IDENTIFICATION OF MULTI DRUG RESISTANT BACTERIA FROM LOCAL FRESH JUICE AND INDUSTRIALLY PROCESSED JUICE

BY

RIFAH TASNIA ABANTI
17326011

FARHANA JAHAN
17326013

MALIHA NAHAR
18126052

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DEPARTMENT OF MATHEMATICS AND NATURAL SCIENCES

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Declaration

It is hereby declared that

1. The thesis submitted is my/our original work while completing my 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 complete 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. I/We have acknowledged all primary sources of help.

Student’s Full Name & Signature:

Rifah Tasnia Abanti
17326011

Farhana Jahan
17326013

Maliha Nahar
18126052

Approval

The thesis/project titled "Identification of multidrug-resistant zoonotic bacteria from local fresh juice and industrially processed juice" submitted by

1. Rifah Tasnia Abanti (ID- 17326011)
2. Farhana Jahan (ID- 17326013)
3. Maliha Nahar (ID- 1826052)

of [Fall], [2022] has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Microbiology on 24th November 2022.

Examining Committee:

Supervisor:
(Member)

Fahim Kabir Monjurul Haque, Ph.D.
Assistant Professor, Mathematics & Natural Science
BRAC University

Co-Supervisor:
(Member)

Akash Ahmed
Lecturer, Mathematics & Natural Science
BRAC University

Program Coordinator:
(Member)

Nadia Sultana Deen, Ph.D.
Associate Professor, Mathematics & Natural Science
BRAC University

Departmental Head:
(Chair)

Professor A F M Yusuf Haider
Professor and Chairperson, Mathematics & Natural Science
BRAC University

Abstract

Fruit juices are widely consumed in many tropical nations and are known for their high nutritional value and mineral and vitamin content. In most cultures, fresh foods like fruits and vegetables are a staple of the average person's diet and are consumed in considerable quantities. The study aimed to detect the presence of pathogens in the fresh and packaged juices that the mass people in Bangladesh are consuming. The presence of *Escherichia coli, Salmonella, Shigella, Klebsiella Pneumoniae* selected from the family 'Enterobacteriaceae' and *Listeria monocytogenes, Pseudomonas*, was the main focus of the study. Among the collected samples, 4 were industrially packaged juices, and four were locally made fresh juices collected from Dhaka city. The samples were collected in sterile test tubes and subjected to total viable count (presence of the targeted organisms) by spread plate method. Later, an antibiogram test was done against ten commonly used antibiotics to determine the susceptibility of the targeted organisms. There were 129 isolates collected from these samples. Among 129 isolated colonies, some notable ones were *Yersinia*(28.0%), *E.coli* (8.0%), *Enterobacter* (6.0%), *Salmonella spp* (6.0%), *Shigella*(2.0%), *Vibrio* (4.0%). Among 50 selected antibiotic-resistant bacteria, 30 MDR were from the local fresh juices, and the rest 20 were collected from the packaged.

This study shows that there are significant medical implications when microorganisms develop resistance to widely used antibiotics and medications. Our results confirmed the presence of pathogens in the juices, significantly deteriorating public health.

Keywords: Zoonotic bacteria; pH; antibiotic-resistant; multidrug-resistant; juice; bacteriological load.

Dedication

"Dedicated to our families and friends who supported and believed in us. And to the better world"

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Abbreviations

Abbreviation	Elaboration
pH	potential of hydrogen
FDA	Food and Drug Administration
AMR	Antimicrobial resistance
MDR	Multidrug resistance
CLSI	Clinical and Laboratory Standards Institute
Et al	And others
mL	Milliliter
XLD	Xylose Lysine Deoxycholate
CFU	Colony forming unit
GHP	Good hygiene practice
ABIS	Automated Biometric Identification System

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CHAPTER 1

1. Introduction

Bangladesh is a riverine country with a tropical climate with high temperatures and high humidity; fruit juice is prevalent throughout the year. In this modern era, packaged fruit juices are famous worldwide. Besides packaged juice, people prefer to have local fruit juices as well. Fruit juice is becoming a part of the modern diet in many communities. They are nutritious drinks and can play an essential part in a healthy diet because they have a fresh taste and have many nutrients naturally found in fruit. They contain many vitamins, minerals (calcium, phosphorus, sodium), and some bioactive compounds (glycoside flavanone, hydroxycinnamic acid, an antioxidant) that help improve human health status, maintain blood lipid profile in patients with hypercholesterolemia, inhibit breast cancer, and urinary tract infection, congestive heart failed (Tasnim et al., 2010; Tambekar et al., 2009, Saenz C and Sepulveda E. 2001, Hyson DA. 2011, FDA. 1999, Basar MA and Rahman SR. 2007). However, fruit juices contain various nutrients, including sugar which can be an excellent source of bacterial nutrients.

On the other hand, they usually have a lower pH (pH < 4.5), so the common feature of potential spoilage agents is that they must be acidophilic. That is why juices are highly prone to contamination. In the process of local fresh juice production, bacterial contamination may occur due to an unsanitary environment, food flies, airborne dust, and long-term storage without proper pasteurization and cooling (Salomão, 2018; Tasnim et al., 2010; Sahu et al., 2014), and polluted water, especially for domestically produced products. Ready-to-drink and packaged fruit juices, factors that can contribute to microbial contamination of fruit can come from several factors, including farm fruit contamination, packaging, transportation, and handling (FDA, 2001). contamination caused by bacteria such as *Escherichia coli* and *Salmonella*, *Staphylococcus* *aureus*, *Enterobacter spp, Klebsiella*, and *Serratia* species which can cause diseases like cause typhoid fever, food poisoning, gastroenteritis, enteric fever, and diarrheal diseases (Mengistu et al., 2021). Fruit juice is consumed directly without further purification. Therefore, the presence of pathogenic, exceptionally multi-drug-resistant bacteria in fruit juice is unacceptable.

Currently, antimicrobial resistance (AMR) is one of the biggest threats to the health of both humans and animals, which is also concerning for public health (James & Panchal, 2021). The Enormous use and misuse of antibiotics and improper hygiene in food production have increased resistant zoonotic bacteria that are commonly transmitted through food. Antibiotic-resistant bacteria spread from animals to humans, a significant public health concern (Bengtsson & Greko,2014). Conventional antibiotics are becoming ineffective due to resistance which is becoming life-threatening for future generations. The insistence on this fact corresponds to diseases that lead to high mortality rates yearly due to factors such as improper antibiotic use by the population, nonessential use in agriculture, and lack of basic hygiene and sanitation care. Studies in several regions of the world have reported the presence of Gram-negative bacteria belonging to the family Enterobacteriaceae produces extended-spectrum beta-lactamases, resistant to many antibiotics, beta-lactams, and non-beta-lactams in various foods, including vegetables and fruit. The rate of drug-resistant bacteria in food is one global phenomenon. It is a significant threat to public health (Rahman Khan et al, 2001) because these organizations have been isolated from a wide range of foods men consume. The relevance of the information obtained on the resistance of bacteria to antibiotics is highly the extent of the problem and establishes baselines for action (Caprioli et al., 2000). An alarming consequence of antibiotic resistance related to the scarcity of new antibiotics is an increase in the frequency of multi-resistant animals (MDRs) that can lead to impaired response to antimicrobial therapy birth or even failure of treatment.

According to Mosupye and Holy (2000), Various reports of illnesses brought on by food-borne illnesses linked to consuming fruit juices at various locations are reported worldwide. For instance, in Ghana, they conducted a study to Identify the microbiological load in commercially and locally produced fruit juice and to ascertain whether any residues of antimicrobials in this fruit juice (Jimma et al, 2022). Also, India, Pakistan, Ethiopia, and Nigeria thoroughly researched this topic. In Bangladesh, some research on identifying pathogenic organisms in juice is seen. For example, Stamford University in Dhaka did a study to assess the risk to the public's health, identify the presence of pathogenic bacteria in freshly pressed fruit juices, and their propensity for drug resistance (Lucky et al., 2017). The packaged fruit juice manufacturing in industries where quality control is the most critical factor in controlling the contamination of zoonotic bacteria. Processing raw materials and produced end products need critical care to maintain a safe level for human consumption. On the other hand, fruit juice from local vendors needs the most hygiene management. The water used in making juice, the equipment used to prepare juice, and the fruits need proper hygiene management as contamination can occur during any of these steps.Nevertheless, a considerable hygiene management gap is present in our country.

This study aims to demonstrate the bacteriological load in locally and industrially processed fruit juice and evaluate the occurrence of multi-drug resistant bacteria.

1.1.Objective of this study

The main objective is to analyze the bacteriological load in locally and industrially processed fruit juice and evaluate the occurrence of multi-drug resistant bacteria around Dhaka city. The specific objective of this study is;

· To assess zoonotic bacterial contamination from freshly prepared juice and industrially processed juice.

· To analyze antibiotic resistance patterns of isolated microorganisms.

CHAPTER 2

2. Material and methods:

2.1. Collection of fruit juice samples:

Fresh local juices and industrially produced juices from Bangladeshi brands were collected from different areas. Along with these, imported juice from Cyprus was collected for the project. The samples were collected in sterile test tubes to avoid contamination while handling. These samples were examined in the microbiology laboratory at BRAC University.

Table 2.1. List of collected samples

Industrially packed juice:	● PRAN Frooto ● Shezan Mango juice ● Frutika Grape Juice ● Fontana Juice (imported from Cyprus)
Local fresh fruit juice:	● Lemon juice ● Mango juice ● Papaya juice ● Mixed fruit juice

2.2. pH measurement:

The pH of the samples was measured after collecting them. 5 ml of samples were used for the pH measurement following the instructions in the handbook.

2.3. Bacteria culture and identification:

For enrichment, industrially produced juices were incubated for 2 hours in the peptone water. (1000 µL of the samples in 5 ml of peptone water). 0.1ml of each dilution was spread on different selective media and incubated for 24h at 37°C. (Geta K. et al, 2019) After incubation, specific bacteria were isolated from the spread plate and streaked separately for pure colonies. (James & Panchal, 2021) The primary identification was made based on colony morphology and gram staining. The different selective media used and the colony morphology is presented in

Table 2.2. The procedure was done following the described methods in the Journal of basic and applied Research. (Fawole and Oso,1988, Okereke and Kanu,2004, Holt et al, 1994)

Table 2.2: Colony Morphology of Specific Bacteria on Selective Media

Organism	**Gram Positive/Negative**	**Media**	**Expected colony morphology**
Escherichia coli	Gram-negative	MacConkey agar	Pink to dark pink, dry and donut-shaped
Serratia	Gram-negative	MacConkey agar	Dark red colonies
Shigella spp.	Gram-negative	XLD agar	Red colonies
Salmonella spp.	Gram-negative	XLD agar	Colorless colonies with a black center
Vibrio cholerae	Gram-negative	TCBS agar	Green colonies
Proteus spp.	Gram-negative	XLD agar	Colonies with black center
Yersinia	Gram-negative	MacConkey agar	Colorless colonies

Enteric group	Gram-negative	MacConkey agar	Pink-red colonies
Citrobacter	Gram-negative	XLD agar	Yellow colonies
Leminorella	Gram-negative	MacConkey agar	Colorless colonies
Moellerella	Gram-negative	MacConkey agar	Bright red colonies
Xenorhabdus	Gram-negative	MacConkey agar	Light pink centered colonies

2.4 Biochemical Test:

Biochemical tests include Catalase, Oxidase, IMViC (indole, methyl red, Voges- Proskauer, citrate), Urease and motility, and gram staining was performed to identify the selected bacteria, and later antibiotic susceptibility test was done.

Table 2.3: Biochemical Test Interpretation for Different Organisms

Organism	Motility	Indole	Urease	Catalase	Oxidase	MR	VP	Citrate utilization	Gram staining
Yersinia	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve

Shigella dysenteriae	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
Salmonella spp.	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve
Enterobacter	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
Vibrio cholerae	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve
Escherichia coli	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve
Proteus spp.	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Citrobacter	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve
Enteric group	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve
Serratia	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve
Leminorella	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
Moellerella	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Xenorhabdus	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve

2.5 Antibiotic susceptibility test:

Commercial discs of antibiotics were used in 10mg (Oxoid) to check the antimicrobial susceptibility. The disc diffusion method was followed to perform this test. (Bauer et al. 1966) enlisted antibiotics were selected based on consumer demand. The bacterial suspension was prepared by adding bacterial colonies into 9 ml of saline solution. The turbidity of the suspension was adjusted, comparing it to the 0.5 McFarland standard. The suspension was lawned, and discs were placed in the Mueller-Hinton agar media. The Petri dishes were kept in incubation for 24 hours at 37°C. After incubation, the diameter was measured following the standard inhibitory zone diameter chart. (CLSI, 2007). The zones were measured precisely using calibrated rulers and classified into susceptible, intermediate, and resistant accordingly. (Tadesse,2018)

Table 2.4: List of Antibiotics used in the Experiment

Serial no	Antibiotic	Group	Effective against	Disc code	Disc potency (µg)	Interpretative Criteria		
						Sensitive mm or more	Intermediate mm	Resistant mm or less
1	Gentamicin	Aminoglycoside	Gram-positive and Gram-negative	GEN	10	15	13-14	12
2	Amoxicillin	Beta-lactamase	Gram-positive and Gram-negative	AMX	10	17	14–16	13
3	Meropenem	Carbapenem	Gram-positive and Gram-negative	MRP	10	23	20–22	19
4	Cefepime	Cephalosporin	Gram-positive and Gram-negative	FEP	30	25	19–24	18
5	Piperacillin tazobactam	Penicillin and beta-lactamase inhibitor	Gram-positive and Gram-negative	PIT	100/10	21	18–20	17
6	Imipenem	Carbapenem	Gram-positive and Gram-negative	IMI	10	23	20–22	19
7	Erythromycin	Macrolide	Gram-positive and Gram-negative	E	15	18	14–17	13

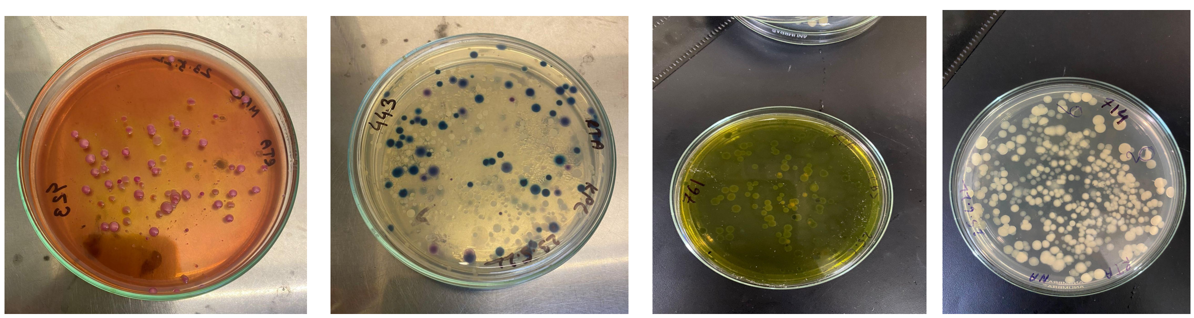
8	Nalidixic Acid	Quinolones and fluoroquinolones	Gram-positive and Gram-negative	NA	30	19	14–18	13
9	Kanamycin	Aminoglycoside	Gram-positive and Gram-negative	K	30	13	14–17	18
10	Colistin	Polymyxin E	Gram-negative	CT	10	-	11-17	-

CHAPTER 3

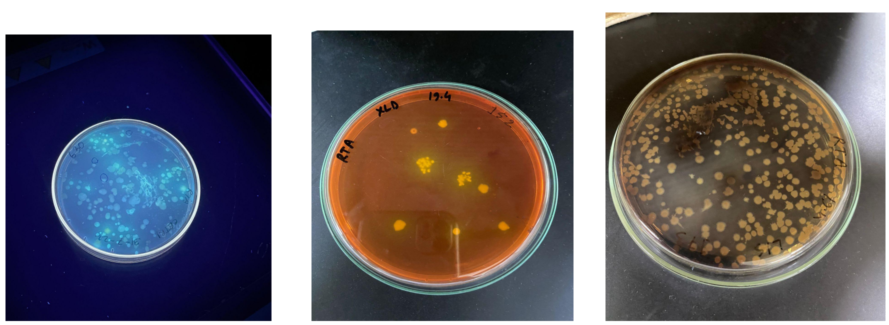
3. Result

3.1. Isolated bacteria from local vendor juice and industrially processed juice.

A total of 8 samples were collected and analyzed. Four of these samples were industrially processed juice, and the other half were local vendor juice. There were 130 isolated colonies obtained. These isolated colonies were obtained randomly depending on the morphological characteristics from 6 selective media and nutrient agar. The six selective media are HiCrome KPC, XLD, MacConkey, Cetrimide, TCBS, and Listeria. The samples were separately cultured in these seven media and incubated in the inverted position for 24 hours at 37℃ by the spread plate method.



A.MacConkey Agar B. Hicrome KPC Agar C. TCBS Agar D. Nutrient Agar



E. Cetrimide Agar F. XLD Agar G. Listeria Oxford Agar

Figure 1. growth of particular bacterial colonies on various selective medium

Figure 1 shows the growth of distinct bacterial colonies on different selective media, which were grown after incubation of 37-degree C.

On TCBS agar, yellow and green colonies were primarily identified as *Vibrio species*. The pink colony was identified as *Escherichia coli, Klebsiella spp* on MacConkey agar. On XLD agar, black colonies were identified as *Salmonella spp*, and red colonies were *Shigella.*

3.2.Bacteriological load of local vendor fruit juices and industrially processed juices

The number of bacteria in the fruit juice samples was calculated based on the number of bacteria per plate, and the serial dilutions were 10^{-1} to 10^{-4} followed by the CFU value. The highest load was found from the local vendor of mango juice. Industrially processed juices have a lower bacterial load than local fresh juice.

The colony count, most of them, was too numerous to count. In local fresh juice, there were more colonies than in industrially processed juice. There were more bacterial colonies in dilution 1 and 2 than dilution 3 and 4. The more juices were diluted the colonies will be less in the agar.

3.3 pH measure

Due to the presence of organic acids, the pH of fruit juices typically ranges from 2.0 to 4.5 and varies depending on the kind of juice (Mettler-Toledo International Inc., all rights reserved, 2022). pH changes may also enable pathogen growth (FDA, 2001). The pH level was measured for the eight local fresh juice and industrially processed juice samples. The pH value of different juices ranges from 1.9 to 6. Industrially processed juice has less pH value than local vendor juice because of its preservative presence.

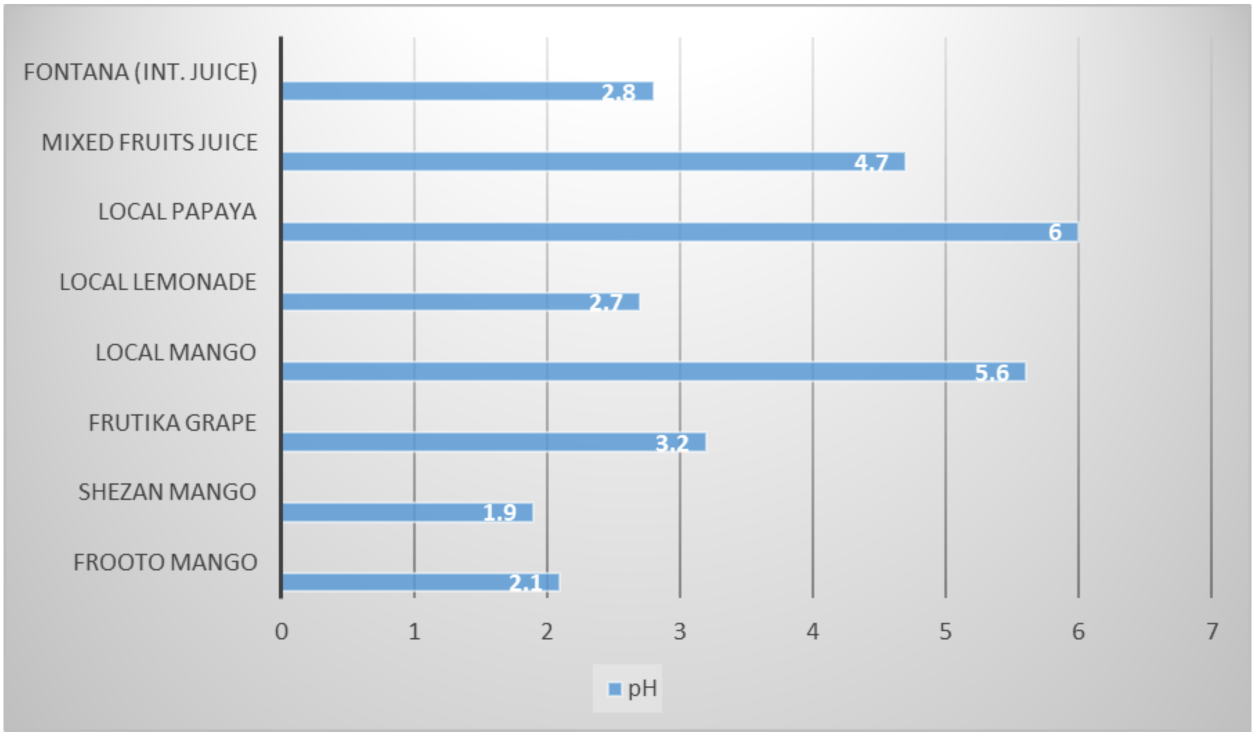


Figure.2: pH of local fresh juice and industrially processed juice.

3.4.Identified bacteria

Different biochemical tests were performed to identify Enterobacteriaceae from these seven media. Various biochemical tests were used for the identified microorganisms, including; the indole test, methyl red test, urease test, motility test, triple sugar iron test, Voges Proskauer test, citrate utilization test, catalase test, oxidase test, and the Gram stain technique.

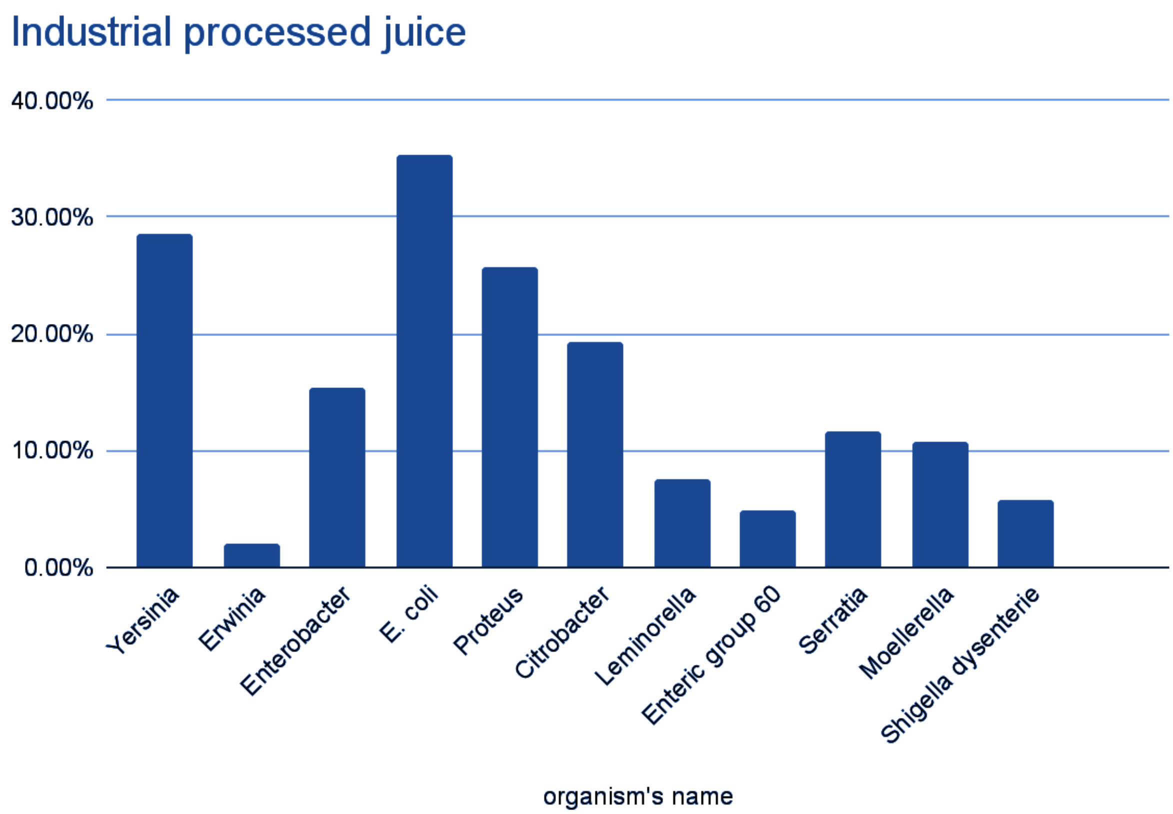


Figure 3: Organisms found in industrially processed juice.

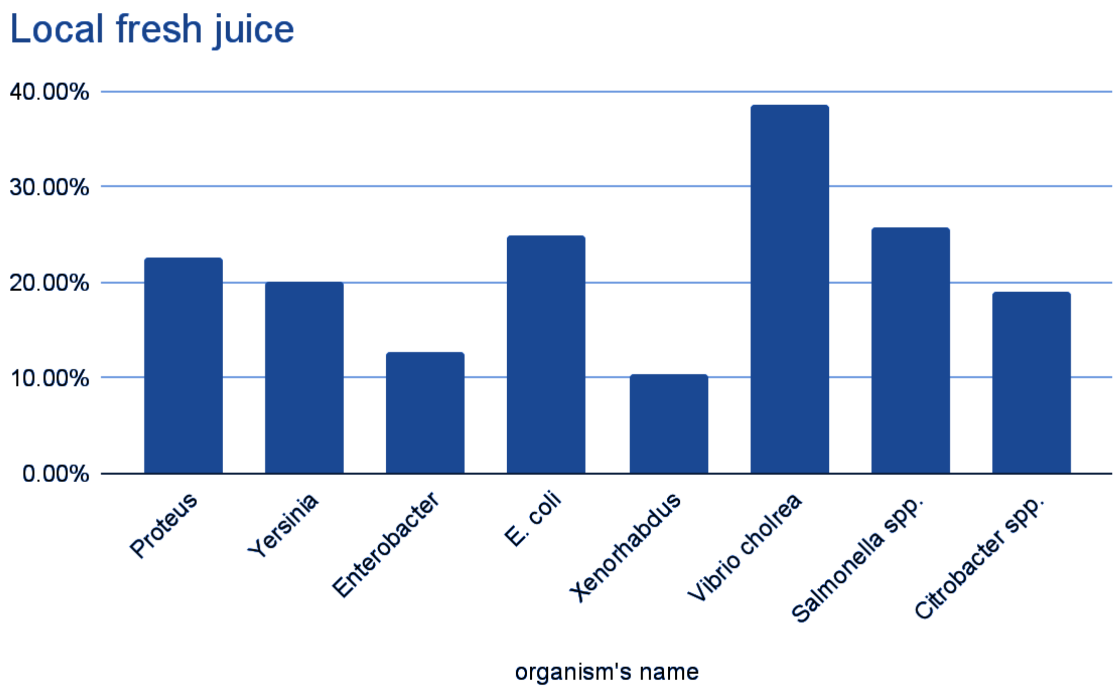


Figure 4: Organisms found in local fresh juice

Fifty isolated colonies were identified through biochemical tests. There are more isolated organisms in local fresh juice than in industrially processed juice. The most organisms in both types of juice are *Yersinia*, *E.coli,* and *Proteus*. After collecting the test results, the ABIS website was used to compare the results for identification. Among various bacteria identified, some notable ones had been, *Yersinia*(28.0%), *E.coli* (8.0%), *Enterobacter* (6.0%), *Salmonella spp* (6.0%), *Shigella*(2.0%), *Vibrio* (4.0%).

3.5.Antibiotic resistance pattern of isolates

In this study, 129 isolates have been precisely selected to identify the antibiotic resistance of different organisms. As previously mentioned, isolates were analyzed against ten standard antibiotics, commonly used by disc diffusion assay on Mueller-Hinton Agar with the antibiotic disc (Bauer et al., 1966; Acharjee et al., 2012). The CLSI guideline was followed to compare the results that were acquired.

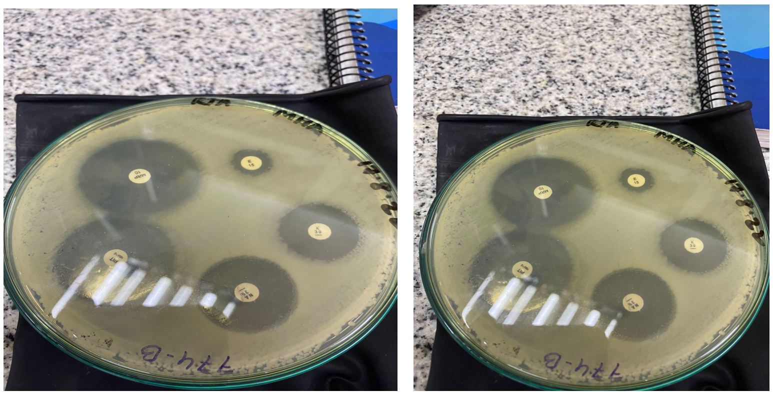


Figure 5. The disk Diffusion method was performed to check antimicrobial susceptibility patterns in selected isolates collected from local fresh juice and packaged juice.

Most isolates were resistant to Amoxicillin, Erythromycin, Imipenem, Nalidixic Acid, Kanamycin, Colistin, Cefepime, and Gentamicin. Piperacillin and Meropenem, both of them, were susceptible.

Antibiotic-resistant microorganisms identified from food and other sources have occasionally become more widespread (Vicas,2010). Erythromycin and Amoxicillin were discovered not to be effective against all microbial isolates.

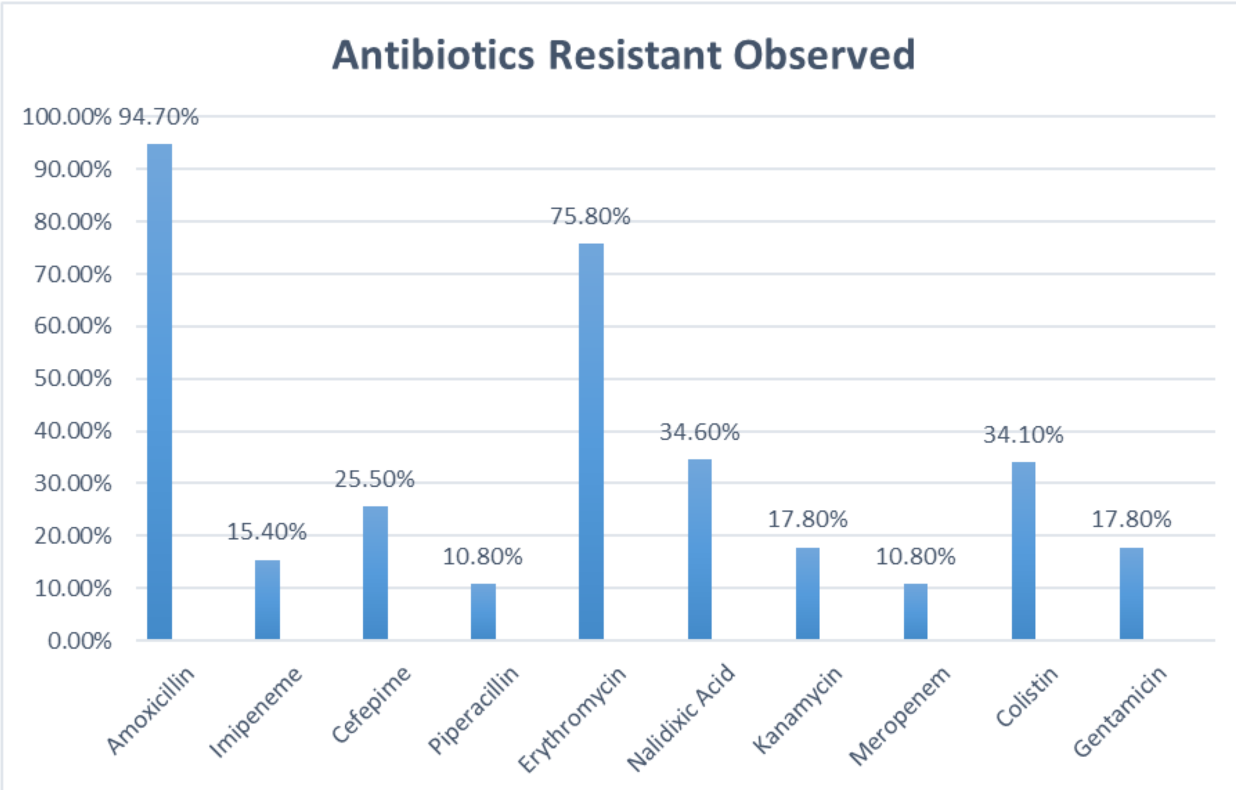


Figure 6: Percentage of resistance observed in all the isolates.

The broad-spectrum antibiotics with the highest resistance levels were Imipenem, Erythromycin, Cefepime, and Kanamycin. The highest resistance level for narrow-spectrum antibiotics was against Amoxicillin, Nalidixic Acid, and Colistin. Some isolates were resistant to all ten of the antibiotic drugs. From this study, there were lots of microbial isolates which were multidrug resistant. The result of the antibiotic susceptibility test was interpreted and presented as the resistance of microbial isolates to the antibiotic and the antibiotic resistance pattern through them. However, a thorough overview of the research was undertaken in this study, which is why the mechanisms of resistance in a few strains of all the bacteria isolated from juices were investigated, which is significant given the global surveillance of antibacterial resistance requirement to recognize and comprehend the scope of bacterial antibiotic resistance (Singleton, 1997) and to build baselines for appropriate response (Caprioli et al.,2000)

Table 3.2: Resistance percentage of antibiotics in different local fresh and industrially processed juice

Sample name	Imipenem	Cefepime	Gentamicin	Amoxicillin	Piperacillin	Erythromycin	Nalidixic Acid	Kanamycin	Meropenem	Colistin
Frooto mango	11%	43.10%	12%	92.20%	1.50%	60.50%	45.58%	36.45%	13.80%	37.86%
Shezan mango	25.73%	22.18%	22.18%	67.86%	1.34%	76.45%	45.73%	18.43%	0	62.79%
Fruitika grape	0	12.45%	0	84.83%	0	60.39%	0	0	0	51.24%
Fontana	75%	65%	65%	100%	65%	75%	100%	75%	75%	75%
Local mango	10%	0	25%	100%	0	70%	5%	0	5%	10%
Local lemonade	58.33%	58.33%	66.67%	100%	66.67%	91.67%	83.33%	58.33%	66.67%	75%
Local papaya	8.33%	8.33%	5%	100%	0	70.83%	54.17%	12.5%	8.33%	5%
Mixed fruit	0	0	0	80%	0	100%	5%	0	0	0

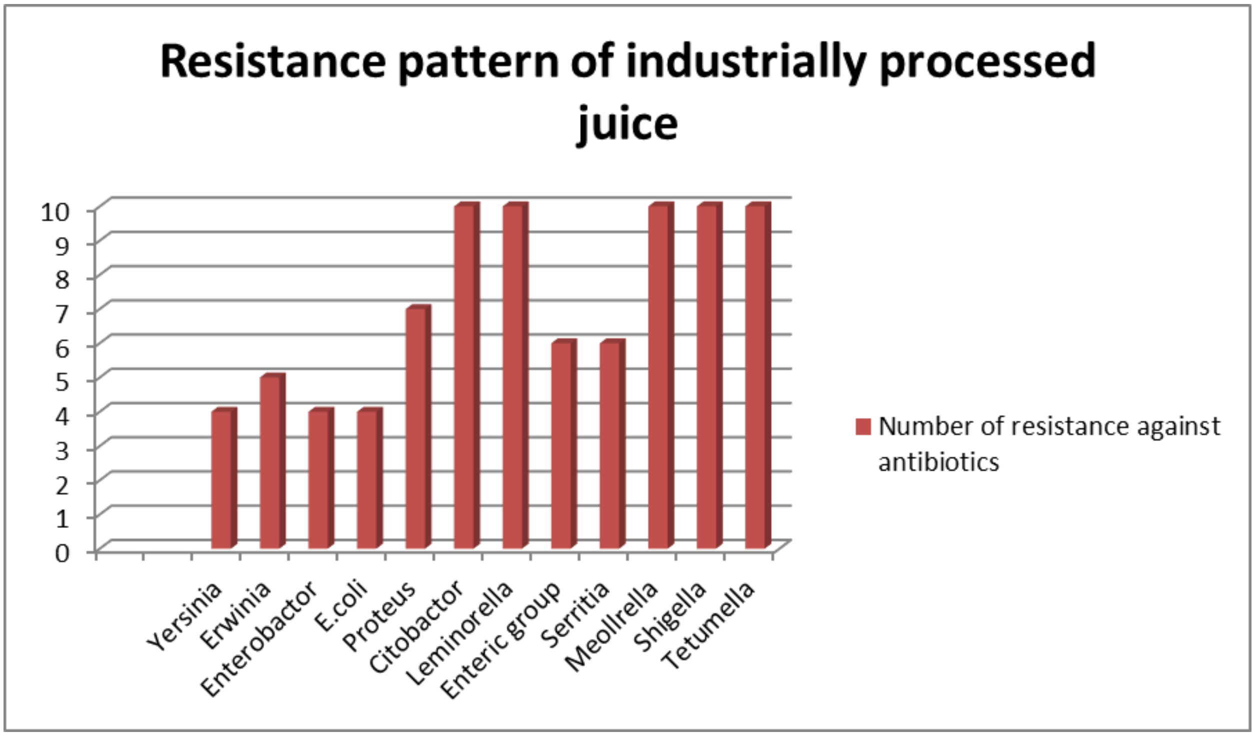


Figure 7: Resistance pattern of industrially processed juice

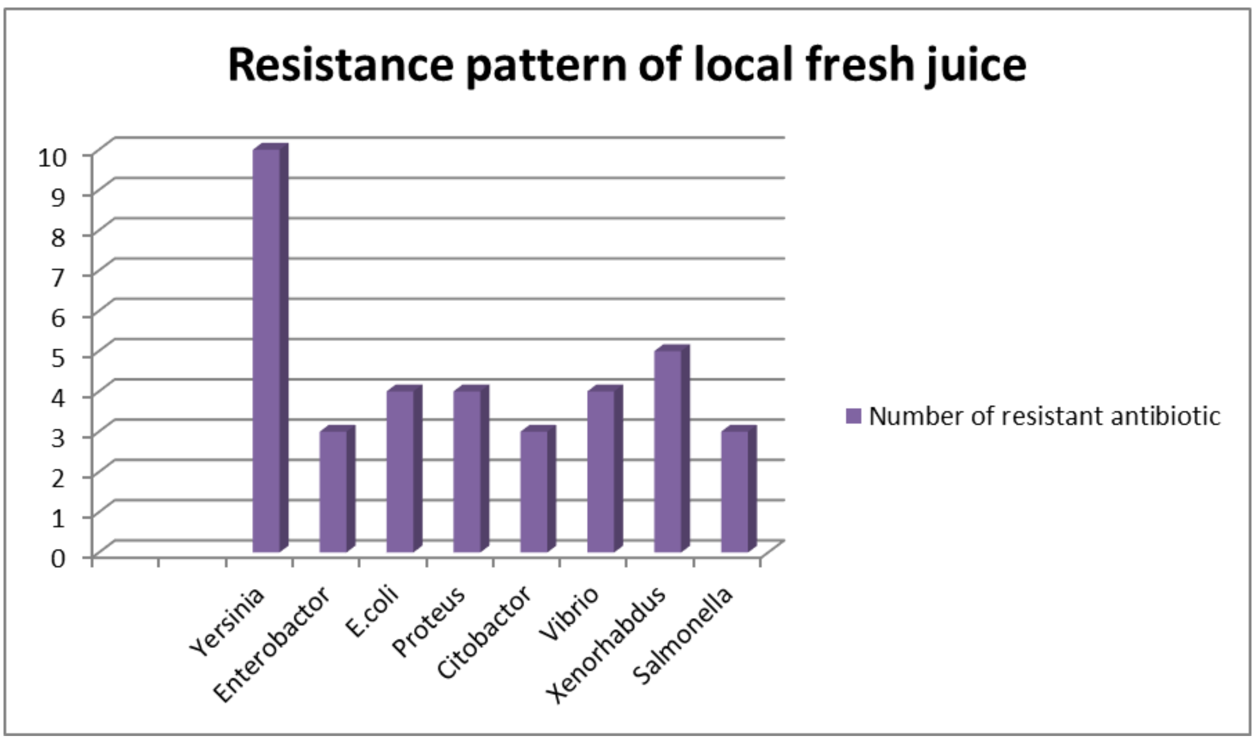


Figure 8:Resistance pattern of local fresh juice

CHAPTER 4

4. Discussion

The presence of multi-drug-resistant bacteria in fruit juice is highly unacceptable. However, it is observed that some of the local companies of Bangladesh need to produce quality juice. Therefore, antibiotic-resistant bacteria have been found in these products. On the other hand, local vendor juice showed signs of a high bacterial count, indicating low hygiene maintenance during fresh juice production. However, the amount of resistant bacteria is equal to the packaged juices. One of the notable regards was that the pH of the observed juices was acidic to a neutral level which is optimal for pathogenic bacterial growth.

Packaged juice from some of the local companies of Bangladesh and juices from local vendors of the Mohakhali and Khilgaon areas were collected. Sample juices were cultured using standard spreading techniques for counting bacterial load and searching for targeted organisms. Among the eight sample-juice collected from industrially manufactured and local vendors, 129 bacteria were selected by morphology (Table 2.2) for processing to find multi-drug resistance and identify the organism. In identifying the selected bacteria, biochemical tests have been performed using the data from a pre-published database (Table 2.3). The results of biochemical tests are compared to known organisms and the ABIS website to identify possible organisms. The standard disk diffusion technique (Table 2.4) has been used for antibiotic susceptibility testing, and 50 bacteria are resistant to three or more groups of antibiotics.

Fruit juices contain lots of organic compounds, which makes them acidic. Usually, the pH of fruit juices ranges between 2.0 and 4.5, which can be used to determine the quality of the juice (Mettler-Toledo International Inc., all rights reserved, 2022). Among the 8 sample juices used in the study, the pH value varied from 1.9 to 6. As seen in Figure 2, industrially manufactured juices have a more acidic pH level which can be less than 4. It is due to preservatives and other artificial ingredients used in packaged juice. On the other hand, the pH of local vendor juices varied between 4 to 6, which is close to neutral. However, one exception can be observed in the case of local lemon juice, which has a pH of 2.7 because lemon is a citrus fruit. The pH level of juice can be an essential factor for the growth of bacteria. Acidic or alkaline pH levels can inhibit bacterial growth. However, the pH level of the consuming product needs to be safe for human consumption. Therefore, consuming excessive acidic juice can have long-term harmful effects on the human body. Bacterial growth in fruit juice, especially in fresh juices, is an indicator of the possibility of spoilage of the product (Lateef et al., 2004). Usually, the water activity (aw) of fruit juices is high, which can assist microbial growth (Harley et al., 1996).

The selected bacteria were further identified using standard biochemical tests (Table 2.3) to identify the organisms to determine the presence of pathogens in the juice. The biochemical tests include indole, methyl red, urease, motility, triple sugar iron, Voges Proskauer, citrate utilization, catalase, oxidase, and the Gram stain technique. After collecting the test results, the ABIS website was used to compare the results for identification. Among various bacteria identified, some notable ones had been, *Yersinia* (28.0%), *E.coli* (8.0%), *Enterobacter* (6.0%), *Salmonella spp* (6.0%), *Shigella* (2.0%), *Vibrio* (4.0%) (Figure3, Figure 4). It shows the concern that the local vendor juices can be more harmful if consumed regularly. Street juices are predominantly connected with diarrheal disease because of unhygienic production (Mensah et al., 2002). The bacteria found in the juices are pathogens that are related to causing gastrointestinal diseases (Durgesh et al.,2008). In our study, the target organisms were *Enterobacteriaceae*, *E.coli*, *Salmonella*, *Shigella*, *and Vibrio*, which are associated with the food-borne disease. The study showed that these pathogens were present both in local vendor juices and packed juices.

Moreover, the identified pathogens were multidrug-resistant, increasing the risk of health damage.

Coliform bacteria are indicator organisms used to detect fecal contamination in the water (Padmadan et al.,2016). It also indicates that the source can be contaminated with pathogens. Therefore, the presence of coliforms in juice indicates pathogen contamination. In the study, coliform such as E.coli was isolated from both industrially manufactured and local vendor juice. The bacterial load count in Macconkey agar plates used for coliform indication was high in number. In the case of industrially manufactured juice, the coliform contamination indicates a probable malfunction in good hygiene practice (GHP). Contamination can occur from the raw materials or the storage where the materials were kept, and the equipment used in the factory was not well maintained.

On the other hand, the local vendor juices can be contaminated through various sources. Often street vendors in our country are seen to be open beside busy roads, which leads to the contamination of pathogens and dust particles in the freshly made juice. Another notable contamination source in local vendor juices is the water used (Lucky et al., 2016). Most of the time, water from contaminated sources, such as rivers, is used without proper purification. The wrong practice of using antibiotics to clean the water is causing antibiotic resistance in the present bacteria.

Antibiotic susceptibility testing was performed to identify the multi-drug resistant organisms from the 129 isolates (Table 2.4). In the study, ten antibiotics of nine different classes, which include Imipenam, cefepime, Meropenem, nalidixic acid, piperacillin, erythromycin, Amoxicillin, gentamicin, colistin, kanamycin been used by disk diffusion method (Bauer et al., 1966; Acharjee et al., 2012). Among the isolated colonies, most were found to be Amoxicillin and erythromycin-resistant. High resistance was also observed for cefepime, erythromycin, kanamycin, colistin, imipenem, nalidixic acid, and gentamicin. Resistance to piperacillin, Meropenem, is relatively less than the rest and, in most cases, susceptible. After thorough analysis, 50 bacteria were found multi-drug resistant, which means they showed resistance against three or more antibiotics, among which 30 MDR were collected from the local vendor juices and the rest 20 were collected from the packaged.

A study by Lateef. A (2004) showed that Amoxicillin had been ineffective against the E. coli strains. Similar results can be seen in this study, where Amoxicillin (98%) is very much effective against microorganisms. One of the notable observations in antibiotic susceptibility testing was that some organisms were resistant to all ten antibiotics, indicating an extreme threat to public health. The resistance pattern of the organisms is a clear indication of the misuse of antibiotics in our country (Umoh et al., 1990; Abbar & Kaddar, 1991; Silva & Hoffer, 1993; Malik & Ahmad, 1994). It can often be seen that health professionals provide broad-spectrum antibiotics without proper identification of the infected bacteria. As a result, bacteria that can be treated using narrow-spectrum antibiotics are being treated with broad-spectrum antibiotics, leading to vast antibiotic resistance. In our study, most resistance patterns found from the MDR for various antibiotics were highest against erythromycin (72.0%), colistin (66.0%), and Nalidixic acid (62.0%). The rest of the antibiotics showed relatively less resistance which was observed for cefepime (54.0%), imipenem and kanamycin (40.0%), and gentamicin (38.0%). The least amount of resistance among all the antibiotics used was observed for piperacillin (28.0%) and Meropenem (26.0%) (Figure 6, Figure 7).

CHAPTER 5

5. Conclusion

Increased antibiotic resistance among pathogenic bacteria is an excellent threat to treating infectious diseases. Shortly, the chance of people losing their lives for being infected by drug-resistant bacteria is rising enormously. This silent pandemic is a significant concern that requires immediate action to prevent further complexity. According to a study by Imperial College London, around 700,000 people die annually from drug-resistant diseases, and by 2050 the number can rise to ten million. With the flow of time, pathogens are adopting available antibiotics at a high pace, and new antibiotics are being produced slowly. As a result, superficial infections caused by antibiotic-resistant bacteria are becoming incurable diseases. Therefore, preventive measures are crucial, along with developing new treatment methods to fight against the issue. In such a situation, antibiotic-resistant bacteria in daily consuming drinks like fruit juice cannot be acceptable. The findings indicate that industries that commercially produce juice in Bangladesh need to be more sensible to keep the quality of products in check. As a result, the tested juice showed the presence of various antibiotic-resistant pathogens in packed juice.

On the other hand, the local vendor juices showed signs of antibiotic-resistant pathogens and a high load of viable pathogenic bacteria which can cause various water-borne diseases. Therefore, strict guidelines must be maintained during antibiotic administration. Local people need more awareness about the impact of the misuse of antibiotics. Moreover, farmed animals should not be treated with the same drug created for human treatment. Lastly, animal waste needs to be adequately treated in potable water. In conclusion, much research and spreading awareness is a must to prevent this fast-growing concern.

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