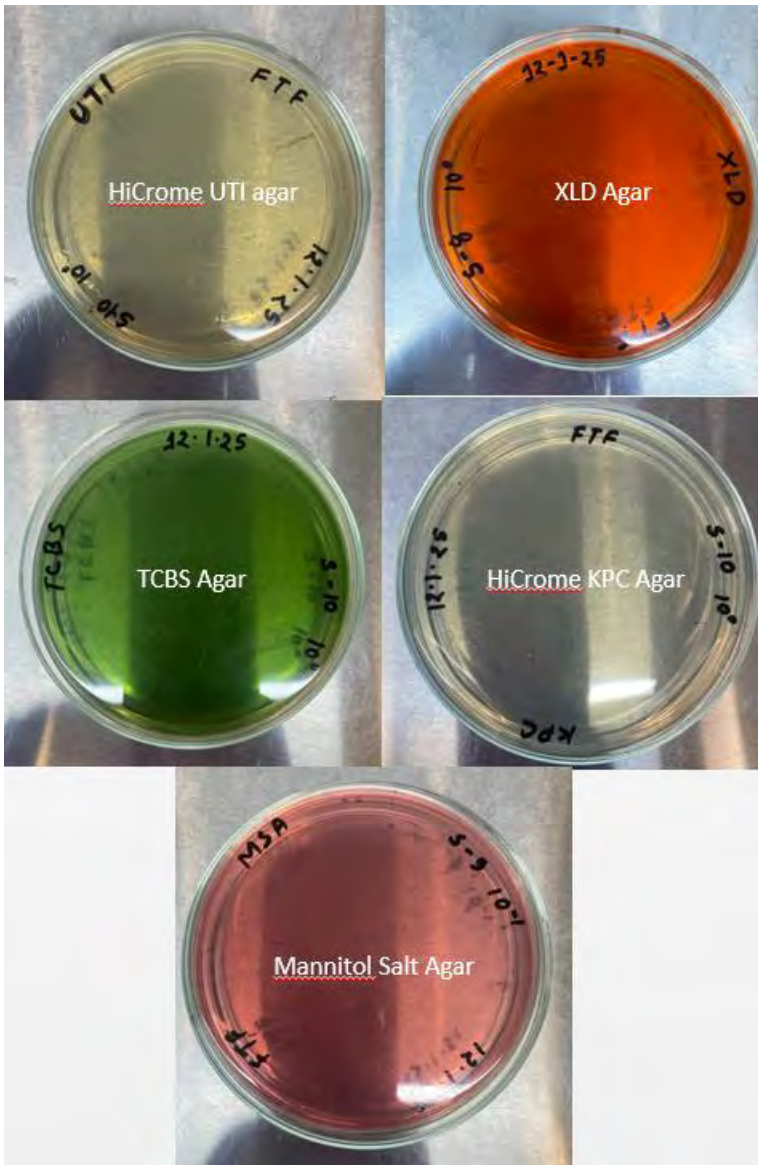

Figure 4: HiCrome KPC agar, HiCrome UTI agar, Mannitol Salt Agar media, XLD Agar and TCBS agar

The Mannitol Salt Agar, HiCrome KPC and HiCrome UTI were sterilized at 121 degree Celsius for 15 minutes at 15 psi before their application. The laminar flow cabinet provided a sterile environment where Petri dishes received media after they were sterilized and cooled. The media then solidified before the inoculation step.

Both raw (undiluted) juice and 10^{-1} diluted samples received 100 µL distribution onto multiple selective and differential media to select and identify target organisms, including *E. coli*,

Salmonella spp., *Staphylococcus* spp., *Klebsiella pneumoniae* and *Vibrio* spp. All media plates were incubated at 37°C for 24 hours.

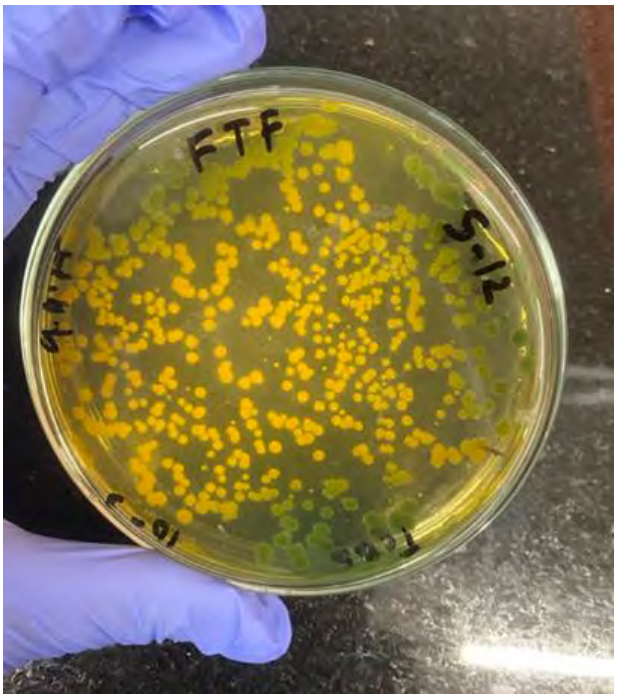

Figure 5: Yellow colored colonies of *Vibrio* spp. in TCBS agar

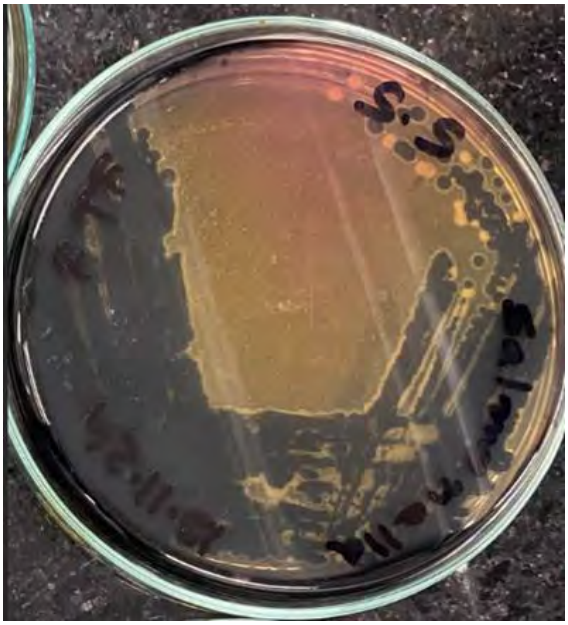

Figure 6: Black colored colonies of *Salmonella* in SS agar

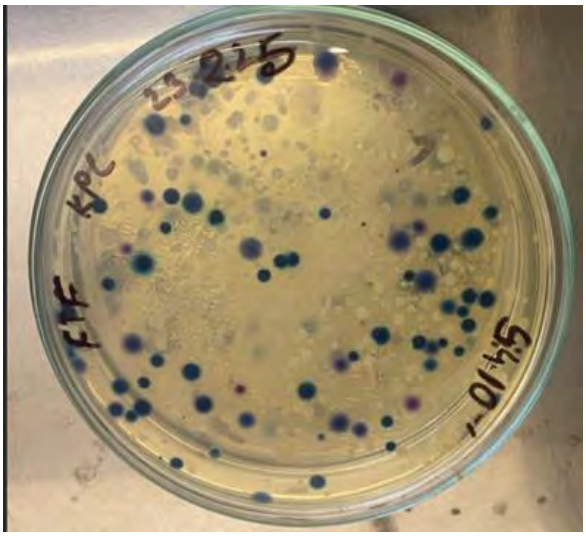

Figure 7: Purpled colored colonies of *Klebsiella pneumonia* in Hichrome KPC agar and purpled colored colonies of *Escherichia coli*

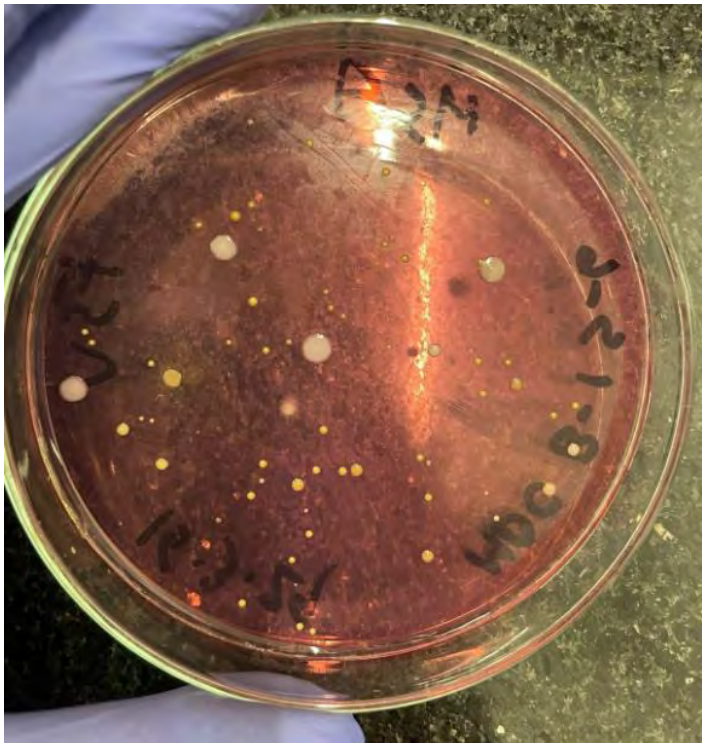

Figure 8: Yellow colored colonied of *Staphylococcus aureus* on MSA

After the incubation period, isolated colonies were selected depending on colony morphology. The morphology is given in Table 2.

Table 2: Colony Morphology of Specific Bacteria on Selective Media

Organism	Gram Positive/ Negative	Media	Expected colony morphology
Escherichia coli	Gram-negative	HiCrome UTI Agar	Purple to pink colonies
Klebsiella pneumoniae	Gram-negative	HiCrome KPC Agar	Blue or light blue colonies (Figure 7)
Klebsiella pneumoniae	Gram-negative	HiCrome UTI Agar	Blue or light blue colonies
Escherichia coli	Gram-negative	HiCrome KPC Agar	Purple to pink colonies (Figure 7)
Staphylococcus aureus	Gram-positive	Mannitol Salt Agar	Colorless or pale colonies with black centers
Salmonella spp.	Gram-negative	SS Agar/ XLD agar	SS agar: Colorless colonies with a black center (Figure 6) XLD Agar: Red colonies with black centers
Vibrio spp.	Gram-negative	TCBS agar	Yellow colonies (Figure 5)

Further Screening of *Escherichia coli* and *Klebsiella pneumonia*

A subculture of *Escherichia coli* and *Klebsiella pneumoniae* isolates was performed on both HiCrome UTI agar and Eosin Methylene Blue (EMB) agar plates. *E. coli* colonies formed purple-colored distinct areas whereas *K. pneumoniae* colonies produced blue-colored features on the

HiCrome UTI agar medium according to their chromogenic identification properties (Figure 10). *E. coli* created a green metallic sheen on EMB agar which demonstrates both strong lactose fermentation and acid production characteristic of this bacterial species (figure 9).

Figure 9: Green metallic sheen on EMB agar

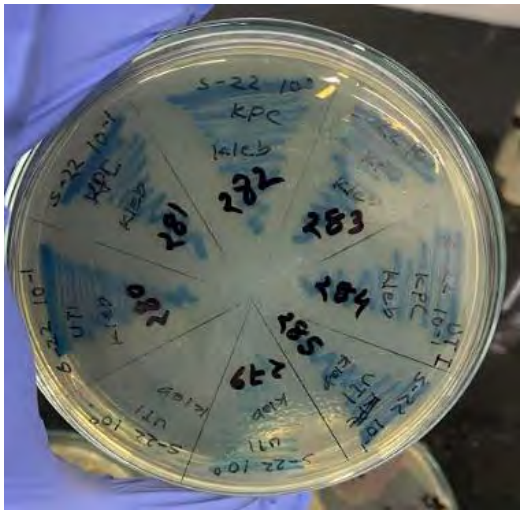

Figure 10: *K. pneumoniae* colonies appeared blue-colored

2.11 Stock Preparation

The production of T1N1 agar stock required mixing 1 g tryptone with 1 g NaCl along with 1.5 g agar in 100 mL distilled water before heating to dissolution. The solution received 2 mL portions before autoclaving at 121°C for 15 minutes under 15 lbs pressure to achieve sterility in sterile glass vials. Sterile glass vials remained upright to enable agar solidification after autoclaving.

A single bacterial colony was chosen using a sterile tool to make a vertical stab into the agar plate when it reached its solid state. The prevention of desiccation during long-term storage was achieved by adding 200 µL of sterile paraffin oil over the solidified agar surface. The vial openings received secure sealing before being placed at room temperature for storage purposes.

2.12 DNA Extraction

The DNA extraction process started with preparing 1X Tris-EDTA (TE) buffer to use as the fundamental solution for cell lysis. The experiment started by depositing 400 µL 1X TE buffer into a clean sterile Eppendorf tube. A loopful from the presumptive bacterial colony was inoculated into the tube that contained TE buffer using aseptic techniques.

A vigorous vortex process secured full distribution of bacterial cells throughout the buffer solution. A centrifugation process using 13,000 rpm for 10 minutes at 25°C separated the bacterial cells into a pellet. The end of the centrifugation process is achieved by removing the supernatant while maintaining the pellet's integrity.

A fresh 400 µL volume of 1X TE buffer was added to the same test tube. A vortexing process homogenized the solution after resuspending the pellet. A proper cleaning process for the cells took place before the lysis step.

The heat block maintained temperatures at 100°C while heating the tube for 10 minutes. A high-temperature treatment was applied to lyse bacterial cells through cell wall and membrane disruption for genomic DNA extraction from the solution.

A five-minute cooling period at room temperature followed the heat treatment to stop DNA thermal destruction. After the sample cooled down it underwent a new centrifugation at 13,000 rpm for 10 minutes to split cell debris with any remaining insoluble contents away from the extracted DNA.

A micropipette carried 200 µL of clear supernatant containing genomic DNA to a new sterile Eppendorf tube.

2.13 Polymerase Chain Reaction (PCR)

A working primer solution of 10 µM concentration was made through pipetting the material into sterile nuclease-free water starting from its original stocks. The primers were transferred to separate tubes which used sterile micro centrifuge tubes to ensure purity without allowing contamination. The reactions for PCR analysis contained 13 µL of volume in separate sterilized PCR tubes for mixture preparation. The PCR reaction contained 2 µL template bacterial DNA from extraction and 6 µL PCR Master Mix, together with 3 µL nuclease-free water and 1 µL of each forward and reverse primer. Single-tube PCR amplification happens with Taq DNA polymerase and deoxynucleotide triphosphates (dNTPs) together with magnesium chloride ($MgCl_2$) inside an optimized reaction buffer to obtain maximum DNA amplification efficiency. The tubes received a quick spin before being introduced into the thermal cycler, where amplification occurred. In Table 3, all the primers that are used in this study are given below:

Table 3: Primers for the targeted Organism

Primer	Primer sequence	Target Gene	PCR conditions	Number of cycles	Amplicon Size	Reference
Klebsiella pneumoniae	KP Pf-F: 5′-ATTTGAAGAGGTTGCAAACGAT-3′ KP Pr1-R: 5′-TTCACTCTGAAGTTTTCTTGTGTTC- 3′	K. pneumoniae 16S-23S ITS*	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.	35	130 bp	(Hartantyo et al., 2020)

Staphylococcus aureus	NUC F: 5'-GCGATTGATGGTGATACGGTT-3' NUC R: 5'-AGCCAAGCCTTGACGAACTAAAGC-3'	Nuc gene	Amplification was done by initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing temperature of primers was 55°C for 45 sec and extension at 72°C for 1 min. The final extension was conducted at 72°C for 10 min	30	275 bp	(Brakstad et al., 1992)
Escherichia coli	16 s-F: 5'-GACCTCGGTTTAGTTCACAGA-3' 16s-R: 5'-CACACGTGACGCTGACCA-3'	ECO (16 s rRNA)	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	35	585 bp	(Thopireddy, 2023)

			at 72°C for 5 min.			
Salmonella	*Salmonella*-F: 5'-GTATTGTTGATTAATGACA TCCG-3' *Salmonella*-R: 5'-ATATTACGCTACGGAAAC ACGTT-3'	invA	The cycling conditions were 5 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 30 s at 60 °C and 1 min at 72 °C, followed by a 10 min hold at 72 °C	35	403	(Ranjbar et al., 2016)
Vibrio spp.	VG C2694352 F46: 5' GTC ARA TTG AAA ARC ART TYG GTA AAG G VG C2694352 R734: 5' ACY TTR ATR CGN GTT TCR TTR CC	Recombinase A	Initial denaturation at 94 °C for 5 min, followed by 25 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, finishing with a final extension at 72 °C for 10 min	25	689	(Kim et al., 2015)

2.14 Agarose Gel Electrophoresis

Agarose gel electrophoresis functioned as the analysis tool to study PCR products through DNA fragment separation made possible by using an electric field across an agarose matrix. The technique allows PCR-amplified products to be visible through ethidium bromide usage since this fluorescent dye binds DNA molecules and produces UV light-induced fluorescent signals. A 1.5% agarose gel prepared with 1X Tris-Acetate-EDTA (TAE) buffer functioned as the running buffer throughout this gel electrophoresis procedure. The gel contained 4 µL of ethidium bromide to allow DNA staining. The 100 bp DNA ladder served as size marker for the PCR products by including it with the loaded PCR samples. The DNA fragments migrated through the gel matrix using 100 volts power for 45–60 minutes duration. The DNA gel went on the UV transilluminator for visualizing and documenting the bands that formed after the run was done.

2.14.1 For *Vibrio* spp.

The solid agarose gel was transferred under UV light to visualize the bands. In case of *Vibrio*, the positive isolates produced a single band at 689 base pairs, observed in lanes 4 to 25. Lane 1 contained the 100 bp ladder, while lanes 2 and 3 were the negative and positive controls, respectively (Figure 11).

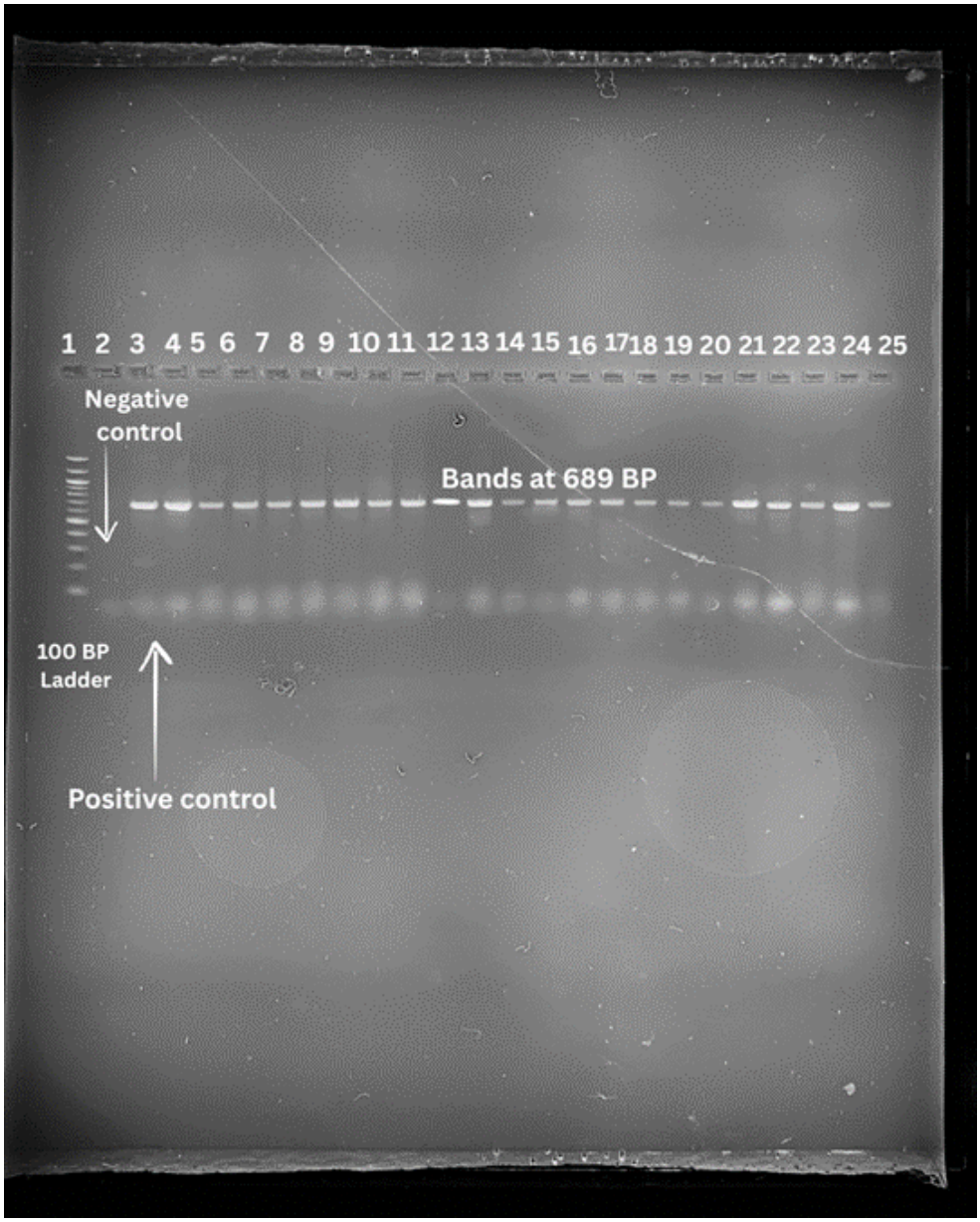

Figure 11: Agarose Gel Electrophoresis of PCR Products of *Vibrio* spp. isolated from Fruit juice sample

2.14.2 For *Salmonella* spp.

The solid agarose gel was transferred under UV light to visualize the bands. During the identification of *Salmonella*, the positive isolates produced a single band at 403 bp, observed in lanes 4 to 7. Lane 1 contained the 100 bp ladder, while lanes 2 and 3 were the negative control and positive control, respectively (Figure 12).

Figure 12: Agarose Gel Electrophoresis of PCR Products of *Salmonella* spp. isolated from Fruit juice sample

2.14.3 For *Klebsiella pneumonia*

The agarose gel was placed under UV light to observe the bands. While *Klebsiella* was identified, a single band at 130 bp was detected in the positive isolates, which appeared in lanes 4 to 39. The position of the 100 bp ladder appeared in lane 1, followed by the negative control band in lane 2 and the positive control appeared in lane 3 (Figure 13).

Figure 13: Agarose Gel Electrophoresis of PCR Products of *Klebsiella pneumonia* isolated from Fruit juice sample

2.14.4 For *Staphylococcus aureus*

Single bands at 275 bp in lanes 4 to 17 suggest that the target gene is present in all tested samples. Lane 1 contained the 100 bp ladder, and lanes 2 and 3 served as the positive control and negative control, respectively. (Figure 14)

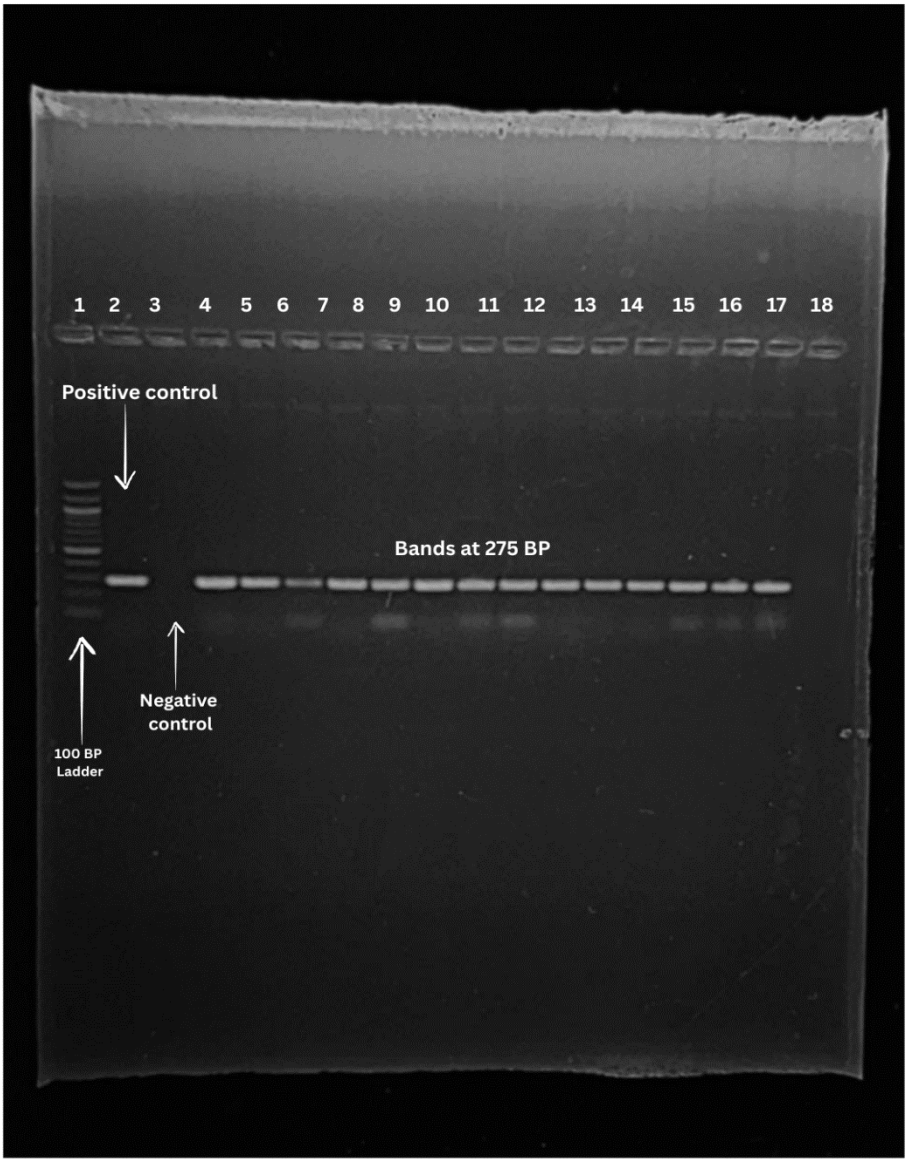

Figure 14: Agarose Gel Electrophoresis of PCR Products of *Staphylococcus aureus* isolated from Fruit juice sample

2.14.5 For *Escherichia coli*

The solid agarose gel was transferred under UV light to visualize the bands. The positive isolates will give single band at 585 bp in lane 4 to 13. Lane 1 contained the ladder, while lanes 2 and 3 were the positive control and negative control, respectively. (Figure 15)

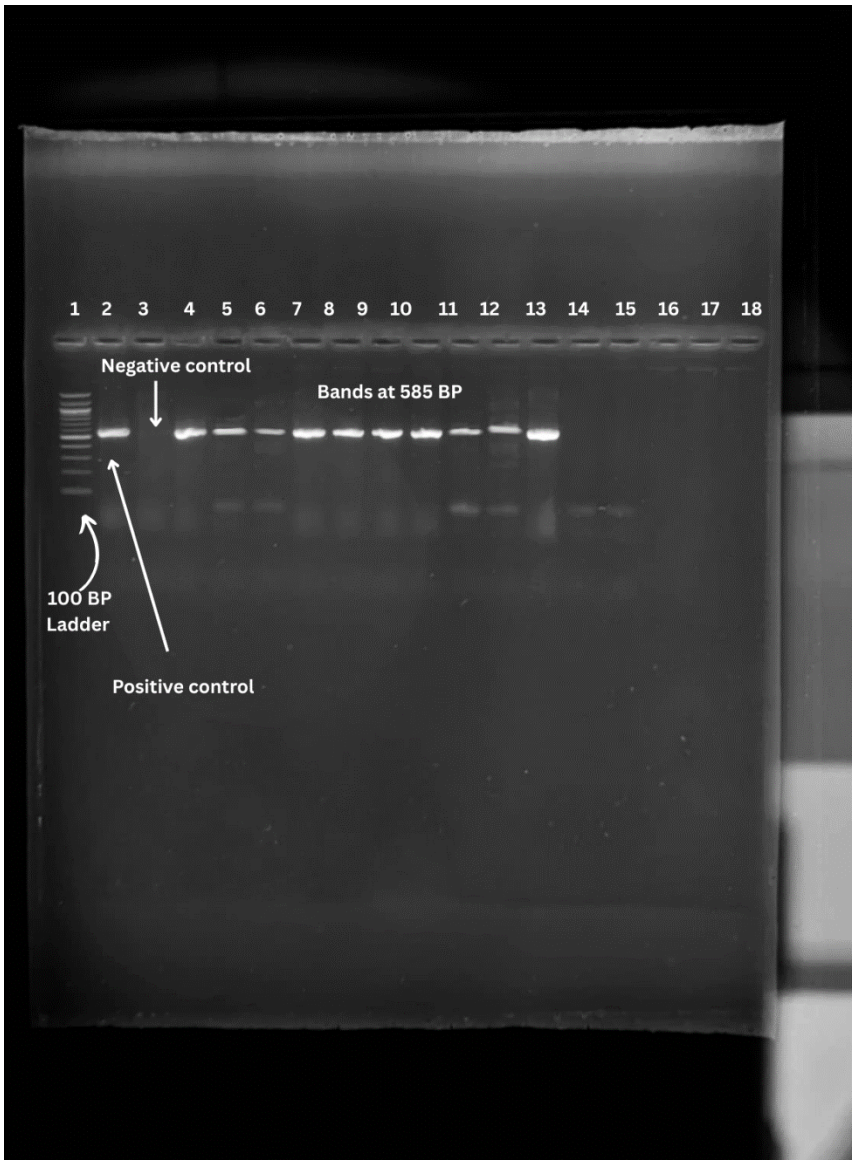

Figure 15: Agarose Gel Electrophoresis of PCR Products of *Escherichia coli* isolated from Fruit juice sample

2.15 Antibiotic Susceptibility Testing (AST)

The antibiotic susceptibility testing of five bacterial species, including *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella* spp., and *Vibrio* spp., was performed through the Kirby-Bauer disk diffusion protocol according to Clinical and Laboratory Standards Institute (CLSI) procedures. The Kirby-Bauer disk diffusion technique remains a leading tool for bacterial resistance pattern evaluation because it delivers simple results with proven reliability across aerobic and facultative anaerobic bacteria. Standardization of bacterial isolates reached a 0.5 McFarland turbidity level. The inoculum was distributed uniformly across Mueller-Hinton Agar (MHA) surfaces because the medium offers both non-selective and non-differential characteristics that yield high reproducibility results. Bacterial toxins get absorbed by starch in MHA as this agar contains a starch component and also supports antibiotic agents to spread evenly during diffusion through its loose structure. Selected antibiotic disks were placed on the MHA plates before the start of 37 degrees Celsius incubation at 18 to 24 hours. The measurement of inhibition zones from antibiotic disks provided sensitivity, intermediate or resistance outcomes using CLSI interpretive standards (Figure 16).

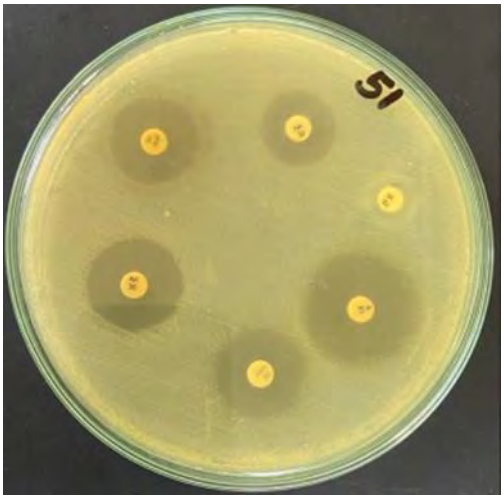

Figure 16: Inhibition zones from antibiotic disks

In this table, a total of 12 antibiotic classes were used to cover a wide spectrum of action and resistance mechanisms (Table 4).

Table 4: Concentrations and diffusion zones of the antibiotics

Antibiotic Class	Antibiotic	Abbr	Susceptible (S)	Intermediate (I)	Resistant (R)
Beta-lactams	Amoxicillin-Clavulanic Acid	AMC	≥18 mm	14–17 mm	≤13 mm
	Amoxicillin	AMX	≥17 mm	14–16 mm	≤13 mm
	Ampicillin	AMP	≥17 mm	15–16 mm	≤ 14 mm
	Cefixime	CFM	≥19 mm	16–18 mm	≤15 mm
	Ceftriaxone	CRO	≥23 mm	20–22 mm	≤19 mm
	Ceftazidime	CAZ	≥18 mm	15–17 mm	≤14 mm
	Cefepime	FEP	≥18 mm	15–17 mm	≤14 mm
	Cefuroxime	CXM	≥ 23 mm	15–22 mm	≤ 14 mm
	Aztreonam	ATM	≥ 28 mm	21–27 mm	≤ 20 mm
Tetracyclines	Tetracycline	TE	≥15 mm	12–14 mm	≤11 mm
	Doxycycline	DO	≥ 16 mm	13–15 mm	≤ 12 mm
Carbapenems	Imipenem	IMP	≥ 23 mm	20–22 mm	≤ 19 mm
	Meropenem	MEM	≥23 mm	20–22 mm	≤19 mm
Aminoglycosides	Kanamycin	K	≥18 mm	14–17 mm	≤13 mm
	Gentamicin	CN	≥15 mm	13–14 mm	≤12 mm
	Amikacin	AK	≥17 mm	15–16 mm	≤14 mm
Fluoroquinolones	Norfloxacin	NOR	≥ 17 mm	13–16 mm	≤ 12 mm
	Levofloxacin	LEV	≥17 mm	14–16 mm	≤13 mm
	Ciprofloxacin	CIP	≥21 mm	16–20 mm	≤15 mm
Macrolides	Azithromycin	AZM	≥18 mm	14–17 mm	≤13 mm
	Erythromycin	E	≥23 mm	14-22 mm	≤13 mm
Sulfonamide	Trimethoprim-Sulfamethoxazole	SXT	≥16 mm	11–15 mm	≤10 mm
Glycylcyclines	Tigecycline	TGC	≥19 mm	15–18 mm	≤14 mm
Amphenicols	Chloramphenicol	C30	≥18 mm	13–17 mm	≤12 mm
Quinolones	Nalidixic Acid	NA	≥19 mm	14–18 mm	≤13 mm
Polymyxins	Colistin	CL		11-17 mm	
Glycopeptides	Vancomycin	VA	≥ 17 mm		

3. Result

3.1 Aerobic Plate count result

The Emirates Authority for Standardization and Metrology (ESMA) has stated that the Gulf Standard No. 1016/2000 allows a maximum APC of 1.0×10^4 CFU/mL in fruit juices. It is commonly used as a safety guideline for fruit juices and drinks that are ready to consume. Bacteria limits being surpassed can show that the food is not safe for those who eat it (ESMA, 2000) (Islam et al., 2025).The test results of juice samples went through comparison with the standard APC value to find harmful samples. The analytical results demonstrated that sample numbers exceeded this threshold limit are 12 samples in total (Table 5).

Table 5: Results of APC Count

Sample	No. of Sample	No. of samples contaminated			Limit of contamination allowed:
		Bacterial load in CFU/g			
		$< 10^4$	10^4	$> 10^4$	
Juice Sample	27	15	0	12	1×10^4 CFU/mL

3.2 Fecal coliform count result

The Bangladesh Food Safety Authority (BFSA) specifies 0 CFU/mL as the maximum allowed FCC value in both food and beverages (Faruk & Akhter, 2012). Most of the analyzed juice samples fell within the set safety threshold according to this study. 18 tested juice samples violated the set maximum standard for FCC while other samples complied with the standards indicating contamination from fecal material. A total of 18 juice samples exceeded the maximum allowed FCC (Table 6). These results show that some juice sellers may not follow proper hygiene, which can lead to contamination and health risks for consumers.

Table 6: Fecal Coliform Count

Sample	No. of Sample	No. of samples are Fecal coliform			Limit of contamination allowed:
		Bacterial load in CFU/g			
		< 100	0	> 100	
Juice Sample	27	16	9	2	0 CFU/mL

3.3 Percentage of Bacterial Presence in Fresh Fruit Juice Samples

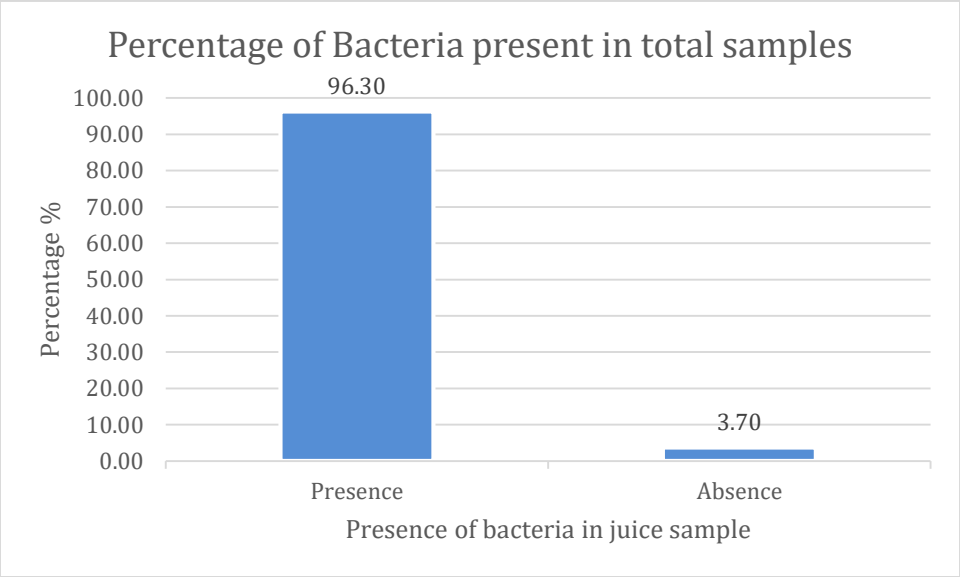

Figure 17: Percentage of Bacteria present in total sample

The percentage of bacterial presence and absence across 27 fresh fruit juice samples. Out of the 27 juice samples tested, 26 samples (96.3%) showed the presence of bacteria, while only 1 sample (3.7%) showed no bacterial growth (Figure 17). This indicates a high contamination rate in locally prepared fruit juices.

3.4 Analysis of Bacterial Diversity in Juice Samples

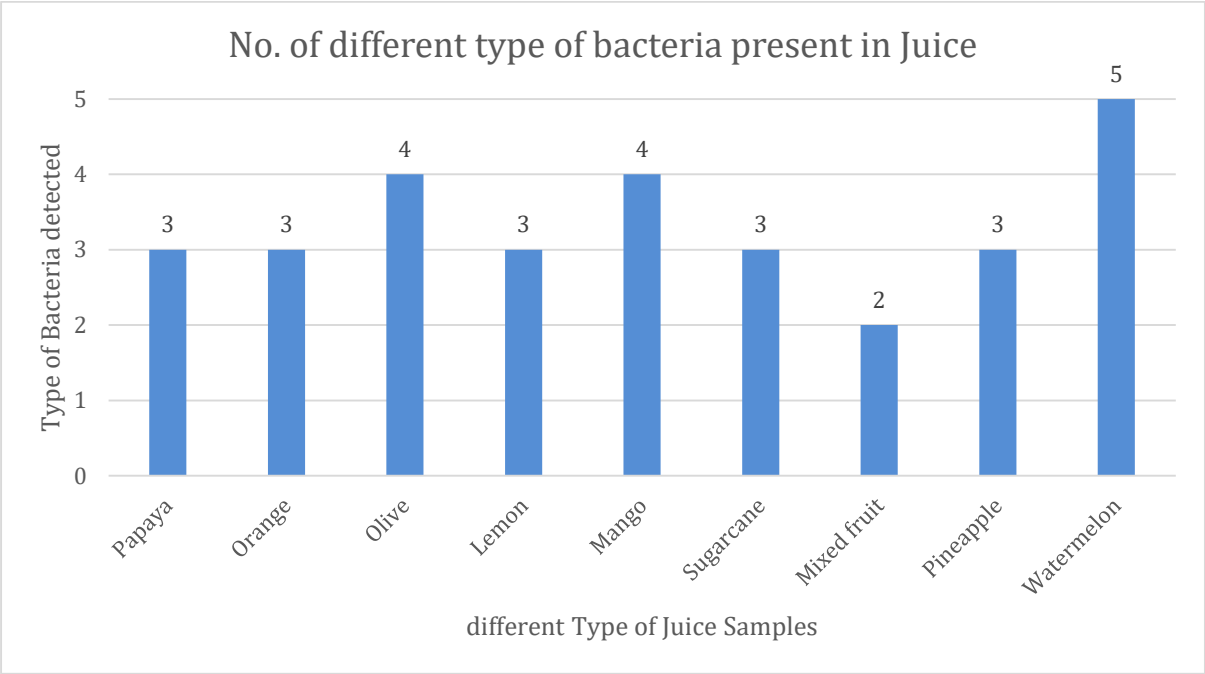

Figure 18: Number of different types of bacteria isolated from various juice samples

Health risks become more severe and removing contamination becomes harder when multiple bacteria are found in one sample. The Watermelon juice has the highest bacterial diversity (5 types), while Mixed Fruit juice has the lowest (2 types). Most other juices like Olive, Mango, and Sugarcane contain 4 types of bacteria. This indicates that watermelon juice is the most contaminated. Watermelon has a seasonal availability, so some vendors keep it in refrigerators, which could lead to bacterial proliferation during storage times.

3.5 Percentage of Bacterial Contamination in Locally Prepared Fruit Juices

Klebsiella is the most common bacteria, being present in the highest number of samples, while *Vibrio* and *Staphylococcus* are moderately prevalent (Figure 19)

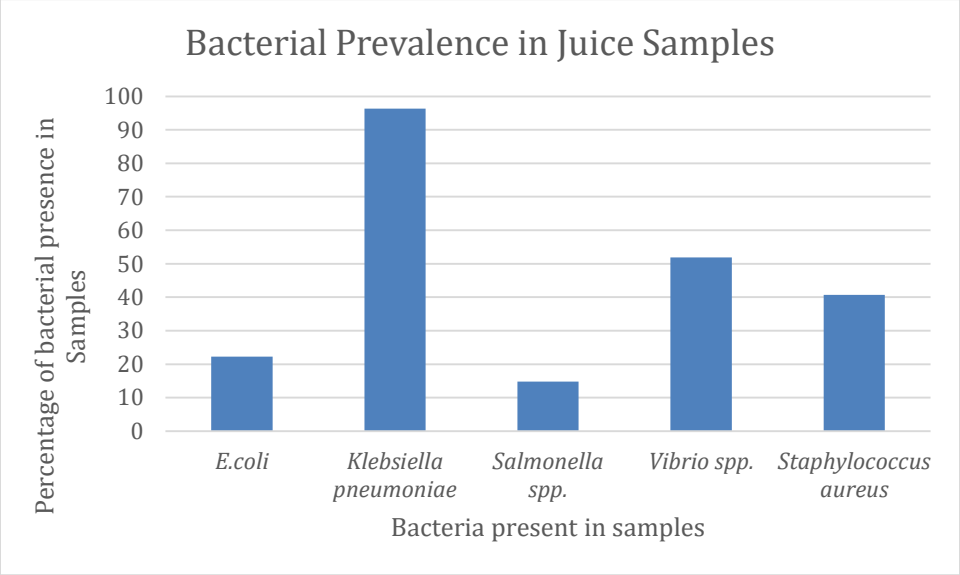

Figure 19: Bacterial Prevalence in Juice Sample

3.6 Location-wise Bacterial Prevalence

Table 7: Number of bacteria in juice sample collected from different location

Sample Location	*Klebsiella pneumoniae*	*Escherichia coli*	*Salmonella* spp.	*Vibrio* spp.	*Staphylococcus aureus*
Badda	2	0	0	2	1
Notun Bazar	3	0	0	3	1
Mohakhali	12	0	0	3	3
Purbachal	13	0	1	6	3
Motijheel	13	2	0	0	2

Baily Road	11	1	1	8	2
khilgaon	8	1	0	2	0
Uttara	7	1	1	0	2
Mirpur	22	5	1	4	0

Bacterial isolates reached their peak count in Mirpur, demonstrating the area's most severe degree of contamination (Figure 20). The hygiene conditions appeared better in Badda and Notun Bazar because these locations showed the lowest number of isolated bacteria.

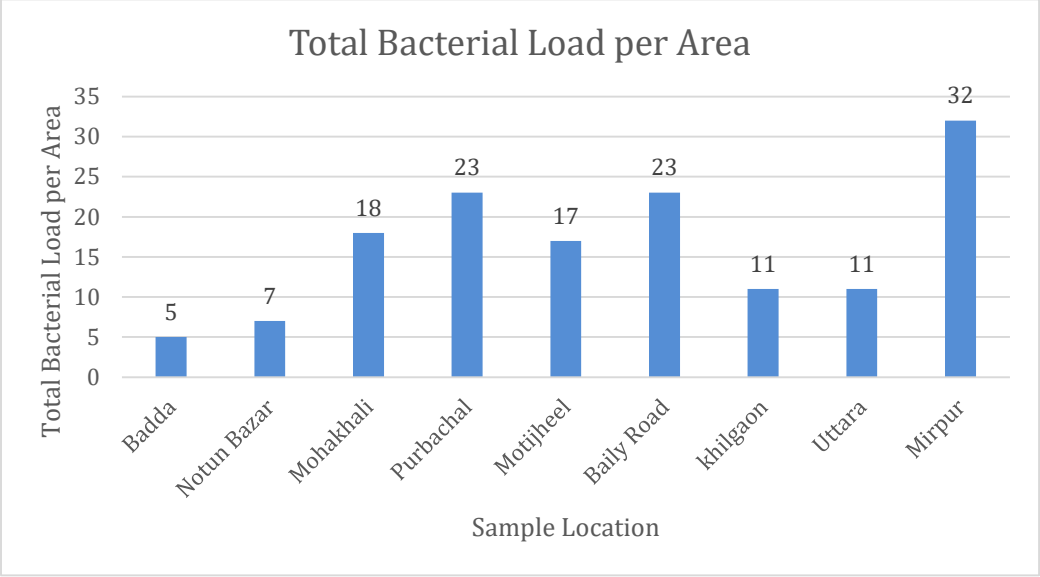

Figure 20: Total bacterial load in sample per area

3.7 Number of isolates found throughout the study from different samples

The studies revealed that mixed fruit juice possessed 21 bacterial isolates, and mango and watermelon juices had the second highest numbers totaling 20 isolates (Figure 21). The contamination levels of orange and olive juices were significant because each had 18 bacterial isolates. A total number of 16 bacterial isolates were found in Pineapple juice and each of lemon

juice and sugarcane juice yielded 12 bacterial isolates. Papaya juice exhibited the least microorganism growth since it had ten bacterial isolates. The analysis reveals mixed and high-sugar juice types tend to experience increased bacterial growth because of more human handling or combined ingredients or incorrect storage practices.

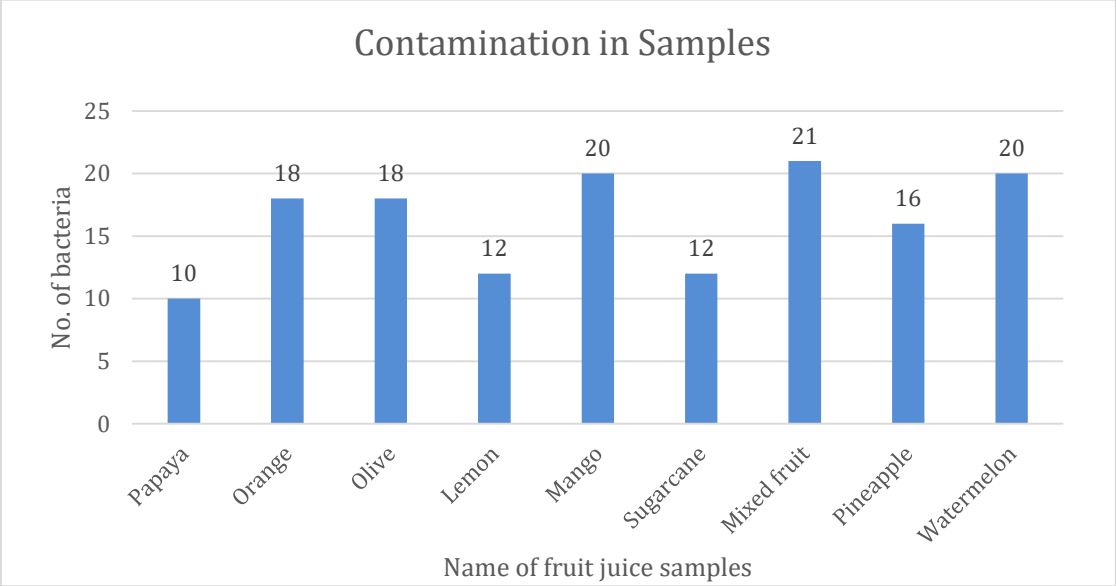

Figure 21: Number of isolates in different samples

3.8 Antibiotic Susceptibility Test Result

The isolation process from the collected samples through selective and differential media yielded a total of 147 presumptive bacterial colonies. *Klebsiella pneumoniae* emerged as the most commonly isolated organism whereby it appeared in 91 isolates from the collected samples throughout the testing process The Kirby-Bauer disk diffusion method was used to test antibiotic sensitivity through Mueller-Hinton agar plates. The results interpretation followed Clinical and Laboratory Standards Institute (CLSI) guidelines to determine Resistant (R), Intermediate (I), or Sensitive (S) categories based on inhibition zone diameters. Results show how different antibiotic treatments perform against identified microorganisms.

3.8.1 Antibiotic Susceptibility Test Result of *Klebsiella pneumonia*

The antibiotic resistance patterns of *Klebsiella pneumoniae* demonstrated high levels of resistance toward both Ceftazidime and Erythromycin, with 88.89% and 69.23%, respectively (Figure 22). The antibacterial response against Cefixime, Amoxicillin and Cefepime substances revealed moderate levels of antibiotic resistance.

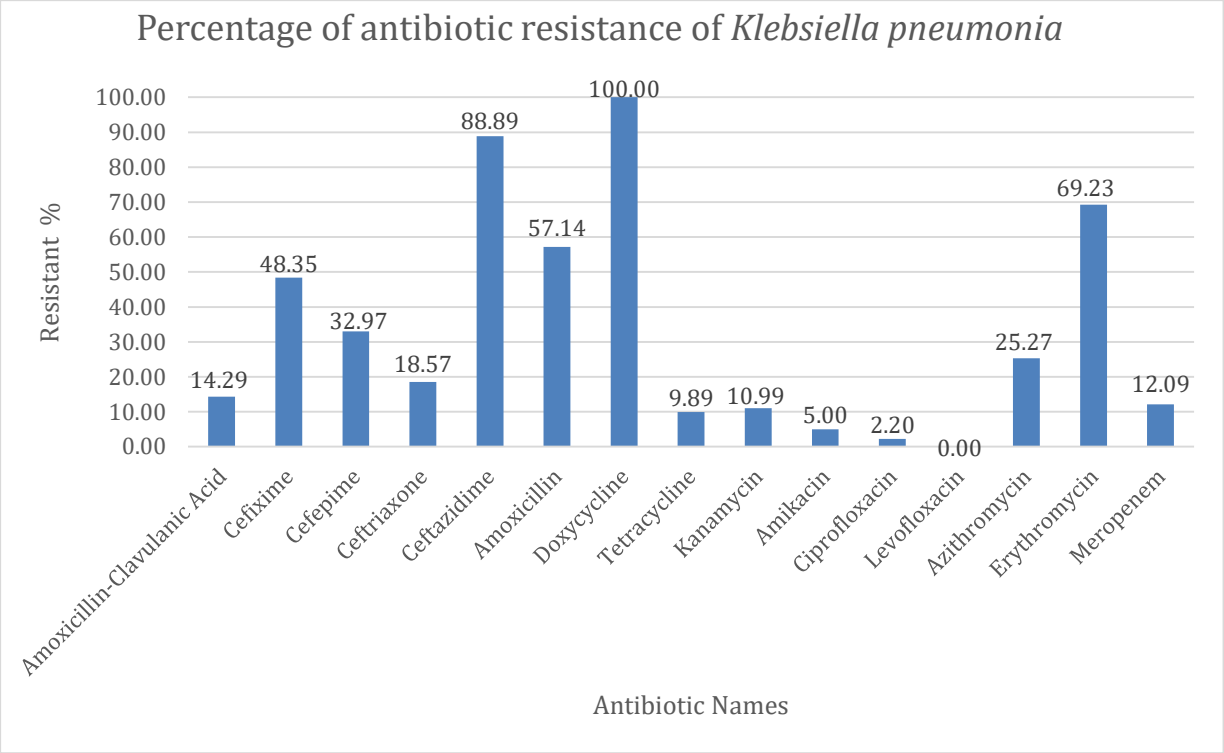

Figure 22: Percentage of antibiotic resistance of *Klebsiella pneumonia*

AST result of *Klebsiella pneumoniae* against different groups of antibiotics

The antibiotic groups fluoroquinolones along with aminoglycoside,s proved optimally effective against *Klebsiella pneumoniae* with susceptibility rates reaching 97.89% and 86.49% respectively. Results with carbapenems revealed 80.22% effective resistance patterns. A higher rate of resistance was identified in macrolides (47.25%) among antibiotic groups with beta-lactams

showing second highest resistance (40.21%). The intermediate susceptibility rate of Tetracyclines reached 62.77%, indicating their variable effectiveness (Figure 23).

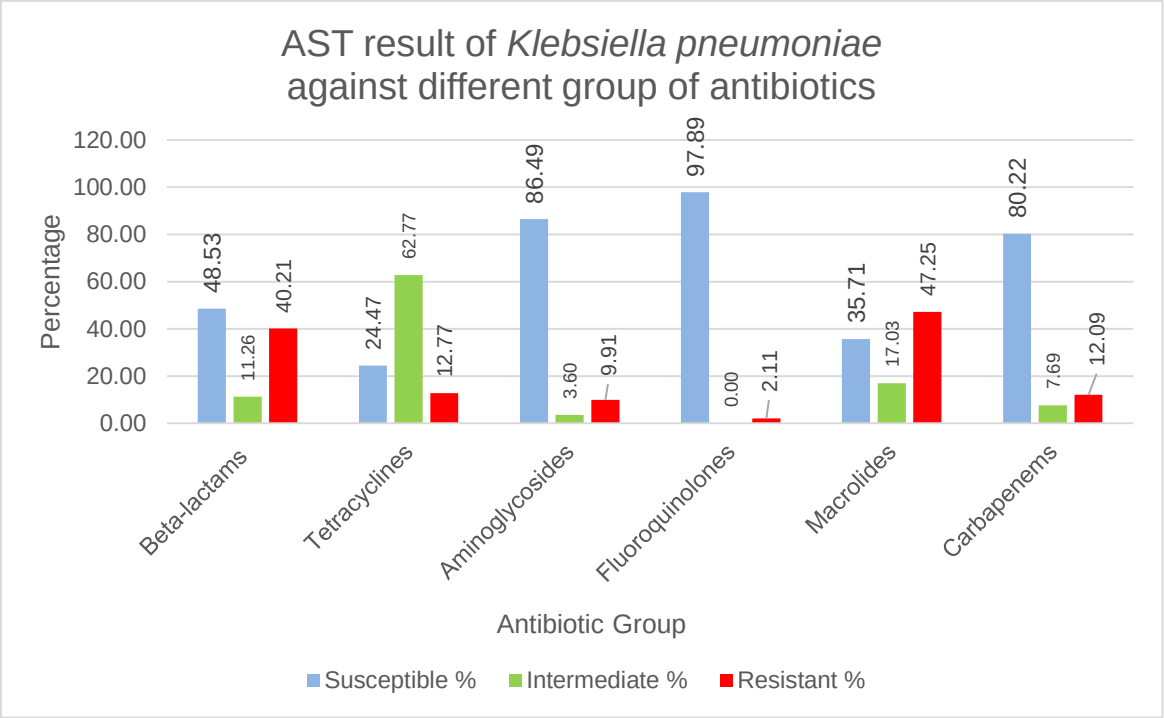

Figure 23: AST result of *Klebsiella pneumoniae* against different group of antibiotics

3.8.2 Antibiotic Susceptibility Test Results of *Vibrio* spp.

The antibiotic resistance test for *Vibrio* spp. showed that all bacteria strains resisted Ampicillin and 96.43% of them were resistant to Ceftazidime which indicates strong antibiotic resistance patterns. A high level of resistance was demonstrated in the case of Doxycycline (85.71%) and Erythromycin (71.43%). (Figure 24) The bacteria demonstrated resistance at a moderate level to Cefuroxime (60.71%) as well as Meropenem (14.29%), yet Ciprofloxacin presented the same resistance rate (14.29%). Amikacin, Norfloxacin and Trimethoprim-Sulfamethoxazole demonstrated no resistance in *Vibrio* strains, thus proving possible as effective treatment options for medical purposes.

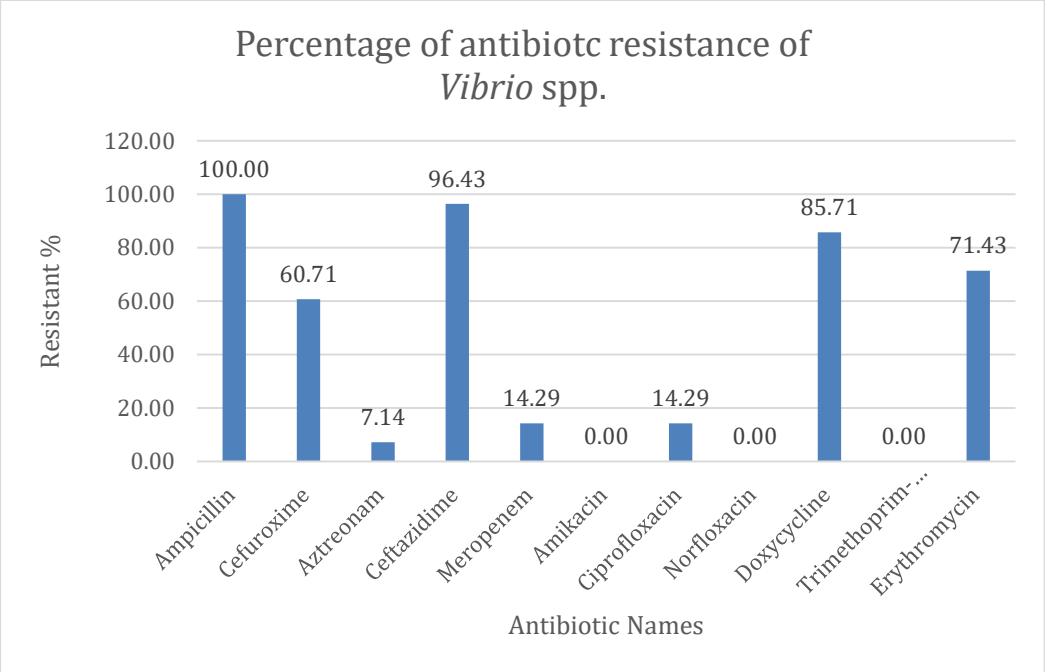

Figure 24: Percentage of antibiotc resistance of *Vibrio* spp.

AST result of *Vibrio* spp. against different groups of antibiotics

The tested antibiotic groups indicated *Vibrio* spp. demonstrated complete susceptibility to aminoglycosides and sulfonamide,s indicating high treatment effectiveness. Fluoroquinolones provided effective results as antibiotics because they had 91.07% susceptibility with 7.14% resistance. Analysis showed tetracyclines exhibited 85.71% resistance while resistance rates of Macrolides reached 71.43% although no susceptible isolates were identified. The susceptibility rate of carbapenems was 75% while the antibiotic resistance ranged at 14.29%. (Figure 25)

The research shows aminoglycosides, along with sulfonamides and fluoroquinolone,s possess the best effectiveness against *Vibrio* infections.

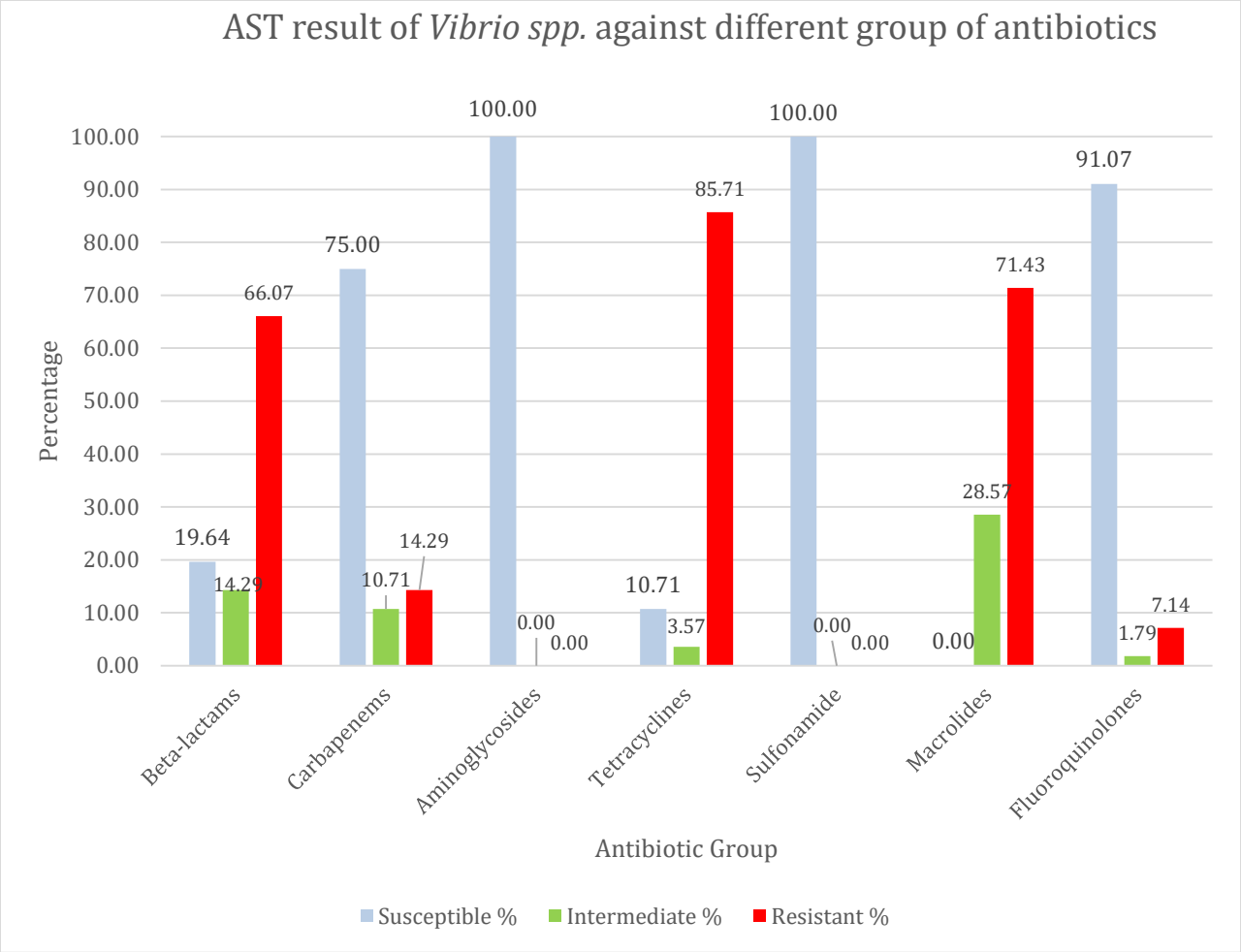

Figure 25: AST result of *Vibrio* spp. against different group of antibiotics

3.8.3 Antibiotic Susceptibility Test Result of *Salmonella* spp.

The resistance rates were 75% for ampicillin and meropenem in this analysis. Ceftazidime along with Chloramphenicol and Tigecycline demonstrated no resistance which indicates their potential use as medical treatments. (Figure 26)

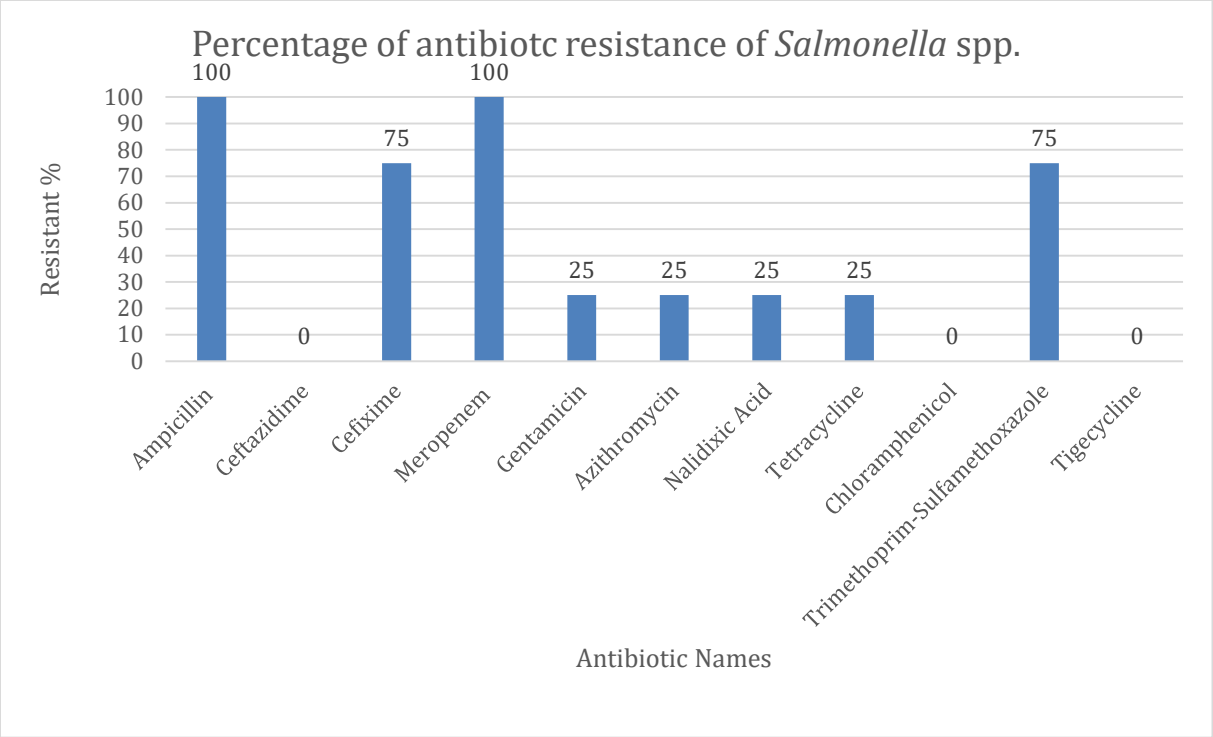

Figure 26: Percentage of antibiotc resistance of *Salmonella* spp.

AST result of *Salmonella* spp. against different groups of antibiotics

The susceptibility tests on *Salmonella* spp. demonstrated diverse results depending on the antibiotics used. Amphenicols demonstrated maximum effectiveness as the drugs tested had 0% resistance along with complete susceptibility but aminoglycosides and tetracyclines trailed behind with 75% and 50% susceptibility rates respectively. *Salmonella* spp. had a 41.67% susceptibility rate toward Beta-lactams alongside a corresponding 58.33% resistance rate (Figure 27).

Salmonella strains exhibited 100% resistance to carbapenems while quinolones remained ineffective with 75% intermediate results against these isolates. The study observed no resistance in glycylcyclines yet all isolates displayed an intermediate reaction pattern.

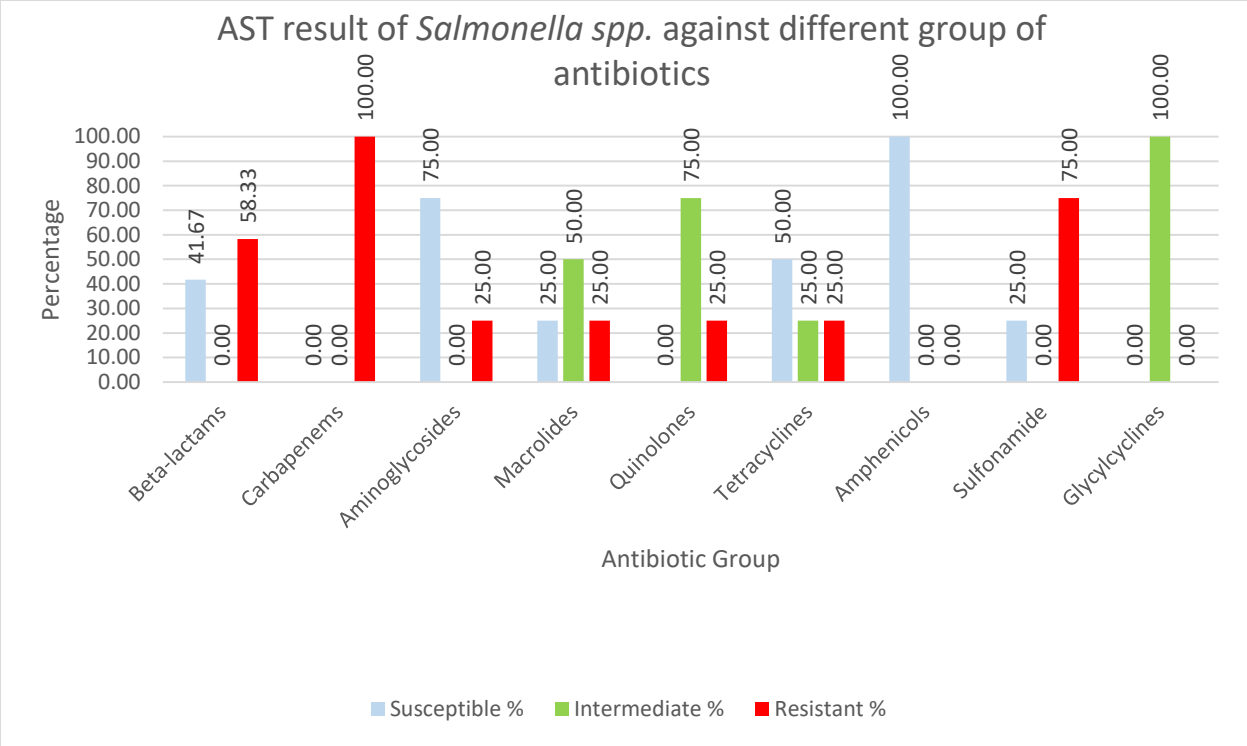

Figure 27: AST result of *Salmonella* spp. against different group of antibiotics

3.8.4 Antibiotic Susceptibility Test Result of *Escherichia coli:*

All *Escherichia coli isolates* demonstrated full resistance against Ampicillin (100%) representing the complete therapeutic failure of this popular antibiotic. The high level of resistance discovered against Colistin (70%) as well. Antibiotic tests demonstrated that Amoxicillin, Cefepime and Meropenem showed resistance at 50% but Ceftriaxone showed a lower resistance of 30%. The effectiveness of Imipenem stood out because it demonstrated resistance rates at 10%. (Figure 28)

The analysis showed no bacterial resistance (0%) to Kanamycin, Amikacin, and Chloramphenicol leading to the conclusion that these antibiotics could effectively treat *Escherichia coli* infections.

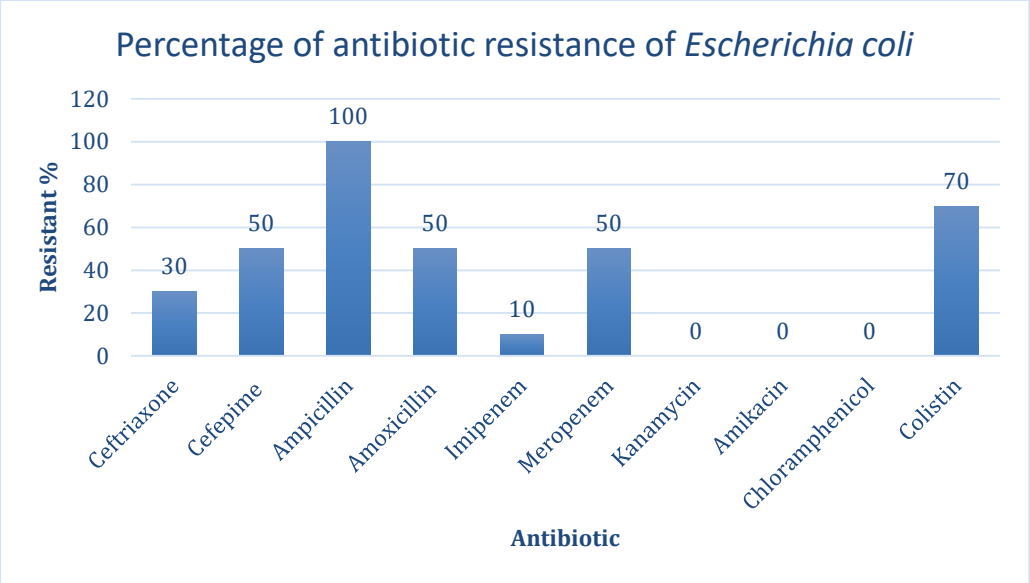

Figure 28: Percentage of antibiotic resistance of *Escherichia coli*

AST result of *Escherichia coli* against different groups of antibiotics

E. coli bacteria demonstrated maximum sensitivity to drugs from the Amphenicols group (100%) and the Aminoglycosides group (95%). The 40% success rate with Carbapenems included 30% cases with drug resistance and 30% cases with intermediate outcomes.

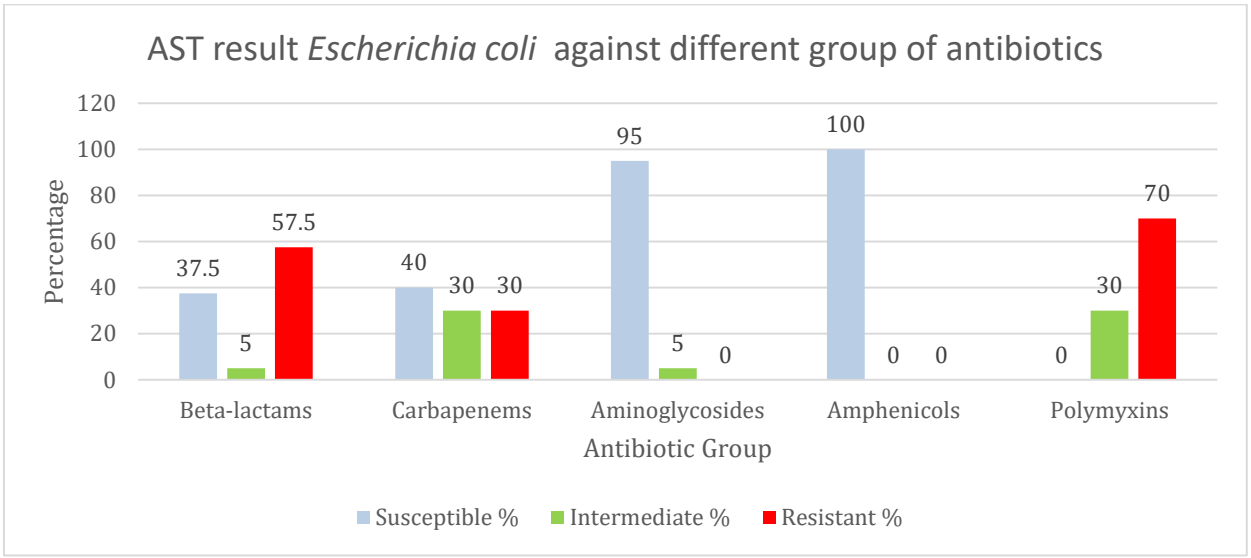

Figure 29: AST result of *Escherichia coli* against different group of antibiotics

3.8.5 Antibiotic Susceptibility Test Result of *Staphylococcus aureus*

Staphylococcus aureus isolates displayed a high level of resistance to Meropenem (85.71%), Vancomycin (71.43%) and Cefixime (100%). These results suggest that these antibiotics have limited effectiveness against the bacteria. Medium-level resistance occurred in the tests of Ceftazidime (78.57%), Tetracycline (21.43%) and Erythromycin (28.57%) and Chloramphenicol (28.57%) (Figure 30). This study established Amikacin as the most effective antibiotic because no isolates showed resistance to it.

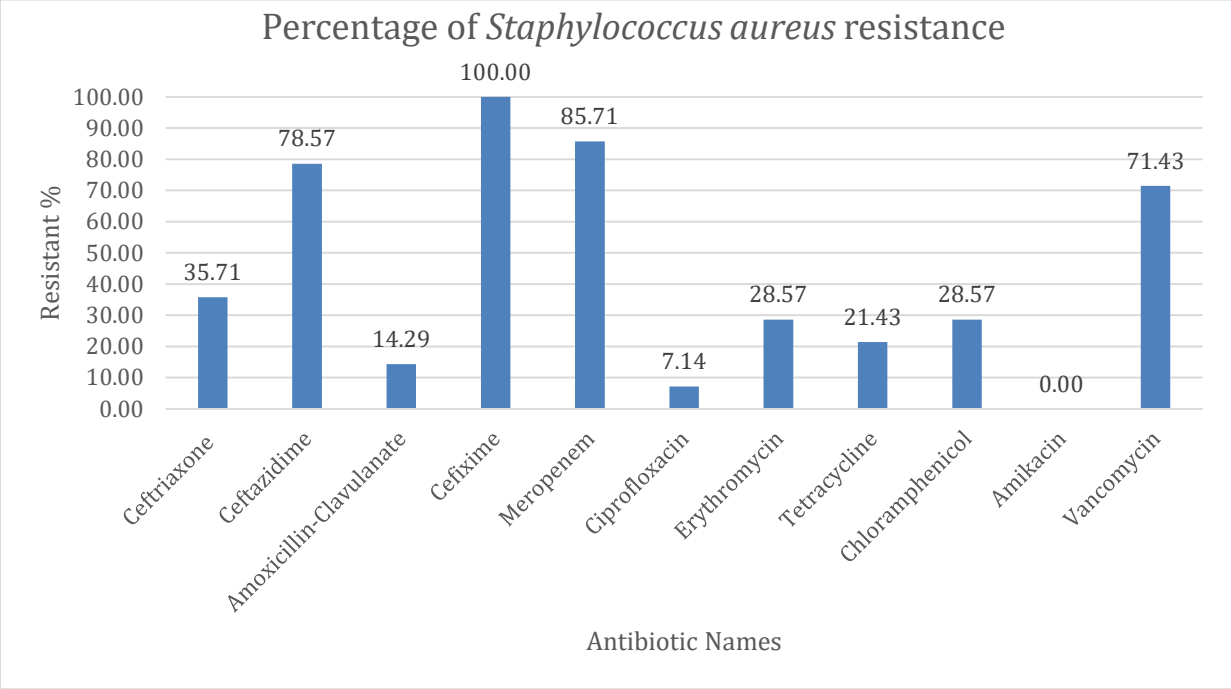

Figure 30: Percentage of *Staphylococcus aureus* resistance

AST result of *Staphylococcus aureus* against different groups of antibiotics

Staphylococcus aureus displayed maximum sensitivity to aminoglycosides because the antibiotic demonstrated 100% susceptibility alongside 0% resistance. The antibiotic group of fluoroquinolones (92.86%,) together with tetracyclines (78.57%) and amphenicols (71.43%) presented strong effectiveness despite their low resistance rates. (Figure 31) The antibiotic resistance data indicate that *Staphylococcus aureus* displays high resistance to carbapenems and glycopeptides alongside beta-lactams which resulted in 85.71% resistance and zero susceptibility.

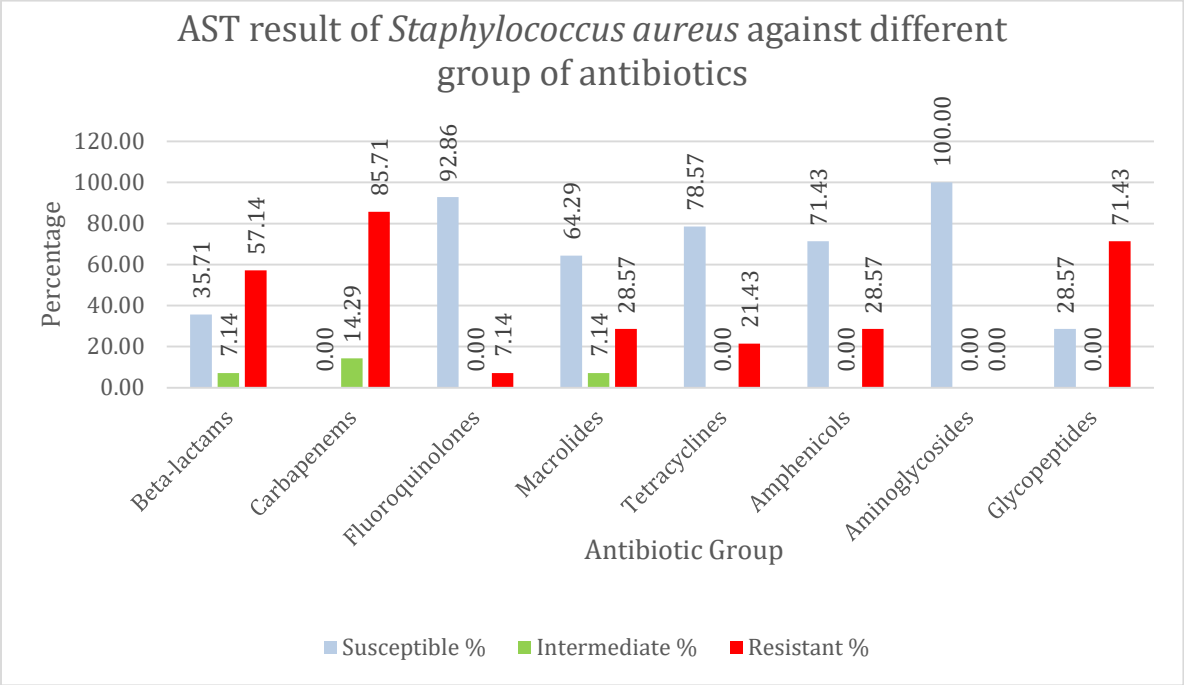

Figure 31: AST result of *Staphylococcus aureus* against different group of antibiotics

3.9 Multi-drug Resistance of the isolates

The study of multidrug resistance (MDR) showed that *Staphylococcus aureus* displayed 78.57% resistance while *Salmonella* spp. had 75.00% resistance and *Vibrio* spp. showed 71.43% resistance. Research results demonstrate an alarming growth in MDR bacterial strain numbers.

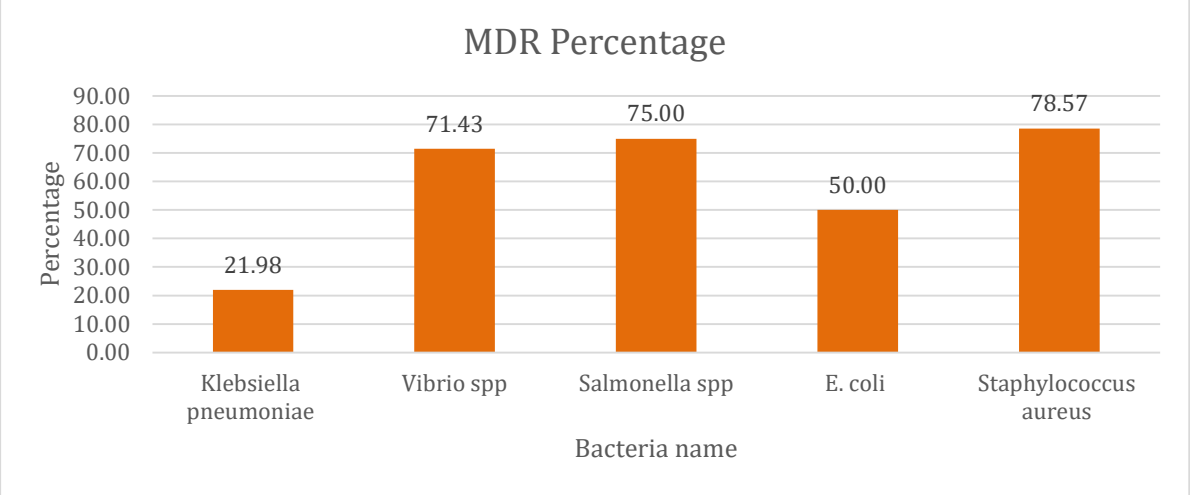

Figure 32: Multi-drug Resistance of the isolates

4. Discussion

This research extensively evaluated the microbiological characteristics of fresh fruit juices distributed across Dhaka city locations by assessing bacteria concentrations and fecal indicators and antibiotic resistance patterns. These popular street beverages exhibit important health threats for public wellness which the results clearly demonstrate. The tested juice samples acquired through the APC analysis showed satisfactory results except for specific samples exceeding the 1×10^4 CFU/mL threshold specified by The Emirates Authority for Standardization and Metrology (ESMA). 12 juice samples exceeded the safe limit by indicating excessive microbial content which created potential health threats. A total of 18 juice samples exceeded the BFSA's 0 CFU/mL maximum standard for FCC in juice samples. The observed deviation indicates possible worsening hygienic practices along with new sources of contamination during the studied period.

The observational survey of nine juice vendors reveals several hygiene and food safety shortcomings. Results from the survey of nine juice vendors displayed numerous unhygienic along with unsafe food handling practices. The survey results showed that 77.78% of vendors performed hand washing with water alone while 66.67% did not possess hand washing facilities. The surveyed vendors only accounted for apron usage by 22.22%, while 55.56% maintained their waste using open bins. Among the practices surveyed 55.56% of vendors stored their juice in refrigerators, whereas 44.44% placed it directly in their squeezing machines. All juicing equipment received daily cleaning but with no more than one daily cleaning cycle (77.78%) and none of the vendors underwent food safety training. The use of unhygienic methods probably led to microbial contamination detected in the juice laboratory analysis.

The antibiotic susceptibility testing of isolated bacteria showed various resistance patterns that were cause for concern. Results confirmed high resistance of *Klebsiella pneumoniae* against Ceftazidime which indicates that this becomes less effective in fighting infections. The resistance patterns of these bacteria represent a fundamental distinction between the previous research results and the present study findings (Uddin et al., 2017). The current study demonstrated 100% resistance against ampicillin in *Escherichia coli* which contradicts previous research that reported only 90% resistance. This indicates complete antibiotic resistance to this commonly used antibiotic. The resistance patterns of *Salmonella* spp. in this study displayed 100% ampicillin resistance when compared with previous reports of 60% and 65% resistance rates. *Vibrio* spp. showed total resistance to Ampicillin during resistance analysis thus rendering this widely administered antibiotic ineffective for treating these strains. Such resistant pathogens create severe therapeutic challenges because they spread in regions with unregulated widespread antibiotic use. This research included a wider selection of antibiotics drawn from distinct pharmacological classes (beta-lactams, fluoroquinolones, aminoglycosides and amphenicols) that previous studies had failed to incorporate.

Study results showing *Klebsiella pneumoniae* presence in almost all tested fruit juice samples should be considered seriously because they match previous findings from Uddin et al. (2017) who discovered similar results in Dhaka street-vended juice samples.

5. Conclusion

The findings of this study present serious public health concerns. This research evaluated both microbiological purity and resistance patterns of bacteria that were isolated from newly produced fruit juices across different parts of Dhaka city. Bacterial contamination of fresh juice samples through *Klebsiella pneumoniae*, *Vibrio* spp., *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus* poses an entirely significant health risk because of antibiotic resistance and unhygienic handling methods. Multidrug-resistant (MDR) bacterial strains pose a substantial treatment challenge since they were identified in both *Staphylococcus aureus* (78.57%) and *Salmonella* spp. (75%). The combination of poor hand hygiene methods along with insufficient food safety training and unfiltered water sourcing and poor storage facilities, develops risks that lead to various food-caused illnesses that affect digestion and spread throughout vulnerable patient groups like children and elderly individuals.

To conclude, the ingestion of contaminated juices will trigger resistant infection outbreaks and drive antimicrobial resistance dissemination through the community while generating healthcare system costs. The quality of fruit juice requires regular supervision to ensure its improvement. Frequent monitoring and supervision should be maintained to stop the consumption of contaminated fruit juice products that create harmful food illnesses.

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