A STUDY ON THE MICROBIAL STATUS OF CONSUMABLE FRUIT JUICES IN DHAKA CITY AND THE COMPARATIVE EFFECTS OF PRESERVATIVES ON DEVELOPING ANTIBIOTIC RESISTANCE

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

Department of Mathematics and Natural Sciences

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DECLARATION

It is hereby declared that

1. The thesis submitted is my 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 that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all of the main sources of help.

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Approval

The thesis/project titled "**A study on the bacteriology of consumable fruit juices in Dhaka city and the comparative effects of preservatives on developing antibiotic resistance"** submitted by

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DEDICATED TO MY PARENTS, MY TEACHERS AND FRIENDS

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Abstract:

This dissertation aims to isolate and identify the various viable bacteria from packaged fruit juices, which is manufactured inside Bangladesh and also abroad. The study focuses on the bacterial morphology and growth patterns in fruit juices with added preservatives. The methodology involves several biochemical tests followed by determining their antibiotic resistance patterns and finally screening for pathogenicity. Three potential pathogens(*Bacillus niacin, Xenorhabdus japonica,*and *Bacillus carboniphilus,*) identified based on the biochemical tests, were detected from two different fruit juices, both made and manufactured in Bangladesh. The DNA of the isolated samples were extracted and amplified using PCR and gel electrophoresis was performed for the confirmation of the presence of DNA. For further confirmation of the pathogenic organisms, DNA sequencing will be done using 16srRNA as a future aspect of this study. The prevalence of the microbes was confirmed by the total CFU count of the different samples. Alongside this study, another study was conducted to determine the effect on antibiotic resistance by growing known organisms in the juice for several days and observing their zone of inhibitions. It provided a clear indication that the more the organisms grew with the added preservatives in the juice, the more resistant they became to certain antibiotics.

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Introduction

INTRODUCTION

1.1 Overview:

The consumption of fruit juices is prevalent and very common all over the world and are almost included every day in our diet charts. The essential minerals, vitamins, and antioxidants present in fruit juices are necessary for the better and improved health of humans and also for the prevention of many crucial diseases Including cardiovascular diseases, diabetes and the onset of cancer. In the 2005 Dietary Guidelines for Americans, it was recommended to consume several cups of fruits and vegetable juice in a day. However, the increasing consumption of fruit juices in recent years has emphasized the need for microbiologically as well as chemically safe and non-hazardous food for the world (Huang, 2009).

1.1.1 Fruit juices:

Fruit juices act as a source of nutritious drinks and also provide great taste and various health benefits. It helps in detoxifying the body and has important medicinal properties as well as nutritional value. These natural properties of fruit juices also play an important role in reducing the risks of various health issues (Noor et al., 2012).

1.1.2 Health benefits of different fruit juices:

Fruit juices comprise of many help promoting nutrients namely Vitamin C, polyphenols, and carotenoids. Many fruits and their juices have shown to have great medicinal impacts on the lives and health of humans. Some of the fruit juices are listed here with their health benefits:

1. Pomegranate juice- This juice prevents all kinds of cardiovascular diseases by working against dangerous LDL-cholesterol in the blood and reduces the pace of cancer. It is rich in Vitamin A, C, E, and Folic acid.
2. Apple juice- It helps to release toxins from the body and helps in the reduction of cholesterol and blood pressure. It is also known for its anti-cancerous and anti-inflammatory properties.
3. Orange juice- Orange juice is rich in Vitamin C and is abundant with antioxidants. It improves the immune system and reduces blood pressure levels. It is also rich in potassium and foliate, thus acting as a good rejuvenating agent.

4. Cranberry juice- Cranberry juice is known to prevent or acts as a treatment for infections associated with the bladder.
5. Mango juice- As the mango has a lot of fiber, it is really good for patients suffering from constipation. Due to the presence of carotenoid, it also helps to prevent colon cancer and gall bladder cancer. This juice also contains a considerable amount of tryptophan and precursors of serotonin.
6. Guava juice-It contains Vitamin C and helps fight against flu, cold, and cough. Guava juice consumption also reduces bad LDL-cholesterol from the body and maintains blood pressure.

1.1.3 Manufacturing and processing of different juices:

Most of the juices are prepared by squeezing by hands or by using juicing machines at large scale in factories for commercially packaged fruit juices. Firstly, the raw materials are sorted for quality and any fruit which seems bad is discarded. The fruits are all washed and dried carefully and yielded for juice by extracting it from the fruit pulp. Other methods of processing and preservation include canning, pasteurization, concentrating, freezing, evaporation, and spray drying. The final juice products are labeled, sealed, and cooled. The process of sterilization is also a very important step (Braide et al., 2012).

1.1.4 Deterioration of fruits and fruit juices:

Most of the fruit juices and drinks sold in the markets are pasteurized. However, a small percentage of juices remain unpasteurized and possess more chances of containing harmful bacteria. Unpasteurized fruit juices have posed serious public health risks in recent years (Alwakeel& Al-Humaidi, 2008).There are several physical factors that results into the deterioration of fruit juices. It could be the fruit's own enzyme action, microbial action, or combination of all of these. In nature, the juices that are being extracted from fruits are acidic. The pH for lemon juice is 2.4 while it is 4.2 for tomato juice. Fruit juices also contain about 2% of sugar content in lemon juices about 17% in some grape juices. That is why mold growth is favored on the surface of these juices. When juices are exposed to high moisture, bacteria and yeast grow faster. By the process of extraction and sieving, solids are removed which makes the oxidation-reduction potential to become higher

which in turn favors the growth of yeast. Also, some bacterial growth is discouraged due to the lack of Vitamin B.

Fruit beverages consist of some bioactive compounds such as ascorbic acid (AA), total phenolic compounds (TPC), total carotenoids (TC) and total anthocyanins (TA) and the degradation of these compounds might serve as a critical factor forthe quality of several fruit-derived drinks. This is true because these substances are very likely to undergo destruction during storage (Trost *et al*., 2008; Barba *et al*., 2012). Depending on the availability of nutrients and the water activity, as well as the medium and pH, among other factors, there may be substantial differences in the microbiota of these products (Kalia and Gupta, 2006). During the growing periods of the fruits in the fields, orchards, vineyards or even in the greenhouses or during their time of harvest, handling the fruits after the harvest and distribution, it is quite possible of contamination of the fruits with microoganisms that might be pathogenic and spoilage causing organisms (Beuchat, 2002). The skin of fresh fruits provides as protective barrier that is natural and acts effectively against most microorganisms that cause plant spoilage and pathogenicity. That being said, during the processing of the fruits this natural barrier and protective skin of the fruits nay be eliminated, thus exposing the naked flesh of the fruit to unfavorable environmental conditions and also to a very high possibility of contaminationwith pathogenic microorganisms including bacteria, viruses, and parasites at the time of the handling, cutting, shredding, and maintenance procedure of the fruits that are freshly cut at the surrounding temperature (Brackett *et al.*,1994; Nguyen-The and Carlin, 1994; Balla and Farkas, 2006).

The colonization of fresh cut fruits are determined by types of factors and are derivatives by microorganisms.

1) Intrinsic factors- The intrinsic factors depend on the composition of food which includes the activity of water, pH of the food, their redox potential, availability of nutrients and the structures as well as the presence of antimicrobial agents.

2) Technological treatments- These mainly focus on the modifications of the initial microbiota.

3) Extrinsic factors- The temperature, relative humidity, and atmosphereare the environmental conditions of the medium.

4) Implicit factors- The implicit factors are dependent on the development of the microbiota and involve the handling of the raw materials that are used and the also the final product during the time of processing and storage (Montville and Matthews, 2001).

1.1.5 Causative agents of microbial spoilage:

Bacteria as well as yeasts and molds can be the causative agents of microbial spoilage in fruits and its derivatives. Fruit juices contain a microflora that is normally present on the surface of fruits during harvest and postharvest processing which include transport, storage, and processing. The microflora is used as a substrate for the growth of many microorganisms such as acid-tolerant bacteria and fungi (molds, yeasts). Before processing, the main flora of fruits is formed by yeast due to the acidic pH. *Candida*, *Dekkera*, *Hanseniaspora*, *Pichia*, *Saccharomyces*, and *Zygosaccharomyces* comprises the major genera. The filamentous fungi namely *Penicillium*, *Byssochlamys*, *Aspergillus*, *Paecilomyces*, *Mucor*, *Cladosporium*, *Fusarium*, *Botrytis*, *Talaromyces*, and *Neosartorya* are most frequently isolated from fresh fruits and juices. Lactic acid bacteria and acetic acid bacteria, among bacteria, have also been isolated from fruit juices (Aneja et al.,2014).

Acetobacter, *Alicyclobacillus*, *Bacillus*, *Gluconobacter*, *Lactobacillus*, *Leuconostoc*, *Zymomona*, and *Zymobacter*are the most commonly reported bacterial genera.The commonly encountered genera responsible for the spoilage of juices, among yeasts are *Pichia, Candida, Saccharomyces* And*Rhodotorula.*Certaincommonmoldssuchas *Penicillium* spp., *Aspergillus* spp., *Eurotium* spp., *Alternaria* spp., *Cladosporium* spp., *Paecilomyces* spp., and *Botrytis* spp. are involved in the spoilage of fresh fruits and some processed fruit derivatives including the thermally processed (Splittstoesser, 1991; Beuchat and Pitt, 1992; Lund and Snowdon, 2000). On the other hand, Jay *et al*., (2005) reported the occurrence of yeasts such as *Saccharomyces* spp. *Cryptococcus* spp., and *Rhodotorula* spp.infreshfruits,and *Zygosaccharomyces* spp., *Hanseniaspora* spp., *Candida* s pp., *Debaryomyces* spp., and *Pichia* spp. in dried fruits. Although both molds and yeasts can grow in fruit tissue, the latter is more often associated with spoilage of cut fruits due to their ability to grow faster than molds.

1.1.6 Effects of preservatives in fruit juices:

Preservatives are natural or synthetic substances that are added to fruits, vegetables, prepared food items, cosmetics, and pharmaceuticals to increase their shelf life and maintain their quality and safety by inhibiting, retarding or arresting their fermentation, acidification, microbial contamination, and decomposition. The major targets for antimicrobials are food poisoning microorganisms (infective agents and toxin producers) and spoilage microorganisms whose metabolic end products or enzymes cause off odors, off flavors, texture problems, and discoloration (Davidson, 2001). The most commonly used preservatives are potassium sorbate and sodium benzoate. However, consumer demand for natural origin, safe and environmentally friendly food preservatives is increasing. Natural antimicrobials such as *bacteriocins*, *lactoperoxidase*, herb leaves, and oils, spices, chitosan, and organic acids have shown feasibility for use in some food products (Gould 2001; Corbo et al., 2009). Some of them have been considered as Generally Recognized As Safe (GRAS) additives in foods.

Artificial preservatives are mostly considered safe, but several have negative and potentially life-threatening side effects. Nitrates, upon ingestion, are converted to nitrites that can react with hemoglobin to produce methemoglobin, a substance that can cause loss of consciousness and death, especially in infants. Proteins in the stomach react with nitrites and produce nitrosamines, substances that are carcinogenic. Researchers claim that there is a substantial link between increased levels of nitrates in food and increased deaths from Alzheimer's, Parkinson's, and Type 2 diabetes. Headache, sweating, redness of skin, nausea, and weakness can occur following the consumption of food containing monosodium glutamate (MSG).

Sulfite containing food preservatives may cause severe allergic reactions and exacerbation of asthma. The toxic paraben chemicals are often used along with methylchloroisothiazolinone and methylisothiazolinone. These are reported to possiblycause neurological damage in rats and are potent irritants and allergens. The use of these toxic chemicals by pregnant women may adversely affect fetal brain development.

Formaldehyde DMDM hydantoin, diazolidinyl urea, and imidazolidinyl urea are all potent skin, eye and lung irritants. High levels of exposure to toxins like these can cause DNA damage to

sperm. Research has shown that the food additives used in hundreds of children's foods and drinks can cause temper tantrums and disruptive behavior (Anand& Sati, 2014).

1.1.7 Antibiotics susceptibility:

Antibiotics are powerful medicines that fight certain infections. They either stop bacteria from reproducing or destroy them. However, Antibiotics aren't effective against viral infections. It is evident that the overuse of antibiotics is contributing to the growing number of bacterial infections that are becoming resistant to antibacterial medications.

Antibiotic resistance occurs when bacteria change in response to the use of these medicines. Bacterial genes due to excessive exposure to the antibiotics, adapt to the new environment, and have a mutation of their gene. The gene that was previously affected by the antibiotic now no longer is recognized by it. It is structurally changing. The mutation might occur due to chemical or radiational exposure or sometimes randomly. The mutated bacteria is more likely to survive in the harsh conditions and continue to multiply. This antibiotic resistance happens when someone is prescribed an antibiotic but he/she fails to complete the entire dosage. They may feel better only halfway through the prescribed dosage and stop taking the antibiotics, but this is where the problem starts. All the bacteria are not completely killed if the entire antibiotic course is not completed. The remaining bacteria inside the body are not enough to make somebody feel bad but they are enough to replicate and multiply to make somebody fall ill again in a few days' time. Unfortunately, if this keeps happening time and again, the antibiotic will stop working efficiently against the bacteria and that is when the bacteria become antibiotic-resistant.

Objectives of the study

The objectives of this study comprises of

- The comparison between different types of packaged fruit juices and determining the microbial quality of these consumable juices.
- To check for pathogenicity for any microorganisms that might be present.
- It also aims to identify and determine the probable effects of additive preservatives on the development of antibiotic resistance.

Chapter II

Materials and Method

Materials and Methods

2.1 Materials

2.1.1 Equipment

- Autoclave machine
- Laminar Air Flow
- Centrifuge Machine
- Incubator
- Shaker Incubator
- Vortex machine
- PCR machine

2.1.2 Media

Media of different types and categories were used for the selective identification, growth, and enrichment of different organisms from the samples

- Nutrient Agar
- MacConkey Agar
- MSA
- MHA
- LB broth
- Different Biochemical mediums

2.1.3 Antibiotic Disc

Eleven different antibiotic discs were used for the antibiogram test of the sample organisms. The table below shows the list of the antibiotics and the diameters of their zones of inhibition

Table 1: List of antibiotics and their zone ranges

No.	**Antibiotic Name**	**Disc Code**	**Disc Potency µg**	**Range**		
				Resistant/ (mm)	**Intermediate/ (mm)**	**Sensitive/ (mm)**
1	Amoxyclav	AMC	30	11	12-14	15
2	Amoxicillin	AMX	30	11	12-14	15
3	Azithromycin	AZM	15	<13	14-17	18>
4	Cephalexin	CL	30	17	-	18
5	Chloramphenicol	C	30	12	13-17	18
6	Cefixim	CFM	5	19	-	20
7	Ciprofloxacin	CIP	5	15	16-20	21
8	Levofloxacin	LEV	5	13	14-16	17
9	Tetracyclin	TE	30	14	15-18	19
10	Vancomycin	VA	30	9	10-11	12
11	Cefuroxime	CXM	30	14	15-17	18

Given names for the 39 isolates from all the ten juice samples

Table 2: Name of all the isolates from the sample juices

Serial no.	**Names of the juices**	**Names of isolates**
1	Acme classic mango juice	AC-1
2	Acme classic mango juice	AC-2
3	Acme classic mango juice	AC-3
4	Acme classic mango juice	AC-4
5	Acme classic mango juice	AC-5
6	Frutica mango juice	F-1
7	Frutica mango juice	F-2
8	Frutica mango juice	F-3
9	Frutica mango juice	F-4
10	Mangolee mango juice	M-1
11	Mangolee mango juice	M-2
12	Mangolee mango juice	M-3
13	Mangolee mango juice	M-4
14	Mangolee mango juice	M-5
15	Mangolee mango juice	M-6
16	Mangolee mango juice	M-7
17	Mangolee mango juice	M-8
18	Pran Apple juice	PA-1
19	Pran Apple juice	PA-2
20	Pran Apple juice	PA-3
21	Pran Apple juice	PA-4
22	Starship mango juice	ST-1
23	Starship mango juice	ST-2
24	Starship mango juice	ST-3
25	Shezan mango juice	SZ-1

26	Shezan mango juice	SZ-2
27	Shezan mango juice	SZ-3
28	Shezan mango juice	SZ-4
29	Shezan mango juice	SZ-5
30	Shezan mango juice	SZ-6
31	Shezan mango juice	SZ-7
32	Shezan mango juice	SZ-8
33	Shezan mango juice	SZ-9
34	Doi Kham guava juice	DK-1
35	Doi Kham guava juice	DK-2
36	Doi Kham guava juice	DK-3
37	Doi Kham guava juice	DK-4
38	Harvey Fresh orange juice	HAR-1
39	Harvey Fresh orange juice	HAR-2

2.2 Methods

2.2.1 Collection of the Samples

A total of ten different fruit juices which were made commercially in both Bangladesh and outside Bangladesh were collected. All of them were store-bought. The name of the ten juices was as follows:

- Acme Classic mango juice
- Frutica mango juice
- Mangolee mango juice
- Pran Apple juice
- Starship mango juice
- Shezan mango juice
- Doi Kham guava juice
- Harvey Fresh orange juice

2.2.2 pH measurement

Two beakers were taken for the test. One was filled with distilled water and the other was used for the juice. The pH meter was already dipped in buffer solution and hence had its pH set at 7. The pH meter was first dipped in the beaker containing distilled water and dried and then dipped in a juice sample. Readings were taken after 30 seconds of dipping the machine in the juice and again rinsed in distilled water, dried and switched off. The same procedure was repeated for all the ten juice samples and all the readings were noted.

2.2.3 Serial Dilutions

Each of the ten juice samples was serially diluted to concentrations of 10^1, 10^2, 10^3, 10^4 and 10^5 times. At first 9 ml of autoclaved saline water (0.9% NaCl) was taken in each of the test tubes and then 1 ml of juice was added to it, making a total volume of 10 ml. This test tube was labeled 10^{-1} dilution. 1ml of this solution was transferred to another test tube containing 9 ml of saline water, and labeled 10^{-2}. All the juice samples were diluted ten folds up to 10^{-5}.

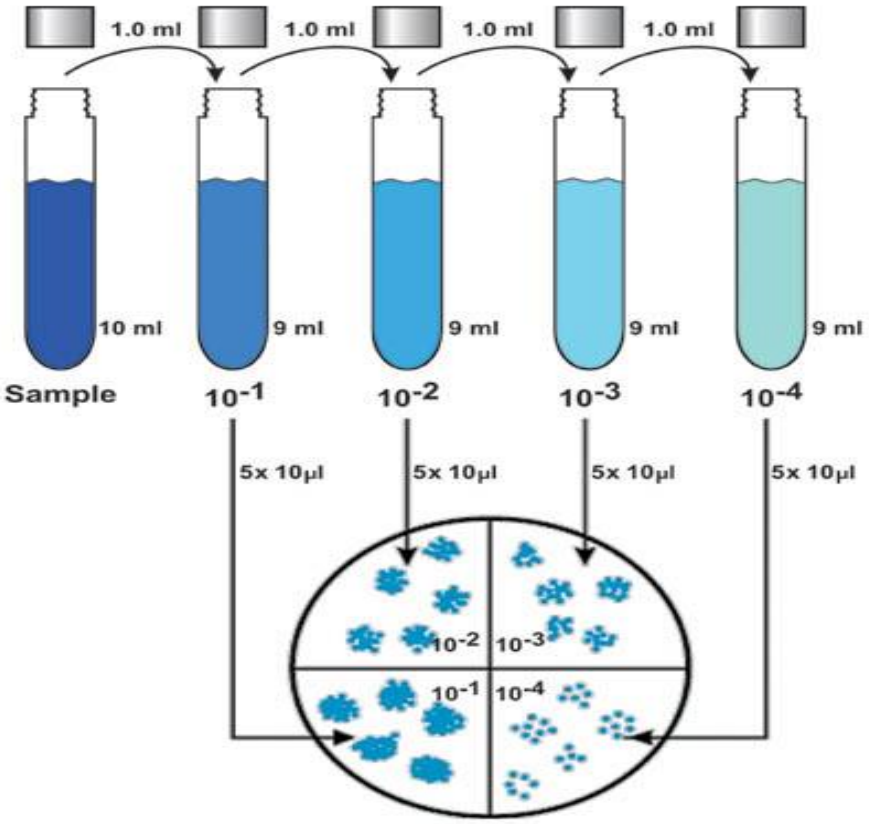

Figure No. 1: Example of Serial dilution

2.2.4 Spread plate method

Five Petri dishes containing NA media were taken for each of the ten juices and were labeled 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Then all the serially diluted samples of the juices were vortexed and 100 µl of the solutions were added onto their corresponding, labeled plates. Using a spreader, the samples was spread onto the plates and incubated for 24 hours at 37°C. After the incubation, the plates were observed for any growth of organisms and were noted and used for further study.

2.2.5 Selection of isolates based on their morphology

Among the numerous colonies growing in the media, only the colonies that had different colony sizes, color, and texture were taken forward for the study. The colonies which looked similar to the naked eyes were discarded. The individual selected colonies were sub-cultured in the NA media.

2.2.6 Microscopic view of the bacteria

Gram stain

It is a differential process that is used for the characterization of bacteria in two major groups, namely the gram-positive bacteria and gram-negative bacteria.

2.2.7 Characterization of bacteria through biochemical analysis

Biochemical analysis is based on the different biochemical activities of the various bacterial species and is used for the identification of these specific species. The several biochemical tests that were carried out during this process are as follows; Starch hydrolysis, MIU test (Motility test, Indole test, and Urease test), TSI test (Triple Sugar Iron Agar test), Citrate utilization test, Methyl Red test, Voges-Proskauer test, and Catalase test.

2.2.7.1 Starch hydrolysis:

It is used to detect the ability of the organisms to hydrolyze starch by secreting the amylase enzyme in the surrounding media. The media was prepared and autoclaved at 121^{o}C for 15 minutes and poured onto the petri dishes and was allowed to solidify. Then using a sterile loop, a single line streak of the unknown organism was streaked across the plate and was incubated at 37^{o}C for 24 hours. After the incubation and growth, the plates were flooded with gram's Iodine and the results were recorded (Aditi *et al*., 2017).

2.2.7.2 MIU test:

The test is based on the motility, indole, and urease production ability of the bacteria. This medium contains peptones that consist of carbon and nitrogen needed for bacterial growth. At the same time, the microorganisms which produce urease, is provided with a source of nitrogen. The pH indicator, which was Phenol Red, has a change in color, from yellow-orange (pH 7.0) to red-pink (pH-8.1). The demonstration of motility is allowed due to the low agar concentration in the media by showing diffused growth away from the stab inoculated line (Westmann, 2018). At first, the media was heated to dissolve completely and sterilized by autoclaving at 121^{o}C ~~psi~~ for 15 minutes. Then the media was cooled to about 50-55°C and 5 ml sterile 40% urea solution per 95 ml basal medium was added to it. Immediately 7 ml of the media was dispensed into sterile test tubes and was allowed to cool and solidify. With a sterile inoculating needle, fresh 24-hour culture of the sample bacteria was stab inoculated and was incubated for 24 hours at 37° C.

2.2.7.3 Triple Sugar Iron Agar test:

The Triple sugar iron test was done to determine and differentiate among organisms based on their ability to ferment different carbohydrates and production of hydrogen sulfide. The fermentation of carbohydrates is indicated by a change in color due change in pH and by the production of gas. The media is prepared by boiling the agar and dispensing them into test tubes. The test tubes were autoclaved at 121°C for 15 minutes and allowed to cool in slanted positions. Using a sterile needle, a 24-hour fresh single colony of the sample bacteria was taken and stab inoculated through the center of the medium till the bottom and then streaked on the surface of the agar slant. The

inoculated test tubes were incubated at 37° C for 24 hours and the results were noted (Aditi *et al*., 2017).

2.2.7.4 Methyl red test:

The methyl red test was performed to detect the production of sufficient acid during the fermentation of glucose. The MR-VP broth is used for both the MR test and VP test but only the reagents used differs. The MR-VP broths of 3.5 ml were dispensed into test tubes and autoclaved at 121°C for 15 minutes. After it was cooled, the test bacteria which were freshly cultured were inoculated into the test tubes. All the inoculated test tubes were incubated for 24 hours at 37°C. For the MR test, about 5 drops of methyl red indicator solution are added to the MR test tubes. A positive reaction is indicated if the color of the broth changes to red with a few minutes (Tankeshwar. 2014).

2.2.7.5 Voges - Proskauer test:

The VogesProskauer test is used to detect the ability of an organism to produce acetylmethyl carbinol from the fermentation of glucose. These tests are particularly useful in the identification of the *Enterobacteriaceae*. To the MR-VP broths after inoculation and incubation for 24 hours with pure culture, 0.6 ml of 5% α-naphthol was added, followed by 0.2 mL of 40% KOH respectively. The tube was gently shaken for exposure of the media to atmospheric oxygen and then left undisturbed for 10-15 minutes. The results were observed for up to a maximum of one hour and not beyond that (Tankeshwar, 2015; Aryal, 2018; McDevitt, 2009).

2.2.7.6 Citrate utilization test:

The citrate utilization test helps to detect the organism's ability to utilize citrate as their sole source of carbon by producing the citrase enzyme. The media was prepared by heating and pouring 2.5 ml of the media into vials and then sterilized at 121°C for 15 minutes. The vials were cooled in a slanted position to create a slanted surface. Using a sterile needle, 24-hour fresh pure culture of the sample bacteria were streaked onto the surface of the slants and incubated for 24 hours at 37°C. (MacWilliams, 2009).

2.2.7.7 Catalase test:

This test was done to demonstrate the production of catalase enzyme by detecting the ability of microorganisms to breakdown hydrogen peroxide and produce oxygen and water. A very small amount of fresh bacterial colony was transferred into a clean and sterile glass slide using a sterile wooden stick. To that, a drop of 3% H_2O_2 was placed on top of the bacterial colony and mixed. For positive results, bubbles were observed in a few seconds (Aditi *et al.*, 2017).

2.2.8 Antibiotic resistance and susceptibility analysis

This analysis aims is to check the performance of antimicrobial susceptibility of certain isolated bacteria for particular infections and as well as the possible drug resistance in pathogenic organisms. It is one of the most basic and important tasks in a microbiology laboratory. This test is performed manually and the possible methods include the disk diffusion and the gradient diffusion methods.

2.2.8.1 Disk diffusion test:

The disc diffusion test is one of the classic microbiology techniques and because of its simplicity, effectiveness, and efficiency, it is still most widely used for determining the antibiotic resistance pattern of microorganisms. At first, Mueller-Hinton agar plates are prepared and a suspension of isolates of approximately 1-2x10^8 CFU/ml, which was prepared to a McFarland standard of 1.0, was spread evenly onto the agar plates. Five different antibiotic discs were placed on the surface of a single plate and were incubated for 16-24 hours at 35°C. The results were observed and their zone of inhibition around each antibiotic disc was measured to the nearest millimeter. The size or the diameter of the no-growth zone is dependent on the inoculum density as well as the diffusion rate in the agar. The size of the zone can be compared with the reference table and can be interpreted whether the organism is susceptible, intermediate, or resistant to a particular antibiotic (Christenson and Relich, 2018).

2.2.9 Pathogenecity test:

Two particular tests were carried out to determine the pathogenicity of the isolates. They were as follows:

2.2.9.1 Blood agar test

The blood agar test was done to differentiate organisms based on their hemolytic properties (β-hemolysis, α-hemolysis, and γ-hemolysis (or non-hemolytic) indicating whether they are pathogenic or not. The blood agar base was prepared which was a nutrient agar base and was autoclaved at 121°C for 15 minutes. The agar base was cooled to 50°C and 5% sheep blood (5 ml sheep blood was added to 95 ml medium) was mixed very gently with avoiding any kind of hemolysis of the red blood cells and any bubble formation. The blood agar media was then dispensed into sterile petri dishes while still liquid and was allowed to solidify. Using a sterile loop, a single colony of 24-hour fresh bacterial sample was taken and streaked onto the media. The plates were incubated at 37°C for 24 hours and later observed for an alpha, beta, or gamma hemolysis (Russell et al., 2006).

2.2.9.2 Dnase test

The Dnase test is used to determine whether an organism can hydrolyze DNA by producing the deoxyribonuclease enzyme and utilize it as a source of carbon and energy for growth. The Dnase media was prepared and autoclaved at 121°C for 15 minutes. After cooling the media a little bit, separately extracted DNA was added to it and mixed and was poured onto sterile plates and allowed to solidify. Then a loopful of 24-hour fresh bacterial culture was streaked in a line, using a sterile loop. The plates were incubated for 18-24 hours at 37°C (Waller *et al*., 1985).

2.2.10 DNA extraction:

DNA was extracted for only the three pathogenic organisms that gave positive results from the pathogenicity tests. For the extraction process, the boiling method was used according to the protocol. The extracted DNA was then stored at -20°C refrigerator for further use.

2.2.11 PCR:

Polymerase Chain Reaction is a process that is used to amplify the extracted DNA using the conventional PCR machine. This step was done so that there was enough PCR product for visualizing by gel electrophoresis and sent for sequencing.The primers used were the Universal PCR primers for bacteria. The primers were the 27F and the 1492R.

2.2.12 Gel Electrophoresis:

Gel electrophoresis is a laboratory method that is used to separate DNA fragments of different sizes and also ensuring if there was any PCR product from the previous method.

2.2.13 Qualitative effects of preservatives in commercially packaged fruit juices

The effects of added preservatives in packaged fruit juices around Bangladesh and also outside of Bangladesh were analyzed.

2.2.13.1 Sterile juice preparation:

The sample juice was autoclaved in seven test tubes,5ml each, at 121 °C for 15 minutes to get rid of all the existing organisms inside the juice, making the juice free from any microorganisms.

2.2.13.2 Inoculum preparation:

Seven known organisms were taken and inoculated into each test tube and were labeled properly.

2.2.13.3 Incubation time:

The seven inoculated test tubes were then incubated for 7 days at 37°C. Every alternate day, that is, day 1, day 3, day 5, and day 7, the test tubes were taken out and cultured in Nutrient agar media with an incubation period of 24 hours at 37°C.

2.2.13.4 Antibiotic susceptibility analysis:

For all the alternate days the cultured juice was tested for antibiotic susceptibility and resistance through the disc diffusion method. The resistance patterns were observed for all the alternate days and the zone of growth inhibition for each of the antibiotics were noted and compared.

Chapter III

Results

Results

Nowadays, fruit juices serve as one of the basic sources of nutrition for most people. It is widely consumed all around the globe due to its great taste as well as the vitamin content and various other health benefits. However, the manufacturing companies are mainly focused on the marketing of their product rather than paying attention to the quality of the juice products. In this study, ten different fruit juices which were commercially manufactured and sold in the markets of Dhaka city were examined and tested for their quality assurance.

3.1 Temperature and pH measurement of all the fruit juices:

Most of the fruit juices consists of organic acids and so have a low pH. The presence of molds and yeasts are natural in a very low pH content. The growth of lactic acid bacteria, acetic acid bacteria, and enteric acid bacteria are also very common but at a pH higher than the pH needed for the growth of molds and yeasts.

In the table below, the pH value results are given for all the ten samples. Most of the samples have a pH value within pH 3-4 and two of the samples have a pH more than 4. None of the samples had a pH of less than 3. The overall range of pH for all the juices was within pH 3-5. The highest being of pH 4.13 and the lowest being of pH 3.50. The temperature for all the juice samples was approximately 24°C.

Table 3: pH value and temperature of different commercially packaged fruit juices

Sample Name	pH	Temperature/ °C
Acme Classic Mango Juice	4.01	24.0
Frutica Mango Juice	3.96	24.0
Mangolee Mango juice	3.86	24.1
Pran Apple Juice	3.50	24.0
Starship Mango Juice	3.68	24.3
Shezan Mango Juice	3.74	24.1
Doi Kham Guava Juice	3.74	24.0
Harvey Fresh Orange Juice	4.13	24.0

3.2 Total viable count of packaged fruit juices:

As per the Gulf Standards, the maximum bacterial load for any fruit juice is 1.0×10^4 (CFU/ ml). The microbial count of different fruit juices was done and recorded in the Table 4 below. The total viable count of commercially packaged fruit juices ranged from (3.00×10^2 – 1.07×10^7) CFU/ ml.

Table 4 : The total viable count (TVC) from different commercially packaged fruit juices

Sample Name	Total Viable Count (CFU/ ml)
Acme Classic Mango Juice	4.60×10^4
Frutica Mango Juice	2.04×10^5
Mangolee Mango juice	2.20×10^4
Pran Apple Juice	1.50×10^4
Starship Mango Juice	3.00×10^2
Shezan Mango Juice	1.07×10^7
Doi Kham Guava Juice	4.90×10^4
Harvey Fresh Orange Juice	3.50×10^4

3.3 Biochemical analysis of the juice samples:

After selecting colonies based on their different morphology and color, several biochemical tests were performed to identify the probable organisms. The following table below illustrates all the biochemical test results of all the selected isolates.

Table 5 : Biochemical analysis of bacterial isolates from different fruit juices

Isolates no.	Isolates		MIU			MRVP		Simmon's citrate	Starch hydrolysis	TSI						Probable organism Interpretation	
			Motility		Urease	Methyl Red	VogesProskauer				Glucose	Lactose	Sucrose	H_2S production	Gas production		
1.	AC-1	-	-		-	-	+	-	-		-	-	-	-	-	*Xenorhabdus japonica Similarity:84.3%*	
2	AC-2	-	+		-	-	-	-	-		-	+	+	-	-	*Xenorhabdus japonica Similarity:84.3%*	
3	AC-3	-	+		-	-	-	-	-		+	-	-	-	-	*Xenorhabdus japonica Similarity:94.1%*	
4	AC-4	-	-		-	+	+	-	-		+	+	+	-	+	*Raoultella (Klebsiella) terrigena, Similarity:80.4%*	
5	AC-5	-	-		-	+	+	-	-		+	+	+	-	+	*Raoultella (Klebsiella) terrigena, Similarity:80.4%*	
6	F-1	-	-		-	-	-	-	-		-	-	-	-	-	*Xenorhabdus japonica Similarity:84.3%*	
7	F-2	-	-		-	-	-	+	-		-	-	-	-	-	*Xenorhabdus beddingii Similarity:84.3%*	
8	F-3	-	-		+	-	-	-	-		-	-	-	-	-	*Xenorhabdus beddingii Similarity:84.3%*	

Table 5 : Biochemical analysis of bacterial isolates from different fruit juices

9	M-1	-	+		-	-	-	+	-		-	-	-	-	-	*Lysinibacillus fusiformis* *Similarity:87.5%*	
10	M-2	-	-		+	-	-	-	-		-	-	-	-	-	*Paenibacillus castaneae* *Similarity:85.6%*	
11	M-3	-	-		+	-	-	-	-		-	-	-	-	-	*Lysinibacillus fusiformis* *Similarity:87.5%*	
12	M-4	-	-		+	-	+	-	-		-	-	-	-	-	*Xenorhabdus nematophilia* *Similarity:84.3%*	
13	M-5	-	-		-	-	-	+	-		-	-	-	-	-	*Xenorhabdus nematophilia* *Similarity:84.3%*	
14	PA-1	-	+		+	-	-	-	-		+	+	+	-	-	*Paenibacillus castaneae* *Similarity:85.6%*	
15	PA-2	-	-		-	-	-	-	-		-	-	-	-	-	*Xenorhabdus japonica* *Similarity:84.3%*	
16	PA-3	-	+		-	-	-	-	-		-	-	-	-	-	*Bacillus farraginis* *Similarity:90%*	
17	ST-1	-	+		-	-	+	-	-		+	+	+	-	-	*Pectobacterium betavasculorum* *Similarity:84.3%*	
18	ST-2	-	-		-	-	-	-	-		-	-	-	-	-	*Xenorhabdus japonica* *Similarity:84.3%*	

Table 5 : Biochemical analysis of bacterial isolates from different fruit juices

19	ST-3	-	+		+	-	-	-	-		+	-	-	-	-	*Paenibacillus residui* *Similaruty:86.7%*	
20	SZ-1	-	+		+	-	-	-	-		+	+	+	-	-	*Paenibacillus antarcticus* *Similarity:81.2%*	
21	SZ-2	-	-		+	-	-	-	-		+	-	-	+	-	*Bacillus farraginis* *Similarity:81.2%*	
22	SZ-3	-	-		+	-	-	-	-		+	+	+	-	-	*Bacillus niacin,* *Similarity:85.1%*	
23	SZ-4	-	-		-	-	-	-	-		+	+	+	-	-	*Clostridium ramosum* *Similarity:82%*	
24	SZ-5	-	+		+	+	-	-	-		+	+	+	-	-	*Clostridium butyricum* *Similarity:81.2%*	
25	SZ-7	-	+		-	-	-	-	-		+	+	+	-	-	*Xenorhabdus japonica* *Similarity:82.7%*	
26	SZ-8	-	+		+	-	-	-	-		+	+	+	-	-	*Paenibacillus antarcticus* *Similarity:81.2%*	
27	DK-1	-	+		-	+	-	-	+		-	-	-	-	-	*Bacillus carboniphilus* *Similarity:85.6%*	
28	DK-2	-	-		-	-	-	-	+		-	-	-	+	-	*Clostridium malenominatum* *Similarity:81.2%*	

Table 5 : Biochemical analysis of bacterial isolates from different fruit juices

29	DK-3	-	+		-	-	-	-	+		-	-	-	-	-	*Xenorhabdus japonica* *Similarity:84.3%*
30	DK-4	-	-		-	-	-	-	+		+	+	+	+	-	*Bacillus carboniphilus* *Similarity:85.6%*
31	HAR-1	-	+		+	-	-	-	-		-	+	+	-	-	*Xenorhabdus japonica* *Similarity:84.3%*
32	HAR-2	-	+		+	-	-	-	-		-	+	+	-	-	*Xenorhabdus japonica* *Similarity:84.3%*

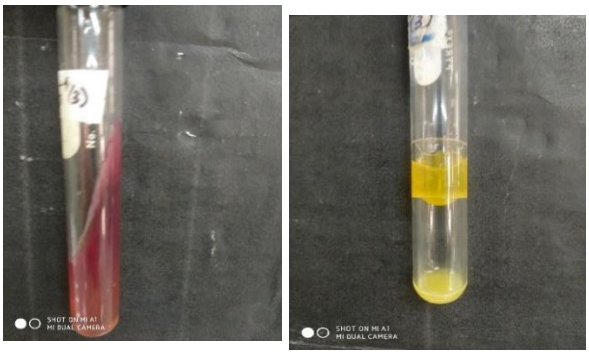

Figure No. 2 : Negative and positive test results for TSI

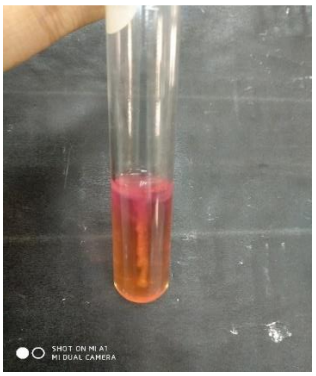

Figure No.3: Positive results for motility and urease test

Figure No 4 : Citrate test positive

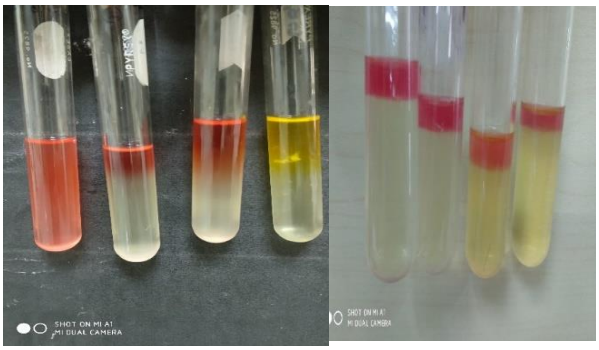

Figure No.5 : Positive results for MR/VP tests

3.4 Antibiotic Susceptibility test:

Thirty-nine different isolates were tested for antibiotic resistance using nine antibiotics. They were analyzed to determine the sensitiveness and the resistance pattern towards specific antibiotics.

All the results are listed in the table below, which shows their zone of inhibition to the nearest millimeters. The diameters of the zone around each antibiotic disc depict whether the organism is sensitive, intermediately sensitive, or resistant for that antibiotic. If the clear zone diameter is larger than the scale of the resistance diameter but smaller the scale of the susceptible diameter, it is called intermediately susceptible. (NCCL)

Table 6: Antibiotic susceptibility of different isolates for different antibiotics

Isolate No.	Antibiotic zone of inhibition (mm)								
	AMC30	AXM30	AZM15	C30	CL30	CIP5	CFM5	TE30	VA30
AC-1	S	R	R	I	S	S	R	R	S
AC-2	S	R	S	S	S	S	R	S	S
AC-3	S	S	S	R	R	S	S	S	S
AC-4	I	R	R	R	I	S	R	R	R
AC-5	R	R	R	R	I	I	S	R	R
F-1	R	R	R	S	S	S	R	I	R
F-2	R	R	S	S	R	S	R	R	R
F-3	R	R	S	R	R	S	R	R	R
F-4	I	R	S	S	R	S	I	R	R
M-1	I	R	R	S	R	S	R	I	R
M-2	I	R	S	S	R	S	R	R	R
M-3	I	R	S	S	R	S	R	I	R
M-4	R	R	S	S	R	S	R	R	R
M-5	R	R	S	S	R	S	R	R	R
M-6	I	R	I	S	R	S	R	R	R
M-7	R	R	S	S	R	S	S	R	R
M-8	I	R	S	S	R	S	S	R	R

Table 6: Antibiotic susceptibility of different isolates for different antibiotics									
Isolate No.	**Antibiotic zone of inhibition (mm)**								
	AMC30	**AXM30**	**AZM15**	**C30**	**CL30**	**CIP5**	**CFM5**	**TE30**	**VA30**
PA-1	S	S	R	S	S	S	R	S	S
PA-2	S	S	R	S	S	S	R	S	S
PA-3	S	S	I	S	S	S	R	S	S
PA-4	R	S	S	S	S	R	R	S	S
ST-1	S	S	R	S	I	S	R	S	S
ST-2	S	S	R	I	S	S	R	S	S
ST-3	S	R	R	S	I	S	S	S	S
SZ-1	S	S	R	I	S	S	R	S	S
SZ-2	S	R	S	R	S	S	R	R	R
SZ-3	S	S	R	S	I	S	R	S	S
SZ-4	S	S	R	R	I	S	R	R	S
SZ-5	S	S	R	S	I	S	R	S	S
SZ-6	S	R	R	S	S	S	R	S	S
SZ-7	S	R	R	S	I	S	S	S	S
SZ-8	S	R	R	R	S	S	R	R	R
SZ-9	S	R	S	S	S	S	S	S	S
DK-1	S	S	S	S	I	S	R	S	S
DK-2	S	R	R	R	I	S	R	R	R
DK-3	S	R	I	I	S	S	R	R	R
DK-4	S	R	I	I	S	S	R	R	I
HAR-1	S	R	R	I	R	S	R	R	S
HAR-2	I	S	R	S	I	I	R	I	S

Table No.6 showed that different isolates are showing antibiotic susceptibility for different antibiotics. Most of the isolates showed an almost equal proportion of resistance and susceptibility for all the antibiotics. Out of 39 isolates, 36 of them were sensitive to Ciprofloxacin and 31 of them showed resistance to Cefixime. A large proportion of isolates also showed resistance for Amoxicillin, Azithromycin, and Tetracycline.

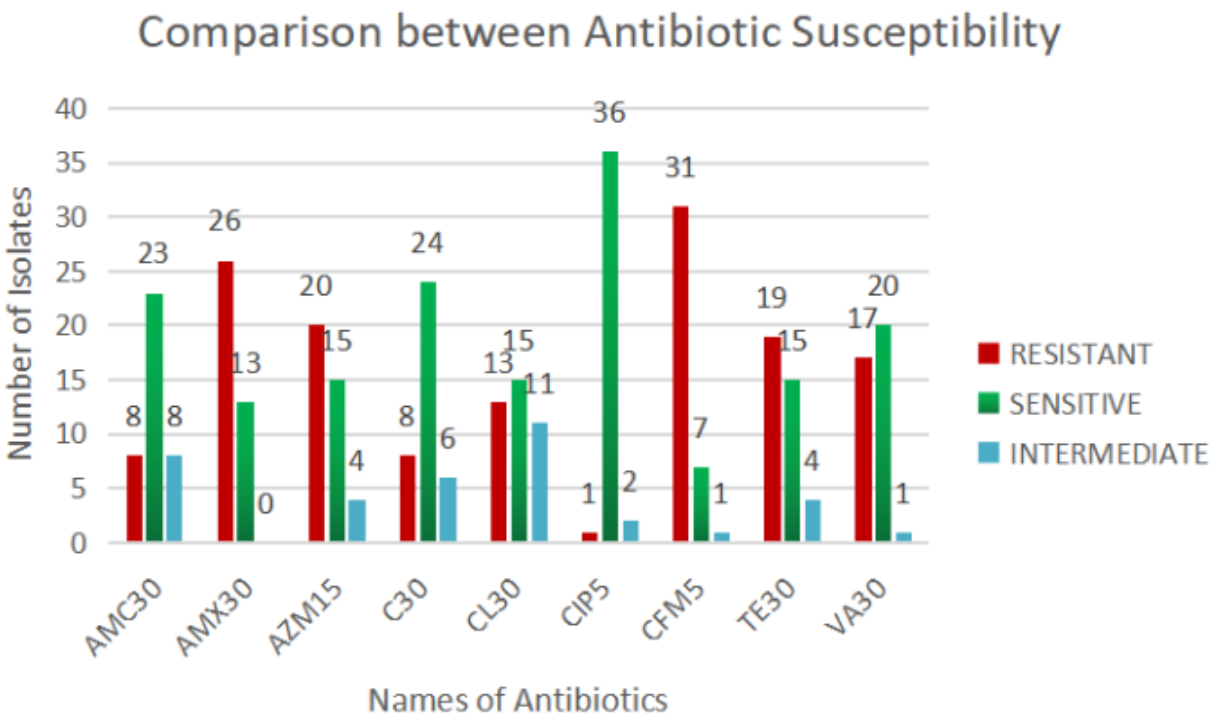

Figure No.6: Antibiotic susceptibility pattern of all the isolates from fruit juices

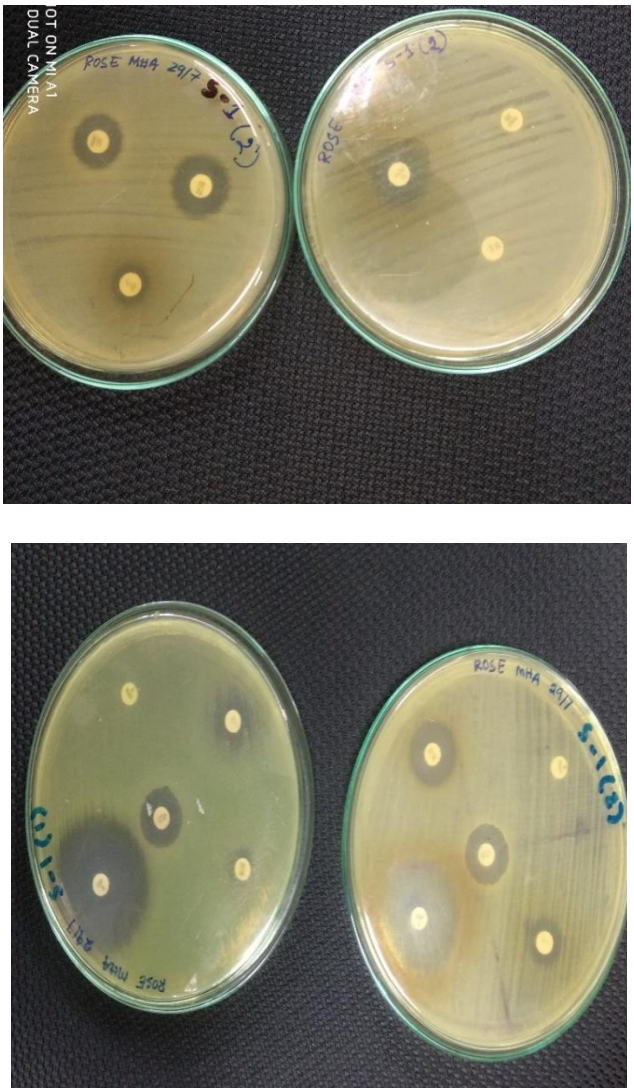

Figure No.7: Organisms showing susceptibility and resistance to different antibiotics on Mueller Hinton Agar plates.

3.5 Pathogenicity tests:

All the bacterial isolates were tested for pathogenicity. Two tests were carried out, which were the Blood agar test and the Dnase test. It was found from the test that three out of the thirty-nine isolates were pathogenic. Two of them were found in guava juice and one was found in mango juice.

Figure No.8: Blood agar test showing positive results for beta hemolysis for three organisms.

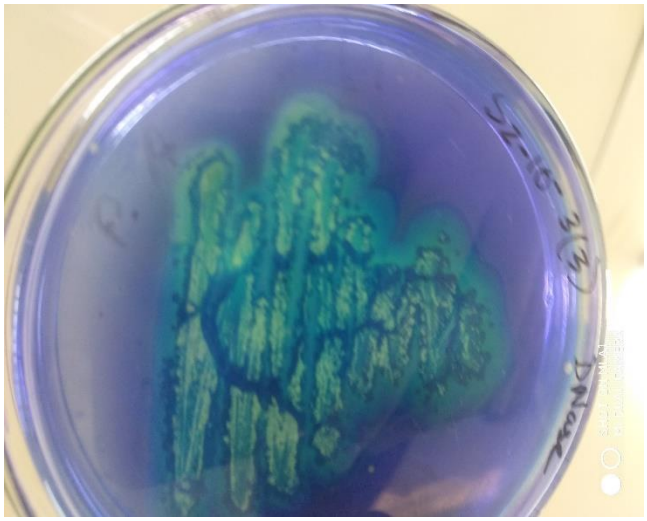

Figure No.9: Dnase test giving positive results for 1 of the 3 organisms

3.6 Gel Electrophoresis

Using the agarose gel 1% and 1Kbp ladder, the gel electrophoresis was performed for the three pathogenic isolates and was viewed under the UV.

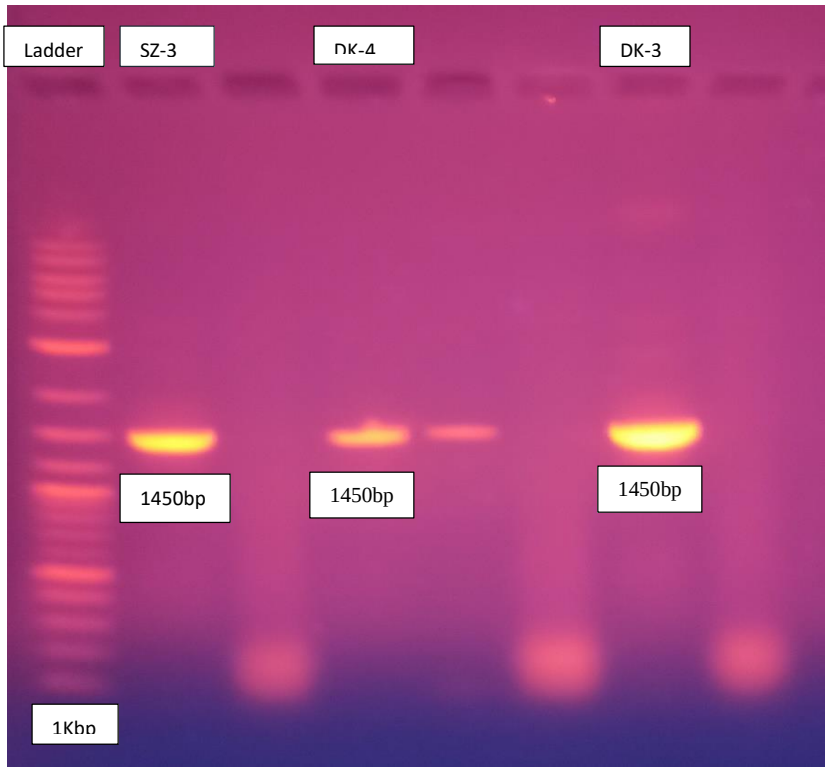

Figure No.10: **Gel electrophoresis showing the bands obtained from the 3 pathogens isolated from two different packaged juices.**

3.6 Probable effects of preservatives in fruit juices

Table 7: Probable effects of preservatives on the resistance pattern of organisms inoculated in the sample named St

	Antibiotic Zone of inhibition (mm)											
Organisms	**AXM 30**	**AM C30**	**AZM 15**	**CL30**	**CFM 5**	**CXM 5**	**C30**	**CIP5**	**LE30**	**TE30**	**VA30**	
ETEC	21	22	_	_	_	_	10.5	26	23	23	8.5	Ref
	_	_	_	_	_	_	_	_	_	_	42	Day 3
	_	11.5	18	_	_	_	26	19	18	23	20	Day 5
	_	10	18	_	_	_	22	18	18	19.5	14	Day 7
EHEC	20	33	16	_	22.5	_	27	31	27	21	_	Ref
	_	26	31	21	_	_	26	28.5	21	21	31	Day 3
	_	10	20	_	_	_	26	18	15	20.5	20.5	Day 5
	_	8	17	_	_	_	24.5	14	15	15.5	18	Day 7

Table 7: Probable effects of preservatives on the resistance pattern of organisms inoculated in the sample named St

Organisms	AXM30	AMC30	AZM15	CL30	CFM5	CXM5	C30	CIP5	LE30	TE30	VA30	
Proteus Vulgarius	18.5	16	_	_	25	_	30	23	20	_	-	Ref
	_	_	48	_	_	_	50	38.5	23	40	41	Day 3
	_	_	20	_	_	_	44	30.5	16.5	28	29	Day 5
	_	_	13	_	_	_	36	29	14	22	22	Day 7
Klebsiella Pneumonae	_	_	12	12	_	_	26	22	_	20	_	Ref
	_	17	45	_	_	_	58	42	21.5	42	42	Day 3
	_	11	29	_	_	_	44	39	21.5	29	31.5	Day 5
	_	11	23	_	_	_	41.5	34	20.5	26.5	25	Day 7
Staphylococcus	30	25	23	21	_	_	24	24	_	23	26	Ref

	Table 7: Probable effects of preservatives on the resistance pattern of organisms inoculated in the sample named St											
	_	19	22	25	_	_	28.5	25	20	24	26	Day 3
	_	9.5	19	_	_	_	11	18	17	21	20	Day 5
	_	9	12	_	_	_	11.5	14	16	18.5	19	Day 7
Salmonella Typhi	_	17.5	23	32	_	_	21	22	_	_	25	Ref
	_	_	45	28	_	_	55	40	19	33.5	41	Day 3
	_	_	20	_	_	_	27	18.5	15	22	21.5	Day 5
	_	_	17.5	_	_	_	22	17.5	15	20	20	Day 7
Shigella	17	_	14	_	_	_	23	30	_	21	-	Ref
	_	_	49	_	_	_	56	39	24	46	41.5	Day 3

	Table 7: Probable effects of preservatives on the resistance pattern of organisms inoculated in the sample named St											
	_	_	15	_	_	_	25.5	19	15	21	20	Day 5
	_	_	11.5	_	_	_	19	10	11.5	20	16.5	Day 7

In the table above, the yellow highlights shows a decrease in the diameters of the zone of inhibition from Day 3 to Day 7 of certain organisms for specific antibiotics.

Chapter IV

Discussion

Discussion:

In several communities and countries, fruit juices are now an integral part of themodern-day human diet because of their highnutritious contents and also and offer a good taste and variety ofnutrients and essential health components (Tasnimet al., 2010, Tambekar et al., 2009). Fruits also come in the forms of beverages that are consumed for their nutritional properties, thirst-quenching properties, and stimulating effect or their medicinal values (Fawole and Osho, 2002). However, fruit juice manufacturers are only concerned about the profit they make from the production of the juices and the marketing of their products rather than maintaining the quality of the product. Though they might maintain a lot of hygiene in their factories, they use a lot of preservatives and chemicals to reduce the growth of microorganisms in the juices and enhance their quality and shelf-life. Aside from their advantages, some of the artificial preservatives may possess life-threatening side effects (Anand and Sati, 2013; Mandal and Mandal, 2011; Seetaramaiah*et al.*, 2011). And these preservatives can cause a thousand times more powerful and harmful diseases to human beings in the long run. Several factors encourage, prevent, or limit the growth of microorganisms in juices; the most evident and important ones are maintaining the pH, controlled hygienic practice and storage temperature, and the concentration of preservatives. Storage of products at refrigerator temperature or below is not always best for the maintenance of the desirable quality of some fruits. Tasnim *et al.*,2010 reported that other microbial contaminants and as well as coliforms may enter fruit juices from the various water sources.

4.1 Total viable count (TVC):

The standard of the total viable count per ml of juice according to the Gulf standard for the maximum bacterial load permitted was (1.0×10^4) CFU/ ml. In this experiment, almost all the juice samples showed a higher colony count than the Gulf standard for fruit juices except for one juice named sample ST. Sample ST had a bacterial load of 3.0×10^2 CFU/ml, which was quite lower than the Gulf standard. The total viable count ranged from (3.0×10^2- 1.07×10^7) CFU/ ml shown in Table 4. The highest bacterial count (1.07×10^7) CFU/ ml was found in a mango juice (sample SZ) while the lowest bacterial count (3.0×10^2) CFU/ ml was also found in another mango juice (sample ST) showed in Table 4. This higher count of the presence of microorganisms means higher chances of contamination and threat of pathogenicity in the fruit juices, indicating bad food safety

conditions. The microbial contamination could be due to low-quality raw materials, contaminated processing equipment and environment, packaging materials, or even untrained workers. Iqbal *et al*. (2015) reported that the mean TVCs among non-pasteurized brands of packed fruit juices (6.80±1.91 CFU/ml) were non-significant with standard permissible limits ($p>0.05$). According to Rashed*et al.*,2013 highest total bacterial count of 2.66 x 10^6 CFU/ ml were found in orange juice and the lowest bacterial count of 1.59 x 10^2 CFU/ ml was found in mango juice. Odu and Adeniji, (2013) reported that total heterotrophic bacterial counts of some fruit juices ranged from 3.0 x 102 CFU/ml to 9.0 x 10^4 CFU/ml. Tasnim*et al.,* 2010 also found that the load of the viable bacteria in processes juice samples was within the standard limit.

4.2 Antibiotic susceptibility:

In this study, of the isolates showed an almost equal proportion of resistance and susceptibility for all the antibiotics that were used. There were 39 different isolated organisms from different fruit juice samples and 36 of them were sensitive to Ciprofloxacin and 31 of them were resistant to Cefixime. A large proportion of isolates were also resistant towards Amoxicillin, Azithromycin, and Tetracyclin. Oladipo et al. (2010) found that 65% of the microorganisms isolated were resistant to the antibiotic used while 35% were sensitive. It was also observed that all the organisms were resistant to Ciprofloxacin and Amoxycillin.

Rashed et al. (2012) found that the *E.coli* isolates were highly resistant against Ciprofloxacin (61%), *Klebsiella spp.* showed higher resistance against Ampicillin (74%), Ciprofloxacin (86%), and Amoxicillin (72%). *Staphylococcus spp.* showed resistance against Ampicillin (93%), Amoxicillin (92%), and Vancomycin (63%). However, in this study, the highest percentage of sensitivity was towards Ciprofloxacin (92%).

4.3 Pathogenicity test:

Among all the 39 organisms isolated, 3 of them were pathogenic which were proved by carrying out two pathogenicity tests; blood agar test and DNase test. Two of the organisms were isolated from a guava juice (sample DK) and one was isolated from a mango juice (sample SZ). Three of these isolates showed beta hemolysis in sheep blood agar and as well as were able to hydrolyze DNA by producing DNase enzymes.

4.4 DNA Extraction:

The DNA was isolated only from the three pathogenic strains of organisms that were found from two different fruit juices using the pathogenicity tests. A DNA extraction kit was used to obtain the DNA from the isolates.

4.5 PCR and Gel Electrophoresis:

To amplify and optimize the DNA extracts, Polymerase Chain Reaction was conducted using the standard protocol for PCR. The universal primers 27F and 1492R primers were used in the reaction. Followed by PCR, gel electrophoresis of the PCR products were performed and 3 visible bands were obtained under the UV lights. This indicated that the PCR was successful and the 3 pathogenic isolates comprised of the DNA which will be further used for rigid identification and specification of the pathogenic strains by genome sequencing.

4.6Effects of preservatives in commercially packaged juice:

A study has been conducted to investigate and find out the probable effects of additive preservatives into fruit juices commercially made in Bangladesh. The juice that was used in this experiment was sample ST. This particular juice sample was chosen among the rest because it had a very low number of viable bacterial counts in the first place. In the study, it was observed that the more number of days the inoculated organisms were in contact with the juice, the smaller was their zone of inhibition that grew around the antibiotics discs that were placed. However, this was not the case for all the antibiotics. Some did not show this pattern in their zone size but most of the organisms did. In the table, *Salmonella Typhi*showed a decrease in the zone size from 33.5mm to 22mm to finally 20mm for day 3, day 5, and day 7 respectively for the Tetracyclin antibiotic. Whereas, for Levofloxacin there was no change in the zone of inhibition diameter and remained at 15mm for day 5 and day 7. Previous studies have reported the absence of any viable microorganisms in fruit juice samples (Ghengesh*et al.*, 2005; Jackson *et al.*, 2010; Odu and Adeniji, 2013). These findings suggested the use of a higher amount of preservatives in fruit juice preparations that had a bacteriostatic effect on microbes. According to Basar and Rahman, (2007) it can be suggested to use a low amount of preservatives during fruit juice manufacturing.

4.7 Conclusion:

From all the data and analysis in this present study, it could be assumed that most of the juices that are commercially manufactured in Bangladesh are very much prone to contamination by microorganisms. The presence of three pathogenic strains indicates the low safety levels and unhygienic management. Even though automated machines and preservatives are being used, the presence of pathogenic organisms proves that the plant management facilities are very poor and not well handled. Proper training of workers, monitoring, and maintaining strict rules and regulations can be implemented to minimize the health risks associated with fruit juice manufacturing industries. Government-authorized institutions can also undertake precautionary investigations to check the microbial quality of these products. The local people can be made aware of the adulteration of fruit juices by campaigns or through social media before they consume these fruit juices.

Reference

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