Microbiological evaluation of natural juices in the city of Benghazi

BY
Munya Ahmed Hasan Bilsheekh

Supervisor
Dr. Ismaeel Hussein Bozakuok

A thesis submitted in partial fulfillment of the requirement of

M.SC. Degree in Botany

Benghazi University

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Declaration

I Munya Ahmed Hasan Bilsheekh confirm that what the thesis contains is the result of my own effort, except for what has been referred to where it was mentioned, and that this thesis has not been submitted before to obtain any academic degree or scientific research.

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بِسْمِ اللهِ الرَّحْمَنِ الرَّحِيمِ

﴿إِنَّا فَتَحْنَا لَكَ فَتْحًا مُبِينًا (1) لِيَغْفِرَ لَكَ اللَّهُ مَا تَقَدَّمَ مِنْ ذَنْبِكَ وَمَا تَأَخَّرَ وَيُتِمَّ نِعْمَتَهُ عَلَيْكَ وَيَهْدِيَكَ صِرَاطًا مُسْتَقِيمًا (2)﴾

صدق الله العظيم

سورة الفتح، الآية (1-2)

Dedication

To the soul of my father Ahmed BilSheekh ... May God have mercy on him

To my support and the source of my strength ..... my dear mother

To my companion ... my dear husband

To whom I am proud ... my supervisors,

Dr. Ismaeel Bozakuok

To my sisters

To all my friends

I dedicate the fruit of my humble effort.

Munya Bilsheekh

ACKNOWLEDGMENTS

Praise be to God, Lord of the worlds, and prayers and peace be upon the Messenger of the Lord of the Worlds, Muhammad al-Sadiq al-Amin, and upon God and his good companions. I extend my sincere thanks and gratitude to my distinguished professor, Dr. Ismaeel Bozakuok, for his preference for me in proposing and supervising the research topic, and for his enlightened and sound guidance provided throughout the research period and for writing the thesis, which was the best example to be emulated in scientific research. I also extend my sincere thanks to all the doctors of the Botany Department for their help and interest, may God reward them on our behalf. I would like to extend my thanks and gratitude to my colleagues and to everyone who helped me with a word or deed, intentionally or unintentionally, may God reward them with good.

Munya Ahmed Bilsheekh

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List of acronyms

AIDS	Acquired immunodeficiency syndrome
Aw	Water activity
C°	It is a unit of measurement for temperature and is symbolized by the symbol ° C or Celsius
CDC	The Centers for Disease Control and Prevention is the national public health agency of the United States
CFU	A colony-forming unit is a unit used in microbiology
CHF	Congestive heart failure is a chronic progressive condition that affects the pumping power of your heart muscle
CNS	Coagulase-negative staphylococci
CO_2	Carbon dioxide
CPS	Coagulase positive staphylococci
EC	The European Commission (EC) Regulation
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
FAO	The Food and Agricultural Organization
FDA	Food and Drug Administration
GAPs	Good agricultural practices
GHPs	Good hygienic practices
HACCP	Hazard Analysis Critical Control Point System
HUS	Hemolytic uremic syndrome is a condition that can occur when the small blood vessels in your kidneys become damaged and inflamed
IARC	International Agency for Research on Cancer
ICMSF	International Commission on Microbiological Specifications for Foods
O3	Ozone is a highly reactive gas composed of three oxygen atoms. It is both a natural and a man-made product that occurs in the Earth's upper atmosphere
PH	"potential of hydrogen" is a scale used to specify the acidity or basicity of an aqueous solution
RTE	Ready-to-eat foods are a group of food products that are pre-cleaned, precooked, mostly packaged and ready for consumption without prior preparation or cooking
SFP	Staphylococcal food poisoning

STEC	Shiga toxin-producing *E. coli*
USFDA	United States Food and Drug Administration is a federal agency of the Department of Health and Human Services
UTI	Urinary tract infections are common infections that happen when bacteria, often from the skin or rectum, enter the urethra, and infect the urinary tract
WHO	World Health Organization is a specialized agency of the United Nations responsible for international public health

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Abstract

Fruit juice contains essential nutrients, minerals, antioxidants, and vitamins for overall health. Products such as fruits and vegetables are normal part of the human diet and are consumed in large quantities in most civilizations. However, food borne diseases related to fruit and fruit products are increasing and became very serious problems in different parts of the world. Therefore, the main objective of this study was to evaluate the bacteriological quality of types of fresh fruit juices available to the consumers in the city of Benghazi. The study was conducted in the end of summer period (September, October 2020 "the first isolation") and (September, October 2021 "the second isolation") for 128 randomly selected samples from sixteen cafes and restaurants in the city of Benghazi. This study was isolate and identify pathogenic bacteria that contaminate fresh juices, for two types of juice most popular among children and adults (strawberry juice and mango juice). All bacteria that were isolated in this study were identified by the appearance of the colonies and through biochemical tests, and some isolates were identified by phoenix 100. The results showed that the bacterial growth rate in the tested samples of fresh juices was (91.41%). This study has revealed that the most predominant pathogens isolated of the fresh juices (strawberry juice and mango juice) were *Klebsiella pneumonia* (36.21%), followed by *fecal Escherichia coli* (31.03%), *Pseudomonas aeruginosa* (12.93%), *Escherichia coli* (7.76%), *Enterobacter aerogenes* (6.89%), *Staphylococcus aureus* (4.31%), *Staphylococcus schleiferi sp.* (1.72%). The study showed that there is a diversity in bacterial isolate between the first and second isolates. For the susceptibility of the isolated pathogens to the antimicrobial agents the result showed the effectiveness of antibiotics on Gram-negative pathogens were resistant to the following antibiotics including Amoxicillin and Amikacin, Clarithomycin, while other antibiotics were more effect. The antibiotics sensitivity for Gram-positive bacteria showed that the pathogen

represented high resistant rate compare to Gram-negative isolates, where the bacterial isolates showed high resistance to the following including Ciprofloxacin, Clarithomycin, Imipenem, Amikacin, Amoxicillin, Oxacillin, Cefixime, Cephalexin.

Chapter 1
1. Introduction

In a very broad sense the term fruit refers to the mature ovary of a plant, including its seeds, covering and connected tissue. This includes both fleshy and dry fruits (IARC, 2003). Fruit juice are defined in the most general sense as the extractable fluid contents or tissues of the fruit or aqueous liquid squeezed or extracted usually from one or more fruit fruits (Bello *et al*., 2014). It is prepared by mechanically squeezing or macerating fruit or vegetable flesh without the application of heat or solvents and which are very nutritive, invigorating and non-alcoholic beverage, which is very popular throughout the world (Fruit juices regulation, England). These drinks are sold all the year round except winter season. Unpasteurized juices are preferred by the consumers because of the "fresh flavor" attributes and low cost. They are simply prepared by mechanically squeezing fresh fruits or may be extracted by water. The final product is an unfermented, clouded, untreated drink, ready for consumption. The drinks are nutritious for man. These are also good medium for the growth of many microorganisms some of which may be pathogenic to man. The pathogenic organism can cause various food borne diseases. Consumption of fresh fruit juices are increasing day by day. In addition to their increasing popularity in consumption patterns, fresh fruits and vegetables have also become increasingly important vehicles in food borne disease statistics (Sivapalasingam *et al*., 2004).

It is important source of bioactive compounds such as phenolics (e.g. flavanone glycosides, hydroxycinnamic acids), vitamin C is naturally present in juices which are essential for the body to form collagen, cartilage, muscle, and blood vessels. It also helps in the absorption of iron, and carotenoid which is an excellent source of bio available antioxidant phytochemicals and it also helpful to improves blood lipid profiles especially the people affected with hypercholesterolemia (Franke *et al*., 2007 and Tasnim *et al*., 2010). They are very scrumptious and palatable and they have most of the minerals like calcium, magnesium, phosphorus, and sodium and vitamins (FDA, 1999 and Ogodo *et al*., 2016). Depending upon further processing fruit juices either unpasteurized or pasteurized. Unpasteurized juice does not undergo further treatment like thermal processing, it is simply made from fruits that are ground and/or pressed or squeezed to extract the juice. This is to maintain its original test and flavor. Unpasteurized fruit juice was considered nonhazardous due to its freshness, acidic

nature (Gahan *et al*., 1996). Pasteurization is relatively mild heat treatment killing vegetative cells of pathogenic microorganisms that impact food safety. Food safety is the assurance that food will not cause any harm to the consumer when it is prepared and/or consumed according to its intended use. Fruit juice is pasteurized to kill those harmful microorganisms and to extend shelf-life (Health Canada, 2007). Food security is a complex issue, which is influenced by a number of factors. The Food and Agricultural Organization (FAO) of the United Nations and the World Health Organization (WHO) state that illness due to contaminated food is perhaps the most widespread health problem in the contemporary world (Edema *et al*., 2005).

In under develop countries juices are sold in the form of either in tetra pack which is pasteurized while streets vendors juices called as fresh juices which provide substantial amount of valuable nutrients at affordable. Most people can enjoy unpasteurized juice and drinks, however, for young children, the elderly and people with weakened immune systems, the effect can be severe or even deadly. Most fruit juices contain sufficient nutrients that could support microbial growth. Several factors encourage, prevent, or limit the growth of microorganisms in juices; the most important are, pH, hygienic practice and storage temperature and concentration of preservative (Esteve *et al*., 2005 and Troller, 1983).

The farmers are attracted toward cultivating different kinds of fruits as well (Abadias *et al*., 2008). Therefore, economical contribution of fruit production by the study area is great enormous. Additionally, the consumption of fruit juices could have both positive and negative effect on the part of consumers. Fruit juices are well recognized for their nutritive value, mineral, and vitamin contents. Fruit juices processed under hygienic condition could play important role in enhancing consumers, health through inhibition of breast cancer, congestive heart failure (CHF), and urinary tract infection (Ketema *et al*., 2008 and Weleni, 2017). On the other hand, there are reports of food borne illness associated with the consumption of fruit juices at several places (Chumber *et al*., 2007). In many communities, fruit juices are becoming an important part of the modern human diet because they are highly nutritious and offer a good taste and variety of nutrients (Tambekar *et al*., 2009 and Tasnim *et al*., 2010). Now a days consumers are more focused towards more nutritious and safe food emphasizing the need for microbiological analysis (Tumane *et al*., 2011). This has led to the popularization of natural fruit juices as an alternative to other beverages. As the food which we eat is either of plant or animal origin thus it is undesirable that our food

supply contains microorganisms in interaction with food. When the microorganism involved is pathogenic, it poses a serious problem as this may lead to food poisoning outbreaks. In the absence of good hygienic practices (GHPs), juice being rich in nutrients favors the growth of microorganisms leading to spoilage of fruit juices and acting as a vehicle for many food borne pathogens and other associated complications (Al-jedah *et al*., 2002 and Tsige Ketema *et al*., 2008).

Water used for preparation of fruit juices can be a major source of contamination (Tasnim *et al*., 2010). Environmental for mites may also make the fruits unsafe and these may have a role in spreading of *Salmonella, Shigella, Vibrio, Escherichia coli,* total *Coliforms, fecal Coliforms,* and other diseases causing as well as fruits spoilage types. Spoilage yeasts, such as *Saccharomyces cerevisiae, Candida lipolytica and Zygosaccharomyces sp*., can tolerate acidic environments (VanDam and Seidell, 2007).

Fruits and vegetables that have been minimally processed have unprotected skin and cell walls, as well as being considered low calorie food, they are rich in fiber and provide a great variety of vitamins, minerals, and other phytochemicals (Sarjo *et al*., 2006). However fruits and vegetables are widely exposed to microbial contamination through contact with soil/dust and water and poor handling at harvest or during postharvest processing. They create favorable condition for diverse range of microorganisms including plant and human pathogens (Nguyen and Carlin, 1994), and the fluid components are easily contaminated by air and microorganisms from the environment (Abbo and Odeyemi, 2006). Food handlers have transient bacteria such as, Staphylococcus aureus resident on their hair, skin, throats, and nasal passages (Centre for food safety fruits for sale or serving in retail outlets, 2006). Bacteria find their way into the juice during processing when no protective apparel is used during food handling (Mihajlovic *et al*., 2013). Bacteria are also introduced into food products due to the frequent immersion of hands in water resulting in soreness and damage to the skin causing wounds (Centre for Food Safety, 2006). Wounds are good sites for bacterial pathogens especially when food handlers use bare hands. When food handlers prepare juices with bare hands, these pathogens come in contact with the juice. Bacterial transfer is facilitated through physical contact when wet hands are used during food preparation (Redmond and Griffith, 2009).

Microorganisms are also introduced through direct contact with animal or human waste or indirectly with contaminated water, soil, processing equipment which can lead to spoilage of the juice (Mihajlovic *et al*., 2013). For economic reasons, water

used in the washing of processing equipment and utensils may be recycled, further contaminating the product resulting into spoilage (Muyanja *et al*., 2011). Juice spoilage leads to deterioration of organoleptic and physicochemical parameters causing rejection of the product by consumers leading to financial losses by the processor. On a more serious note, contamination may also be a potential microbiological health hazard to the consumer. Increase in pH from 6 to 7 is optimum for the growth of mesophilic bacteria. Increased numbers of bacteria lead to the accumulation of metabolic by products leading to bio deterioration of juices and possibly spoilage. Vital nutrients such as antioxidants, vitamins A, C, E, decreased shelf life due to deteriorative reactions such as microbial spoilage, development of off flavors, change in color, texture, or appearance leads to degradation of the product, making it unacceptable to consumers (Amiri Sedigheh, 2008). Consumers in universities, offices, schools, shops, markets, roadside stalls, motorists, and even travelers may fall victim to contaminated juice resulting in foodborne illnesses. In addition, vendors do not adhere to good manufacturing practices and some lack proper personal hygiene during juice processing. High microbial numbers in fresh unpasteurized juices from unhygienic processing techniques increase consumers exposure to health hazards.

Epidemiological data indicate that cross-contamination during food preparation contributes notably to the occurrence of food borne diseases. *Cholera* transmission was associated with consumption of Street Vended beverages in Peru and Guatemala (ICMSF, 1980 and Ries, 1992). The unpasteurized juices have been shown to be a potential source of bacterial pathogens notably, *Salmonella, E. coli O 157:H7* (Koo *et al*., 1996; Ryu *et al*., 1998 and Uljas *et al*., 1998). Nowadays, ready to eat (RTE) foods like vegetable salad and fruit juices constitute a suitable and convenient meal for today's lifestyles, because they need no cooking or further preparation. Contamination of food products can result in many health problems ranging from mild bloating and gas to serious incidents of food poisoning and dehydration. Unsafe and non-hygienic fruits consumptions cause serious outbreaks of food borne illness (Sivapalasingam *et al*., 2004). There have been some notable outbreaks of illness in recent years that demonstrate the increasingly important role of fresh fruits and vegetables in foodborne disease (Asghar *et al*., 2018; Parish, 1997; Sandeep *et al*., 2001 and Tasnim *et al*., 2010). There are several reports of illnesses due to the food borne diseases associated with the consumption of fruit juices at several places around the globe (Chumber *et al*., 2007; Mosupye and Holy 2000 and Muinde and Kuria 2005). Usually raw materials,

equipment's, hand of the handlers, containers etc., are responsible for contamination. Contamination from raw materials and equipment's, addition a processing conditions, improper handling, prevalence of unhygienic conditions contributes substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables (Oliveira *et al*., 2006 and Nicolas *et al*,. 2007).

Many microorganisms are found in fresh fruits and fruit juices as environmental or raw material contaminants, but actually, very few of them can survive the acidic nature of the juices. Low oxygen levels also play an inhibitory effect on contamination of packaged products. Thus, contamination risk of unpackaged products with pathogen microorganisms is more likely than the contamination of processed products. Yeasts are the most significant group of microorganisms associated with spoilage of fruits and fruit juices. Their metabolic by products, for example, CO_2, acid, and tainting compounds can cause alterations in taste, smell and appearance of the products. Even though the most spoilage is caused by yeasts and mold species in fruit-related products, acid tolerant bacteria can also play a minor role in spoilage of such products (Hocking and Jensen, 2001 and Jay and Anderson, 2001). Microorganisms initially observed on whole fruit and vegetable surfaces are soil inhabitants. For example, human and animal enteric pathogens (except soil borne spore formers such as *Bacillus cereus* and *Clostridium perfringens*) are usually absent from fresh vegetables and fruits at harvest unless they have been fertilized with human and animal wastes or irrigated with contaminated water with such wastes (Bryan, 1997). Microbial profile of fruits and vegetables are direct reflection of the sanitary quality of the cultivation, harvesting, transportation, storage, and processing of the produce (Andrew and Harris, 2000 and Janisiewicz and Korsten, 2002). The difference in the microbial profiles of fruits and vegetables also result largely from unrelated factors like resident micro-flora in the soil and nonresident micro flora through animal manures, sewage or irrigation water, transportation and handling by sellers (Ofor *et al*., 2009 and Ray and Bhunia, 2007).

The nutrient contribution of consuming minimally processed products like juices is very important, making it necessary to ensure the quality and safety of these products, since their management and preparation makes them highly perishable due to the presence of fungi and yeasts (Das *et al*., 2010). The presence of coliforms and antimicrobial resistant pathogens have been identified in fresh foods and unpasteurized fruit juices have been reported as vehicles of foodborne outbreaks of *Escherichia coli*, *Staphylococcus aurus*, *Cryptosporidium*, *Listeria monocytogenes*, *Campylobacter*

jejuni, Candida, sp., and *Acetobacter*, among others (Reinders *et al*., 2001; Hanashiro *et al*., 2005; Ramos *et al*., 2010; Guven *et al*., 2010; Baragón *et al*., 2013; Aneja *et al*., 2014; Callejón *et al*., 2015 and Hossen *et al*., 2020). Fruit juices are mostly contaminated with *Staphylococcus aureus, Entrobactersp, Klebsiella sp., E. coli, Salmonella typhi* and *Serratia sp. Escherichia coli* is one of the most common human pathogen that cause several diseases such as diarrhea, kidney failure, pneumonia, skin infection, respiratory disease, meningitis, food poisoning etc. This is mostly in immune compromised people (Heaton and Jones, 2008). In the absence of good manufacturing and hygienic practice the nutritional richness of fruit juices makes the product good medium for bacterial growth (Al-jedah *et al.*, 2002).

The practice of consuming fruit and vegetable juices cannot be stopped on unhygienic grounds or prohibited from selling such items, since it is a source of their livelihood (Tambekar *et al*., 2009). Despite of the potential benefits offered by fruit juices, concerns related their quality and safety have been raised; as freshly prepared juices have no preliminary steps or process to minimize microorganisms if they are contaminated (Saroj *et al*., 2006).

1.1.1 Hypothesis

Fresh juice products are safe for local consumption.

1.1.2 Alternative Hypothesis

Studies have shown high rates of microbes in fresh juices and therefore should checked for pathogens presence.

1.2 Aims of the study

1.2.1 Isolation of foodborne pathogens that contaminate fresh juices (strawberry juice and mango juice).

1.2.2 Frequency and prevalence of the bacteriological load (bacterial contamination) into different types of fresh made natural juices.

1.2.3 Antimicrobial susceptibly testing.

Chapter 2
2. Literature Review

2.1 Fruit juice

In simple words, juice is the extractable fluid contents of cells or tissues. It is defined as fermentable but unfermented juice, intended for direct consumption, obtained by the mechanical process from sound, ripe fruits, preserved exclusively by physical means. The juice may be turbid or clear. The addition of sugars or acids can be permitted but must be endorsed in the individual standard (Bates *et al*., 2001; Bevilacqua *et al*., 2011 and ICMSF, 2005). Fruits and vegetables form a versatile and complex substance group category of foods. The relevant substance groups are carbohydrates, acids, minerals, polyphenols tannins, including the colourful anthocyanins, water soluble vitamins, amino acids, aroma compounds, carotenoids, fibers and other bioactive substances. During processing, they are essentially transferred into the pressed juice or into the puree (Bates *et al*., 2001).

Mitchell *et al*., 2020 reported that 100% natural fruit juice contains over 5% of the recommended dose of Vitamin C, folic acid, magnesium, and potassium. In addition, street foods have acquired relevance in consumption for its freshness, pleasant flavor and low cost (Asiegbu *et al*., 2016; Cortese *et al*., 2016; Ferrari *et al*., 2021; Gupta *et al*., 2018 and Soon, 2019). Fruit, in botanical terms is freshly or dry ripened ovary of a plant, which encloses the seed or seeds. The fleshy component, which is normally the portion eaten, serve to protect and eventually nourish the seed as part of the natural development of the original plant's progeny (FAO, 2001). Fruit juices, either fresh or processed, form an important part of our daily diet, and demand is increasing in all over the world. Recent advances in agricultural technology have contributed significantly to the production of fruits throughout the world. Fruits are very perishable in nature because they are living beings and carry out transpiration, respiration, ripening and other biochemical activities which adversely affect the quality. In addition, because of their high moisture content (in an average 85%) fruits are inherently liable to deteriorate, especially under tropical conditions, and finally become unmarketable (Titarmare *et al*., 2009). Fruits are not only colorful and flavorful components of our diet, but they also serve as a source of energy, vitamins, minerals, dietary fiber and antioxidants. They are very low in fats and proteins but high in sugar as they contain large amount of glucose, fructose, and sucrose. In addition, most fruits are often consumed fresh due to their cherished flavor, pat ability and they contribute immensely

to nutrients intake. On the other hand, the increased consumption of fruits and vegetables in recent years has been found to be accompanied by an increase in the number of human infections and outbreaks (Olaimat and Holley, 2012). As these can serve as reservoirs for pathogens or opportunistic pathogens (Berg *et al*., 2014). Fruits and vegetables can be contaminated with spoilage or pathogenic bacteria at any stage from production to consumption (Zahra *et al*., 2016). Although their micro flora is dominated by spoilage bacteria, yeasts, and molds, fruits and vegetables can harbor pathogenic bacteria such as *Salmonella*, *Escherichia coli*, *Bacilluscereus*, *Campylobacter sp*., *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Clostridium botulinum*, as well as some viruses and parasites (Beuchat, 2002).

In Royal Hospital, Oman, May 2008, *B. cereus* caused a nosocomial outbreak with gastroenteritis and affected 58 individuals. *B. cereus* and its toxin were found in different foods including vegetables (Al-Abri *et al*., 2011). A more serious outbreak, May–July 2011, was caused by *Shiga* toxin producing *E. coli O104:H4* in Germany where 2987 cases of gastroenteritis, 855 cases of hemolytic uremic syndrome, and 53 deaths were reported. Fenugreek sprouts were found to be contaminated with the causative agent (Hauswaldt *et al*., 2013). Bean sprouts were linked to two outbreaks in USA, in 2014; one was caused by *Listeria monocytogenes* in August while *Salmonella Enteritidis* caused the other one just a month later. *L. monocytogenes* was also involved in another outbreak that was linked to caramel apples in October 2014 in USA (CDC, 2014). Opportunistic pathogens can cause life-threatening infections mainly in immunocompromised people but they may have positive effects on the health of immunocompetent individuals by stimulating immune functions and priming the immune system continuously (Berg *et al*., 2014). Nonpathogenic microbes associated with fruits and vegetables may have various consequences on the quality of the produce by affecting the rate of the food spoilage. Fruits and vegetables seem also to be the sources that disseminate many microbes to food preparation areas (Leff and Fierer, 2013).

2.2 Health benefits of fruit juices

Fruit juice based diets have been very popular recently. However, well designed controlled research studies with clinical outcome measures providing scientific evidence of potential health benefits of juice only diets are limited (Horne *et al*., 2015). The consumption of vegetable, fruit juice during the abstinence from food provides essential nutrients and improves compliance. Fruit and vegetables are rich sources of several

biologically active components that contribute to general health and decrease the risk of chronic diseases such as cardiovascular disease (Tome and Visioli, 2015). They are the most ubiquitous source of phenolic compounds (Abuajah *et al*., 2015). Polyphenols exert a variety of physiological effects in vitro including antioxidative, immunomodulatory and antimicrobial activities (Li *et al*., 2014). The absorption of polyphenols in the small intestine is limited and considerable amounts of these polyphenols can be found in the colon. There the colonic bacteria metabolize polyphenols to smaller compounds, which in turn alter the abundance of bacteria in the intestinal microbiome. In addition, fruit and vegetable are rich in fermentable fiber with prebiotic activity. High fiber intake is associated with decreased risk of cardiovascular disease, type two diabetes and some forms of cancer (Dahl *et al*., 2017). Fiber is composed of oligosaccharides, which resist digestion in the small intestine and are transported to the colon where they provide energy for gut bacteria. Growing evidence is demonstrating the role of the microbiota in the health benefits of dietary fiber consumption. In the human intestine the gut microbiota is an important contributor to human health and has been implicated in the development of obesity and obesity related diseases such as diabetes and cardiovascular disease. The two most abundant bacterial phyla in humans and in mice are Firmicutes (40–60%) and Bacteroidetes (20–40%) with lower abundance of *Actinobacteria, Fusobacteria, Proteobacteria* and *Verrucomicrobia*. Recent studies show that dietary interventions with polyphenol rich extracts and foods, including dealcoholized red wine polyphenols, cocoa-derived flavanols, quercetin and grape anthocyanins, modulate the human gut microbiota by decreasing the abundance of Firmicutes and increasing Bifidobacteria, *Lactobacillus* and *Verrucomicrobia*, which is also a key difference in the gut microbiota found in obese and lean individuals (Susanne *et al*., 2017).

Consumption of fruits and vegetables helps to prevent many degenerative diseases such as cardiovascular problems and several cancers. Decades of research have found that fruits and vegetables are crucial dietary components that can help to reduce the risk for numerous chronic diseases which, in many cases, have been shown to be initiated by long term inflammation. Fruit juices contain low sodium and high potassium which help in maintaining normal blood pressure and absence of fat in fruit juices is beneficial for the cardiovascular system. Many reports have revealed that fruit juices may play an important role in slowing the progress of Alzheimer's disease and

development of cancer (Cutler *et al*., 2008; Dai *et al*., 2006; Delichatsios and Welty, 2005; Holt *et al*., 2009; Kyle *et al*., 2009).

Fruit juice are defined in the most general sense as the extractable fluid contents or tissues of the fruit or aqueous liquid squeezed or extracted usually from one or more fruits (Bello *et al*., 2014). Fruit juices are prepared mechanically by squeezing or macerating the pulp of fresh fruits or vegetables without application of heat or solvent to give an unfermented cloud, un clarified and untreated juice ready for consumption. A common practice like diluting or blending in fruit juices preparation determine the strength of acidity or flavor (Asha *et al*., 2014). Depending upon further processing fruit juices either unpasteurized or pasteurized. Unpasteurized fruit juice does not undergo further treatment like thermal processing, it is simply made from fruits that are ground and/or pressed or squeezed to extract the juice. This is to maintain its original test and flavor. Often it can be prepared or purchased as freshly from local market, orchards, farmers and juice houses. Unpasteurized fruit juice was considered free from bacteria due to its acidic nature (Gahan *et al.,* 1996).

Pasteurized fruit juices, Pasteurization is relatively mild heat treatment killing vegetative cells of pathogenic microorganisms that impact food safety. Fruit juice is pasteurized to kill those harmful microorganisms and to extend shelf life (Health Care Canada, 2007). Not only the locally prepared, fresh fruit juices but also some times pasteurized juices are important problem in resulting food borne illness. A study conducted in Kumasi, Ghana, on the fresh minimally processed fruit juices and vegetable salad, it is microbial profile indicate significant increase in bacteria load in the apple and mango fruit juices as they stayed for a long period in shelves (Abadias *et al*., 2008).

2.3 Microbial contamination of fresh food

Fresh fruit and vegetables are an essential component of a healthy and balanced diet. Their consumption has increased worldwide in recent years (Betts, 2014) as a result of the promotion of healthier lifestyles. In many cases, these commodities are ready-to-eat (RTE). Consumers often do not subject these foodstuffs to any processing step prior to consumption to ensure the effective removal or inactivation of contaminants such as chemical residues or pathogenic microorganisms. Their increased consumption, allied with the globalization and large scale of production of RTE foodstuffs (Olaimat and Holley, 2012), has resulted in longer distribution times and greater distribution distances, which increases the complexity and importance of food

safety management (Kirezieva *et al*., 2015). Notably, the number of produce related disease outbreaks has risen in recent years (Critzer and Doyle, 2010; Hoelzer *et al*., 2012 and Olaimat and Holley, 2012). Reports by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) show an increasing trend in the implication of foodstuffs of non-animal origin on the total burden of foodborne outbreaks in Europe. In 2006, foodstuffs of non-animal origin accounted for 5.2% of the total number of foodborne outbreaks with confirmed etiology (EFSA and ECDC, 2007), and this figure had increased to 20.2% in 2010 (EFSA and ECDC, 2012). In 2016, these foodstuffs accounted for 13.1% of the total reported foodborne outbreaks (EFSA and ECDC, 2017). Similarly, the same trend is observed in data published by the Centers for Disease Control and Prevention (CDC), regarding foodborne outbreaks in the United States. RTE foodstuffs (specifically fruits and nuts, leafy vegetables, root vegetables, sprouts, and vine-stalk vegetables) were responsible for 6.7% of total foodborne disease outbreaks reported by the Foodborne Disease Outbreak Surveillance System between 1998 and 2008 (Gould *et al*., 2013). The contribution of these foodstuffs to reported foodborne disease outbreaks had increased to 9.7% in the time period between 2011 and 2015 (CDC, 2014a, 2014b, 2015, 2016, 2017).

Microbial contamination of fresh produce has been widely reviewed in the literature (Alegbeleye *et al*., 2018; Berger *et al*., 2010; Gutierrez-Rodriguez and Adhikari, 2018; Julien-Javaux *et al*., 2019; Miceli and Settanni, 2019 and Mritunjay and Kumar, 2015). When considering the farm-to-fork chain, microbial contamination of fresh produce can occur at multiple steps (Matthews, 2013).

Contamination during cultivation can be caused by contaminated soil. Soil intended for crop production is often amended with treated or untreated animal manure/human bio solids applied as fertilizers, which serve as a cost effective nutrient source, although able to harbor pathogenic microorganisms (Gutierrez-Rodriguez and Adhikari, 2018 and Julien Javaux *et al*., 2019). Transport of microorganisms from contaminated soil to produce can be mediated by splashing originating from water droplets. Both rain and irrigation water droplets have been shown to carry over soil particles to the surface of plants, leading to produce contamination (Allende *et al*., 2017 and Girardin *et al*., 2005). Direct contact of plant surfaces with manure is also a source of contamination (Alegbeleye *et al*., 2018).

Water can be an important source of microbial contamination, water used for irrigation of produce, as well as pesticide application, cooling, or protection of crops from frost, can be sourced from municipal water supplies, groundwater, recovered rainwater, surface water, or reutilized wastewater (Jongman and Korsten, 2018). Water sources of poorer microbiological quality (surface water and reutilized wastewater) can be a source of contamination of fresh crops. Furthermore, the type of irrigation utilized in a given crop also influences the potential for microbial contamination of fresh produce (Alegbeleye *et al*., 2018). Meteorological conditions are an important factor to consider regarding microbial contamination of produce. The influence of extreme weather events is well documented and reviewed in literature. Extreme rain can lead to flooding of terrains or run off events that can result in contamination of crops. On the other hand, extreme drought conditions can lead to the utilization of water of lower microbiological quality, due to the lack of potable water, increasing the chances of contamination (Yeni and Alpas, 2017). Microbial contamination of produce has also been shown to be dependent on growth season (Marine *et al*., 2015). The survival of microorganisms in produce may be dependent on factors such as air temperature, solar radiation, or humidity. Survival of *Escherichia coli* and *Listeria innocua* in lettuce plants has been demonstrated to be greater in colder weather conditions (fall winter) than in warmer conditions (spring summer), in similar growth practices (Oliveira and Abadias, 2011 and Oliveira and Abadias, 2012). However, it is relevant to mention that the influence of weather conditions on plant contamination may be specific for each microorganism, plant, weather condition combination, meaning that a definite trend cannot be established (Ward *et al*., 2015).

Focusing on the post cultivation phase of the farm-to-fork chain, the general manipulation of foodstuffs in the field is a particular concern. Handling of contaminated soil or produce can result in transference of pathogenic microorganisms to workers' hands. Furthermore, the survival of microbial pathogens such as *E. coli O157* and *Salmonella* on nitrile and latex gloves after transfer from contaminated produce has been demonstrated (Erickson *et al*., 2018), which may increase the probability of further contamination of foodstuffs. Practices such as trimming and coring of lettuces in the field upon harvest can result in plant contamination. Field core harvesting knives can act as contamination vectors, allowing for the transfer of pathogenic microorganisms present in contaminated soil to the exposed edible part of plants (Yang *et al*., 2012). Importantly, produce handlers with infectious illnesses can become a risk of

contamination themselves (Julien-Javaux *et al*., 2019). The environment where these foodstuffs are manipulated (that is, food contact surfaces or equipment used in processing) may also play a role in the contamination of food items (Gaul *et al*., 2013; Stephan *et al*., 2015). For example, reusable crates have been shown to be a potential source of cross-contamination among different batches of product (Murray *et al*., 2017).

Washing of fresh produce may prove ineffective to remove microorganisms, as they may remain attached to plant surfaces (DiCaprio *et al*., 2015), or become internalized in the edible parts of the plant (Franz and van Bruggen, 2008), and thus, are not accessible for efficient removal. In addition, the water used to wash fresh produce can be a source of microbial contamination (CDC, 1998; Hedberg *et al*., 1999). Washing water may be reutilized, and generally large washing tanks are used, promoting the contact of large volumes of produce with the water. This can lead to the spread of pathogenic microorganisms between clean and contaminated plants (Baert *et al*., 2009; Gil *et al*., 2009 and Holvoet *et al*., 2012 and Luo, 2007). Cross contamination may occur due to the transport of microorganisms via washing water, particles present in the water (that is, soil and small plant fragments), or plant to plant contact (Gombas *et al*., 2017). Factors such as the configuration of the washing system, type and quality of product, product to water ratio, and the type and concentration of antimicrobial treatment, if used, will influence the ability of a given product washing system in preventing cross contamination (Gombas *et al*., 2017). The efficiency of antimicrobial treatments used can also be affected by the presence of high organic matter content in washing water, resulting from the accumulation of soil, debris, and cut-produce tissue fluids from RTE crops (Allende *et al*., 2008; Luo, 2007). The maintenance of cold storage conditions throughout processing, shipping, storing, and retail of fresh produce is of crucial importance, as low temperatures will hinder the proliferation of bacterial pathogens during these steps (Castro *et al*., 2017). Failing to maintain these conditions during storage and/or transportation of these commodities will favor proliferation of bacteria if they are already present on produce (Zeng *et al*., 2014). Furthermore, the quality of water used for spraying (cooling) or making ice (shipping) is also a risk to consider, particularly when the water source is not treated or of poor microbiological quality, enabling pathogenic microorganisms to contaminate foodstuffs and cause disease (CDC, 1999). Moreover, hydrocooling practices can favor the internalization of pathogenic microorganisms (Murray *et al*., 2017). Finally, manipulation of RTE

foodstuffs in kitchens, either from the final consumer or in commercial settings such as canteens, restaurants, or catered events, should be highlighted as a risk, due to possible cross contamination with other pathogen sources, such as raw meats and eggs (Del Rosario and Beuchat, 1995 and De Waal *et al*., 2000).

2.4 Food Safety versus Food Quality

Food safety is the assurance that food will not cause any harm to the consumer when it is prepared and/or consumed according to its intended use, and refers to practices and conditions that preserve food quality to prevent contamination and food-borne illnesses during preparation, handling, and storage. Examples of Food Safety procedures and policies: personal hygiene, personal presentation and preparation, pest control, waste management, cleaning and sanitizing, temperature control and measurement, food safety hazard identification.

These are only a few examples of Food Safety procedures that should be in place in a food-handling environment. Food Safety procedures vary from company to company and industry to industry. Whereas food quality the quality characteristics of the food that is acceptable to consumer. This includes external factors as appearance (size, shape, color, gloss, and consistency), texture, and flavor (FAO/WHO,1997). But both food safety and quality assurance in fresh produce should be ongoing processes that incorporate activities from the selection and preparation of the soil in agricultural operations through the final preparation and consumption of the food.

Food borne disease outbreaks involving agents such as *Escherichia coli*, *Salmonella* and chemical contaminants highlight problems with food safety and increase public anxiety that modern farming systems, food processing and marketing do not provide adequate safeguards for public health. Factors which contribute to potential hazards in foods include improper agricultural practices; poor hygiene at all stages of the food chain; lack of preventive controls in food processing and preparation operations; misuse of chemicals; contaminated raw materials, ingredients and water; inadequate or improper storage, etc. Specific concerns about food hazards have usually focused on: microbiological hazards, pesticide residues, misuse of food additives, chemical contaminants, including biological toxins, adulteration.

The list has been further extended to cover genetically modified organisms, allergens, veterinary drugs residues and growth promoting hormones used in the production of animal products. Consumers expect protection from hazards occurring along the entire food chain, from primary producer through consumer (often described

as the farm to table continuum). Protection will only occur if all sectors in the chain operate in an integrated way, and food control systems address all stages of this chain (FAO and WHO, 2003). As no mandatory activity of this nature can achieve its objectives fully without the cooperation and active participation of all stakeholders e.g. farmers, industry, and consumers, the term Food Control System is used in these Guidelines to describe the integration of a mandatory regulatory approach with preventive and educational strategies that protect the whole food chain. Thus an ideal food control system should include effective enforcement of mandatory requirements, along with training and education, community outreach programmers and promotion of voluntary compliance. The introduction of preventive approaches such as the Hazard Analysis Critical Control Point System (HACCP), have resulted in industry taking greater responsibility for and control of food safety risks. Such an integrated approach facilitates improved consumer protection, effectively stimulates agriculture and the food processing industry, and promotes domestic and international food trade.

2.5 Factors affecting shelf life of juices

The shelf life of a food can be defined as the time period within which the food is safe to consume and/or has an acceptable quality to consumers or shelf life is also defined as the time to reach a microbial population of 6 log cfu/ml which determined experimentally (Andres *et al*., 2004). The shelf life of juices is affected by both the intrinsic and extrinsic factors. Intrinsic factors include pH, oxidation–reduction potential, water activity, availability of nutrients, the presence of antimicrobial compounds, and competing microflora.

Extrinsic factors encompass storage temperatures and times, relative humidity conditions during storage and packaging material characteristics (Aneja *et al*., 2008; Lawlor *et al*., 2009).

2.5.1 Intrinsic factors

2.5.1.1 Moisture content

Microorganisms need water in an available form to grow in food products. The control of the moisture content in foods is one of the oldest exploited preservation strategies. Food microbiologists generally describe the water requirements of microorganisms in terms of the water activity (aw) of the food or environment. Water activity is defined as the ratio of water vapor pressure of the food substrate to the vapor pressure of pure water at the same temperature (Jay, 2000). The food describes the

degree to which water is "bound" in the food, its availability to participate in chemical/biochemical reactions, and its availability to facilitate growth of microorganisms. Most fresh foods, such as vegetables, and fruits, have aw values that are close to the optimum growth level of most microorganisms (0.97- 0.99). The aw can be manipulated in foods by a number of means, including addition of solutes such as salt or sugar, physical removal of water through drying or baking, or binding of water to various macromolecular components in the food.

Water activity of juices is associated with ºBrix. In juices, ºBrix is used to indicate the percentage of soluble solids and is one of the most important factors for grading the quality of juice. Microorganisms cause fruit juice spoilage by fermentation of sugars, and can therefore increase the ºBrix value owing to the conversion of complex sugars into monosaccharaides (Lawlor *et al*., 2009 and Rivas *et al*., 2006).
Microorganisms respond differently to aw depending on a number of factors. Microbial growth, and in some cases, the production of microbial metabolites, may be particularly sensitive to alterations in water activity (aw). Microorganisms generally have optimum and minimum levels of aw for growth depending on other growth factors in their environments. One indicator of microbial response is their taxonomic classification. For example, Gram negative bacteria are generally more sensitive to low aw than Gram positive bacteria. It should be noted that many bacterial pathogens are controlled at water activities well above 0.86 and only *Staphylococcus aureus* can grow and produce toxin below aw 0.90 (Moral *et al*., 2017).

2.5.1.2 Nutrient content

Microorganisms require certain basic nutrients for growth and maintenance of metabolic functions. The amount and type of nutrients required range widely depending on the microorganism. These nutrients include water, a source of energy, nitrogen, vitamins, and minerals (Jay, 2000 and Mossel *et al*., 1995). Amino acids serve as a source of nitrogen and energy and are utilized by most microorganisms. Some microorganisms are able to metabolize peptides and more complex proteins. Other sources of nitrogen include, for example, urea, ammonia, creatinine, and methylamines. Examples of minerals required for microbial growth include phosphorus, iron, magnesium, sulfur, manganese, calcium, and potassium. In general, small amounts of these minerals are required; thus a wide range of foods can serve as good sources of minerals. In general, the Gram positive bacteria are more fastidious in their nutritional requirements and thus are not able to synthesize certain nutrients required for growth

(Jay, 2000). For example, the Gram positive food borne pathogen *Staphylococcus aureus* requires amino acids, thiamine, and nicotinic acid for growth (Jay, 2000; Moral *et al*., 2017).

2.5.1.3 Biological structure

Plant derived foods, especially in the raw state, have biological structures that may prevent the entry and growth of pathogenic microorganisms. Examples of such physical barriers include taste of seeds, skin of fruits and vegetables, shell of nuts. Plant foods may have pathogenic microorganisms attached to the surface or trapped within surface folds or crevices. Intact biological structures thus can be important in preventing entry and subsequent growth of microorganisms.

Several factors may influence penetration of these barriers. The maturity of plant foods will influence the effectiveness of the protective barriers. Physical damage due to handling during harvest, transport, or storage، as well as invasion of insects can allow the penetration of microorganisms (Jay, 2000 and Mossel *et al*., 1995). During the preparation of foods, processes such as slicing, chopping, grinding, and shucking will destroy the physical barriers. Thus, the interior of the food can become contaminated and growth can occur depending on the intrinsic properties of the food. For example, *Salmonella sp*. has been shown to grow on the interior of portions of cut cantaloupe, watermelon, honeydew melons (Golden *et al*., 1993), and tomatoes (Lin and Wei, 1997) given sufficient time and temperature. Fruits are an example of the potential of pathogenic microorganisms to penetrate intact barriers. After harvest, pathogens will survive but usually not grow on the outer surface of fresh fruits and vegetables. Growth on intact surfaces is not common because food borne pathogens do not produce the enzymes necessary to break down the protective outer barriers on most produce. This outer barrier restricts the availability of nutrients and moisture. One exception is the reported growth of *E. coli O157:H7* on the surface of watermelon and cantaloupe rinds (Del Rosario and Beuchat, 1995). Survival of food borne pathogens on produce is significantly enhanced once the protective epidermal barrier has been broken either by physical damage, such as punctures or bruising, or by degradation by plant pathogens (bacteria or fungi). These conditions can also promote the multiplication of pathogens, especially at higher temperatures (Moral *et al*., 2017).

2.5.1.4 pH and acidity

Used preservation method consists of increasing the acidity of foods either through fermentation processes or the addition of weak acids. The pH is a measure of

the product acidity and is a function of the hydrogen ion concentration in the food product. It is well known that groups of microorganisms have pH optimum, minimum and maximum for growth in foods. Bacteria normally grow faster between pH ranges of 6.0-8.0, yeasts between 4.5-6.0 and molds between 3.5-4.0. An important characteristic of a food is its buffering capacity, *i.e.* its ability to resist changes in pH. Foods with a low buffering capacity will change pH quickly in response to acidic or alkaline compounds produced by microorganisms, whereas foods with high buffering capacity are more resistant to such changes (Valero *et al*., 2012).

The pH of a food is one of several important factors that determine the survival and growth of microorganism especially bacteria during processing, storage and distribution. The acidity of a food may occur naturally as in citrus fruits, apple, strawberries or it may be produced in foods through microbial fermentation. High acidic fruit juices (pH 3.0 – 4.0) could not support survival and growth of bacteria pathogens. However, a number of documented outbreaks of human infections associated with the consumption of raw fruits, and unpasteurized fruit juices increased in recent years (Buck, 2003). Although growth is unlike at low pH, it is well documented that pathogenic bacteria may survive in fruit juices, become adapted to the acidic environment, and cause outbreaks of food borne illness (Parish, 2009).

Citrus juices are characterized by their low (acidic) pH; however, human pathogens have managed to break that barrier, in addition to the fact that their characteristic acidity makes them ideal for the growth of certain kinds of fungi and yeasts (Food Drug Administration, 2021).

2.5.2 Extrinsic factors

2.5.2.1 Temperature

Microorganisms, individually and as a group, grow over a very wide range of temperatures. Therefore, it would be well to consider at this point the temperature growth ranges for organisms of importance in foods as an aid in selecting the proper temperature for the storage of different types of foods. The lowest temperature at which a microorganism has been reported to grow is -34C˚; the highest is somewhere in excess of 10OC˚. It is customary to place bacteria into three groups based on their temperature requirements for growth. *Psychrotrophs* that grow well at or below 7C°, *mesophiles* that grow well between 20C˚ and 45C°, *thermophiles* that grow well at and above 45C°, because of these levels of temperatures, bacteria have the ability to grow (Jay *et al*., 2005).

The quality of the food product must also be taken into account in selecting a storage temperature. Although it would seem desirable to store all foods at refrigerator temperatures or below, this is not always best for the maintenance of desirable quality in some foods. For example, bananas keep better if stored at 13-17C˚ than at 5-7C˚. A large number of vegetables are favored by temperatures of about 100C˚, including potatoes, celery, cabbage, and many others. In every case, the success of storage temperature depends to a great extent upon the relative humidity of the storage environment and the presence or absence of gases such as CO_2 and O_3.

Temperature also influence the shelf life of juices. The shelf life of freshly squeezed, unpasteurized orange juice is less than 20 days at 1C˚. Low temperature is necessary during manufacturing and storage of juice. The primary purpose of low temperature storage is to increase the shelf life by slowing down depredatory reactions and limiting microbial growth. Therefore the combination of reduction in chemical, biochemical and microbial kinetics, can increase the shelf life of fresh and processed foods (Bates *et al*., 2001; Hartel and Heldman, 1997 and Raccach and Mellatdoust, 2007; Sandhu and Minhas, 2007).

2.5.2.2 Relative Humidity of Environment

The relative humidity of the storage environment is important both from the standpoint of aw within foods and the growth of microorganisms at the surfaces. When foods with low water activity values are placed in environments of high relative humidity, the foods pick up moisture until equilibrium has been established. Likewise, foods with a high water activity lose moisture when placed in an environment of low relative humidity. There is a relationship between relative humidity and temperature that should be borne in mind in selecting proper storage environments for foods. In general, the higher the temperature, the lower the relative humidity, and vice versa. Foods that undergo surface spoilage from molds, yeasts, and certain bacteria should be stored under conditions of low relative humidity (Jay, 2005).

The storage of fresh fruit and vegetables requires very careful control of relative humidity. If it is too low then many vegetables will lose water and become flaccid. If it is too high then condensation may occur and microbial spoilage may be initiated (Adams and Moss, 2008).

2.5.2.3 Gaseous Atmosphere

Oxygen comprises 21% of the earth’s atmosphere and is the most important gas in contact with food under normal circumstances. Its presence and its influence on redox

potential are important determinants of the microbial associations that develop and their rate of growth .The inhibitory effect of carbon dioxide (CO_2) on microbial growth is applied in modified-atmosphere packing of food and is an a advantageous consequence of its use at elevated pressures (hyperbaric) in carbonated mineral waters and soft drinks. Carbon dioxide is not uniform in its effect on micro-organisms. *Moulds* and oxidative Gram negative bacteria are most sensitive and the Gram positive bacteria, particularly the lactobacilli, tend to be most resistant. Some yeasts such as *Brettanomyces sp*. also show considerable tolerance of high CO_2 levels and dominate the spoilage microflora of carbonated beverages. Growth inhibition is usually greater under aerobic conditions than anaerobic and the inhibitory effect increases with decrease of temperature, presumably due to the increased solubility of CO_2 at lower temperatures. Some microorganisms are killed by prolonged exposure to CO_2 but usually its effect is bacteriostatic (Adams and Moss, 2008).

Ozone (O_3) is the other atmospheric gas that has antimicrobial properties, and it has been tried over a number of decades as an agent to extend the shelf life of certain foods (Jay *et al*., 2005).

2.5.3 Food spoilage microorganisms

Chemical reactions that cause offensive sensory changes in foods are mediated by a variety of microbes that use food as a carbon and energy source. These organisms include bacteria, yeasts and molds. Some microbes are commonly found in many types of spoiled foods while others are more selective in the foods they consume; multiple species are often identified in a single spoiled food item but there may be one species (a specific spoilage organism) primarily responsible for production of the compounds causing off odors and flavors. Within a spoiling food, there is often a succession of different populations that rise and fall as different nutrients become available or are exhausted. Some microbes, such as *lactic acid* bacteria and molds, secrete compounds that inhibit competitors (Gram *et al*., 2002). Spoilage microbes are often common inhabitants of soil, water, or the intestinal tracts of animals and may be dispersed through the air and water and by the activities of small animals, particularly insects (Rawat, 2015).

A spoiled food has lost the original nutritional value, texture or flavor and can become harmful to people and unsuitable to eat. The microbial spoilage of food products constitutes an important economic problem, as it results in high economic losses for the food industry, especially under incorrect refrigeration conditions. Thus,

spoilage bacteria are able to grow in large number in food, decompose the food and cause changes in the taste, which affect the quality of the products. Spoilage bacteria normally do not cause illness; however, when consumed in high concentration, they can cause gastrointestinal disturbance (Blackburn, 2006). There are different bacterial species that can cause spoilage in food products and the spoilage microbiota depends in great part on the processing and preservation method. Storage temperature also plays a key role in the growth of undesirable microbiota in food (Böhme *et al.*, 2012).

The causative agents of microbiological spoilage in fruits and fruit juices can be bacteria, as well as yeasts and molds. The main spoilage agents can be considered as due to the low pH of most fruits. Some bacteria such as *Campylobacter sp., E. coli O157:H7, Salmonella sp., Listeria monocytogenes, Staphylococcus aureus, Shigella sp., Erwinia sp., Enterobacter sp., Alicyclobacillus sp., Propionibacterium cyclohexanicum, Pseudomonas sp.,* and *lactic acid* bacteria can cause spoilage in fruit and fruit juices (Walker and Phillips, 2008). Certain common molds such *as Penicillium sp., Aspergillus sp., Eurotium sp., Alternaria sp., Cladosporium sp., Paecilomyces sp.,* and *Botrytis sp.,* have been shown to be involved in the spoilage of fresh fruits (Lund and Snowdon, 2000).

2.6 Bacteriological Quality of Fruit Juices

Microorganisms (bacteria, virus, fungi, and parasites) are a group of naturally occurring living organism that can initially in all food crop plants starting from pre harvest up to consumptions. They are found in a wide range of foods around the world. Their presence or absence in the food is considered as one quality. This quality is sometimes affected by the presence of microorganisms that are resident and nonresident the soil. This mainly occurs in fruit which grow with contaminated irrigating water and human and animal faces, animal grazing area etc. Studies reveled that bacterial qualities are fluctuating throughout most food commodities (Burnett and Beuchat, 2001). This lead to food poisoning due to food borne pathogens which is a major public health issue associated with food hygiene and overall food safety. In developing countries, bacterial quality problem is common for some foods that are important part of the diet. *Salmonella* and some strains of *E. coli*, such as *E. coli O157:H7*, the most common food poisoning bacteria (Mead *et al*., 1999).

There is in every possibility of contamination of fruit juice at any stage of processing. Contamination may be occurred by spoilage organisms and/or by food borne pathogens. Food borne disease outbreaks have been documented in different

countries (Parish, 2009 and Sandeep *et al*., 2001). Specially Yeast and molds are the dominant microorganisms in juice because they can thrive the high acidic conditions of the juice. Some examples of them are species of the genera *Cladosporium, Candida, Dekkera, Pichia, Saccharomyces, Aspergillus, Zygosaccharomyces, Penicillium, Byssochlamys, Hanseniaspora, Paecilomyces, Mucor, Fusarium, Botrytis*, and *Neosartorya Talaromyces etc*. Some lactic acid and acetic acid bacteria may be present in fruit juices. Some pathogenic bacteria like *Escherichia coli O157, Salmonella*, and *Cryptosporidium, fecal Streptococci* and some spore formers like *Clostridium pasteurianum* and *Bacillus coagulans* may be present in fruit juice if the juice is not processed adequately (ICMSF, 2005 and Noor and Feroz, 2015).

Bacterial growth in fruit juice depends on pH, storage temperature, types of packaging material, humidity, water activity, sugar contents, quality of water used in juice, quality of the machines used in the juice industries, quality of fruits etc. Mainly improper washing of fruits with poor quality water used in the juice preparation are the major source of contaminating microbes found in the fruit juices (Basar and Rahman, 2007 and Feroz *et al*., 2013). Fruit juices are stored in cold temperature, When juices are kept in humid condition and at ambient temperatures (for example during summer seasons), spoiling microorganisms often get stimulated to grow and cause spoilage and change in odor, taste, visual change *etc*. (Tasnia *et al*., 2018).

2.7 Food borne pathogens

Fruit juice associated disease outbreaks Food borne pathogens if present at sufficient number in fruit juice may cause illness in the consumers. Some microbes only survive in the juice and multiply within the host after consumption and start the disease. Some microbes which can multiply within the juice may yield off odor in juice and make juice unfit for human consumption. *Clostridium perfringens, Staphylococcus aureus, Bacillus cereus, Clostridium botulinum* able to produce toxins in fruit juice and after drinking the juice can cause food poisoning. Fruit juice contaminating pathogens and symptom caused by the mare listed in table (2.1).

Listeria monocytogenes, a notorious food borne pathogen is found in unpasteurized apple juices. *Salmonella sp., Escherichia coli O157:H7*, and *Cryptosporidium sp*., were also found in unpasteurized juices. *Salmonella sp*. associated food borne disease outbreak occurred before the 20th century in Sarasota County, Florida and United States. Hepatitis A was occurred in Egypt in 2004 due to intake of fruit juice (Frank *et al*., 2007). In India, a large population of all income and age groups

consume freshly squeezed fruit and vegetable juice, but the presence of pathogenic microorganisms in street vended fruit juices have been reported in various parts of India such as Vishakhapatnam (Lewis *et al*., 2006), Mumbai (Mahale *et al*., 2008), Amravati (Tambaker *et al*., 2009), Nagpur (Titarmare *et al*., 2009), Kolkata (Mukhopadhyay *et al*., 2011), and Tirumula (Suneetha *et al*., 2013).

Food borne pathogens such as *Escherichia coli* and *Salmonella* survive in acidic environment of fruit juices due to acid stress response (Ghenghesh *et al*., 2004; Tribst *et al*., 2009 and Ray Baudi Massilia *et al*, 2009). Some strains of *E. coli*, *Shigella* and *Salmonella* may survive for several days and even weeks in acidic environment by regulating their internal pH that maintained at neutral pH by combination of passive and active mechanisms (Vantarakis *et al*., 2011). *Shigella flexneri* and *S. sonnei* survive in apple (pH 3.3) and tomato juices (pH) 4.0 at 70C for at least 14 days (Van Opstal *et al*., 2006). Sospedra *et al*.,(2012) reported the presence of *Salmonella sp*., and *Staphylococcus aureus* in orange juice extracted by squeezing machine used in restaurants. Because of the presence of pathogens in fruit juices, the food borne outbreaks associated with consumption of fruit juices have been increased (CDC ,2007; Ray *et al*., 2009; Sospedra *et al*., 2012; Van Opstal *et al*., 2006 and Vantakratis *et al*., 2011). *Serratia sp*., are gram negative, bacilli shaped, facultative anaerobe, motile bacteria that belongs to the family *Enterobacteriaceae.* These bacteria grow well on standard media and produce a red to dark pink pigment that aids in identification. (Kayla, 2004). Although *Serratia marcescens* was considered to be an innocuous, non-pathogenic organism, over the last two decades they have become an opportunist pathogen causing nosocomial infections. A broad range of hospital acquired infections caused by *S. marcescens* include respiratory tract infections, urinary tract infections (UTI), septicemia, meningitis, pneumonia, conjunctivitis wound and eye infections, osteomyelitis, keratoconjunctivitis, keratitis, endophthalmitis and endocarditis (Hejazi, 1997).

Table (2.1) : Pathogens and symptom they cause.

Bacteria	Symptom
Salmonella sp.	Abdominal pain, diarrhea, chills, fever, Nausea.
Shigella sp.	Abdominal pain, diarrhea, fever, nausea .
Clostridium botulinum	Nausea, vomiting, fatigue, dizziness, dryness of mouth and throat, muscle paralysis, difficulty swallowing, double or blurred vision.
Escherichia coli 0157:H7	Bloody diarrhea, abdominal pain, hemolytic uremic syndrome (HUS), kidney failure.
Listeria monocytogenes	Gastroenteritis, childbirth in pregnant women, septicemia, meningitis

2.7.1 Bacterial diversity and most frequent pathogens in food material related to the contamination

Escherichia coli pathogens are gram-negative bacilli of the family *Enterobacteriaceae*, they are facultative anaerobes and nonsporulating. are very common bacteria in the gastrointestinal tract, and part of the normal bacterial flora. However, some *E. coli* strains are able to produce a toxin that could produce serious infection. The main reservoir of such *E. coli* strains is grass-feeding animals, cattle in particular. Their meat might become contaminated by fecal matter due to poor processing methods during slaughter, and their faces might end up contaminating other foods (e.g. milk, vegetables, fruits) and water. Diarrhea genic *E. coli* strains are worldwide in distribution. The route of infection is fecal-oral, predominantly via contaminated water and food. STEC, especially *E. coli O157:H7*, is shed in feces of cattle, sheep, deer, and other ruminants. Human infection is acquired via contaminated food or water or via direct contact with an infected person. Outbreaks have been linked to ground beef, exposure to animals in public settlings (petting zoos), contaminated apple cider, and contamination of water in recreational areas. The incubation period for most *E. coli* strains is 10 hours to6 days. For *E. coli O157:H7*, the incubation period is usually 3to 4 days. *E. coli* strains with the K1 capsular polysaccharide antigen cause approximately 40% of cases of septicemia and 80% of cases of meningitis. Different strains of *E. coli* are associated with a number of distinctive diarrheal illnesses. Among these are the enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and *Shiga* toxin–producing *E. coli* (STEC). Of the STEC, *E. coli* O157:H7 is the prototypic strain. Each class of *E. coli* has distinct somatic (O) and flagellar (H) antigens and specific virulence characteristics (WHO, 2018).

Escherichia coli serotype O157:H7 can be differentiated in that it is unable to ferment sorbitol efficiently, unlike most generic *E. coli*. *E. coli* O157:H7 is specifically adapted for survival in the gastrointestinal tract of host organisms. Some strains produce curli fimbriae that facilitate attachment of cells to surfaces (Jay *et al*., 2005). This is notable in that it not only facilitates attachment of cells to host organisms, but also can aid in attachment of cells to abiotic surfaces where biofilm formation will further facilitate persistence and survival of the pathogen (Torres *et al*., 2005 and Uhlich *et al*., 2010). *E. coli* O157:H7 is most well known as a causative agent of Hemolytic Uremic Syndrome (HUS). Its virulence is due to production of non-heat stable *shiga*-like toxins, responsible for HUS which attack epithelial and renal cells, causing lysis, leading to

bloody stool or blood in urine. The protein toxin attaches to specific receptors on these cells and disrupts protein synthesis. It is important to note that HUS is only caused when a population of *shiga*-toxin *E. coli* (STEC) is able to grow in a host organism, as the toxin is a protein, produced more readily at 37C˚ than at room temperature (Abdul Raouf *et al*., 1994), though the pathogen is able to survive in a wide range of conditions including lower temperatures associated with storage of meat products. Traditionally associated with ground beef, *E. coli* O157:H7 has also been found in nonmeat foods such as radish sprouts in Japan in 1996 and even hazelnuts in the Great Lakes region in 2011 (Centers for Disease Control and Prevention j Outbreak Net, 2011).

As is the case with most foodborne pathogens, *E. coli O157:H7* is of greatest concern to at-risk groups: the very young, the very old, and immunocompromised people. People with a healthy immune system may experience gastroenteritis and diarrhea without developing HUS. Scallan *et al*., (2011) reported that *E. coli O157:H7* could be responsible for over 60,000 cases of illness per year as well as being responsible for up to 20 deaths on average. In addition to the health consequences and threats posed by the pathogen, continued presence of the pathogen and resurgence in the food supply undermines consumer confidence in commercial food production and processing (Viazis and Diez-Gonzalez, 2011). Controlling the organism and incidence of illness is beneficial from an economic, public relations, and a public health standpoint.

Salmonella sp. is a genus of rod-shaped (bacillus) Gram-negative bacteria of the family *Enterobacteriaceae*. The two species of *Salmonella* are *Salmonella enterica* and *Salmonella bongori. S. enterica* is the type species and is further divided into six subspecies that include over 2,600 serotypes (CDC, 2007 and Voetsch *et al*., 2004). *Salmonella* was named after Daniel Elmer Salmon (1850–1914), an American veterinary surgeon (CDC, 2007). *Salmonella sp*., are food-borne pathogens of major public health concern in most countries of the world. *Salmonella enterica* is an important zoonotic pathogen that causes an estimated 1.4 million illnesses, 16,000 hospitalizations, and between 400 and 600 deaths annually in the United States alone (El-Safey, 2013). *Salmonella* infections have become one of the most important groups of bacterial diseases affecting poultry, and, according to domestic poultry constitutes the largest single reservoir of salmonella organisms existing in nature. Unpasteurized fresh fruit is a traditional product that is produced and consumed in the regions of the world, particularly during the fall harvest season. However, this

product has been implicated as the vechle for food-borne diseases. *Salmonella* species are responsible for the highest number of documents cases of food poisoning in the developed country (El-Safey, 2013). A variety of foods, including poultry, eggs, meat, milk, fruits, and vegetables, have been implicated as vehicles of one or more of these pathogens in outbreaks of food-borne illness. *Salmonella* is an enterinvasive bacterium and causes infections that may have one of five different clinical presentations (El-Safey, 2013). Gastroenteritis is the most common presentation in industrial countries and is considering as an emergent foodborne pathogen caused disease it's a self-limited illness of brief duration, usually characterized by diarrhea and fever. *Salmonella Heidelberg* is an important human pathogen, which causes diarrhea diseases. There are two main diseases caused by *Salmonella sp*. and they are Salmonellosis and typhoid fever. *Salmonella enteritidis* or *Salmonella typhimurium* causes Salmonellosis and Typhoid fever is caused by *Salmonella typhi*. People who eat food contaminated by *Salmonella* can become ill with salmonellosis.

Shigella is classified in the family *Enterobacteriaceae*. It is gram negative bacilli, non-spore forming, non-motile, 0.5-0.7µm in size and facultative anaerobic pathogen that are closely related to *Escherichia coli*. It is differentiated from *Escherichia coli* on the basis of serology, pathogenesis and physiology. *Shigella* species usually ferment sugars without production of gas and lactose, urease and oxidase negative (Ud-Din and Wahid, 2014). The *Shigella* genus is divided into four species that are *Shigella dysenteriae* (serogroup A), *Shigella flexneri* (serogroup B), *Shigella boydii* (serogroup C) and *Shigella sonnei* (serogroup D). According to biochemical characterization and serological properties, these species are further distributed into several serotypes, as *Shigella dysenteriae* have 15 serotypes, *Shigella flexneri* have 14 serotypes and subserotypes, *Shigella* boydii have 20 serotypes and *Shigella sonnei* with a single serotype (Livio *et al*., 2014). The causative agent of human shigellosis, *Shigella* causes disease in primates, but not in other mammals (Ryan *et al*., 2004). It is only naturally found in humans and gorillas (Bardhan *et al*., 2010 and Wen *et al*., 2012). During infection, it typically causes dysentery. The symptoms can range from mild watery diarrhea to severe inflammatory dysentery with the passage of mucoid and bloody stools. The other clinical manifestation includes abdominal cramping, fever, nausea, malaise, vomiting and convulsions. Other complications of shigellosis include septicemia, dehydration, joint pains, hypoglycemia, hemolytic

uremia and neurological complications (Marteyn and Sansonetti, 2012). Mode of transmission is via fecal-oral route and by direct contact with an infected individual. The *Shigella* species are highly infectious, as only 10-100 organisms are enough to cause disease and the bacteria is more resistant to stomach acid and can easily pass through the gastric acid barrier (Patil and Lava , 2012). A combination of antibiotics and oral rehydration can lead to the rapid resolution of disease (Saima *et al*., 2018).

Shigella is one of the leading bacterial causes of diarrhea worldwide, causing an estimated 80–165 million cases (Bowen, 2016). The number of deaths it causes each year is estimated at between 74,000 and 600,000 (Mani *et al*., 2016). It is one of the top four pathogens that cause moderate-to-severe diarrhea in African and South Asian children. *Shigellosis* or *Bacillary dysentery*, a gastrointestinal disease caused by *Shigella* species, is recognized as a serious health problem throughout the world. It is mostly found in developing countries due to improper waste management, poor hygienic condition and unsafe drinking water. In industrialized nations, it is mostly due to travel to unindustrialized countries and consumption of contaminated food material (Saima *et al*., 2018). Globally, mortality and morbidity due to shigellosis were found to be highest in children under five years old. In Africa, it is estimated that more than 8 million *Shigella* infections occur per year, whereas in Asia 91 million cases and 414,000 deaths occur annually (Anon, 2005). The first report on the *Shigella* isolation and characterization was published by Kiyoshi Shiga in 1897 (Yabuuchi, 2002).

Pseudomonas sp. are aerobic, Gram-negative, non-spore-forming bacteria; some strains produce pigments, *i.e*., the yellow-green fluorescing pigment called pyoverdine (especially some *P. fluorescens* and *P. aeruginosa* strains) and the blue-green pigment called pyocyanin (Carminati *et al*., 2019). *Pseudomonas sp*. produce thermotolerant lipolytic and proteolytic enzymes that reduce the quality and the shelf-life of raw and processed milk (Foods, 2021). These bacteria are inactivated by the thermal processes currently applied through the production of most dairy products, but they can also enter the food production chain as post-process contaminants, due to the contact of the final product with soil, water or raw material (Carrascosa *et al*., 2015).

Pseudomonas are also recognized to be able to colonize environmental production as well as equipment and facilities for long periods, thanks to their ability to produce persistent biofilms (Rossi *et al*., 2018). Psychrotolerant *Pseudomonas sp*. are among the most common bacteria implicated in spoilage, especially of refrigerated food

with a prolonged shelf life, where they are likely selected (Spanu *et al*., 2018). *Pseudomonas aeruginosa* is one of the opportunistic human pathogen that preferentially infects patients with cancer or AIDS, immunocompromised patients by surgery, cytotoxic drugs or burn wounds, people with cystic fibrosis, eye, ear and urinary tract infections (Senturk *et al*., 2012). *P. aeruginosa* can produce any of the opportunistic extra-intestinal infections caused by members of the *Enterobacteriaceae* and may progress to bacteremia (Kayser *et al*., 2005). Septicemia and endocarditis may occur in patients who are debilitated due to concomitant infection, malignancy or immunosuppressive therapy (Gellatly and Hancock, 2013 and Kumar, 2012). In some cases of *P. aeruginosa* bacteremia, cutaneous papules develop that progressto black, necrotic ulcers (Gellatly and Hancock, 2013). *P. aeruginosais* also one of the most common causes of infection in environmentally contaminated wounds, *eg*: osteomyelitis after compound fractures or nail puncture and body wounds (Kayser *et al*., 2005). The success of *P. aeruginosa* in diverse environments is attributed to its impressive arsenal of virulence factors, which include multiple cell-associated factors such as alginate, lipopolysaccharide, flagella and pili, and secreted virulence factors, including toxins, elastases, alkalin protease, hemolysin, pyocyanin, as well as small molecules that include phenazines, rhamnolipid, and biofilm formation (Govan, 2007). Alkalin protease it is an extracellular protease that is produced by *P. aeruginosa* and it plays an important role during acute infection, however details of their actions are sometimes unclear (Lidija Izrael *et al*., 2010). Alkaline proteases are referring to proteolytic enzymes which work optimally in alkaline pH (Abdulnasser *et al*., 2007).

Pseudomonas aeruginosa It was isolated from many samples of foods and juices in many previous studies, including (Das *et al*., 2010**;** Batool et al., 2013; Braide *et al*., 2012 and Iqbal *et al*., 2015).

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on MacConkey agar. Although found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human and animal lungs if aspirated, specifically to the alveoli resulting in bloody, brownish or yellow colored jelly like sputum (Ryan *et al*., 2004). In the clinical setting, it is the most significant member of the genus *Klebsiella* of the *Enterobacteriaceae. K. oxytoca* and *K, rhinoscleromatis* have also been demonstrated in human clinical specimens. In recent years, *Klebsiella* species have become important pathogens in nosocomial infections.

Klebsiella pneumoniae is an opportunistic bacterium found in various microbiological niches such as soil; the skin, intestines, and feces of mammals; and food. *K. pneumoniae* has been documented to cause bacteremia, pneumonia, and urinary tract infection (Siu *et al*., 2011); the gastrointestinal carriage of *K. pneumoniae* has been said to be a predisposing factor for liver abscess (Fung *et al*., 2012), and hyper virulent *K. pneumoniae* strains have emerged as a predominant cause of pyogenic liver abscess in Asia (Lübbert *et al*., 2014). Studies have reported that most pathogens in liver abscess are susceptible to broad-spectrum antibiotics such as fluoroquinolones and third- and fourth-generation cephalosporin's (Fung *et al*., 2012 and Siu *et al*., 2012), but over the years, antibiotic resistance in *K. pneumoniae* has been increasingly observed in both nosocomial and community settings. Reports of *K. pneumoniae* having developed an acquired resistance to last-line antibiotics (*i.e*., carbapenems) (Arnold *et al*., 2011 and Pitout *et al*., 2015) have been most concerning. The interplay between mechanisms of antibiotic resistance and virulence in *K. pneumoniae* is not well established. It is generally accepted that antibiotic resistance comes with a fitness cost and decreased virulence, but recent studies have suggested otherwise the development of antibiotic resistance in *K. pneumoniae* has been reported to augment virulence (Kidd *et al*., 2017), and increased virulence is said to naturally evolve in response to, or potentially be shared among, bacteria, leading to acquired resistance (Shroeder *et al*., 2017). Although *K. pneumoniae* is more commonly associated with nosocomial infections, food has also been reported to be a possible transmission vector. *K. pneumoniae* has been isolated from raw meat (Davis *et al*., 2015 and Guo *et al*., 2016), raw vegetables (Falomir *et al*., 2013 and Puspanadan *et al*., 2012), fruit juice (Ghenghesh e*t al*., 2004 and Sultana *et al*., 2019), and ready-to-eat (RTE) food (Takeuchi *et al*., 2017). The WHO has classified carbapenems resistant and third-generation cephalosporin resistant *Enterobacteriaceae* (including *K. pneumoniae*) as critical priority pathogens in its list of antibiotic resistant bacteria needing new treatments.

Staphylococcus aureus is a non-motile Gram-positive facultative anaerobe. Cells are spherical, single and often form grape-like clusters. They form spherical clusters in two planes and have no flagella. The bacteria are about 0.5 – 1.0 μm in diameter. The organism produces catalase and coagulase. Staphylococci form clusters while streptococci forms chains, a character used to distinguish the two bacteria. Colonies of *S. aureus* on solid medium appear as golden or cream white depending on the culture media. The genus *Staphylococcus* is taxonomically in the bacterial family

Staphylococcaceae that include three lesser known genera, *Gamella, Macrococcus* and *Salinicoccus. S. aureus* are able to grow in a wide range of physical conditions for example optimum temperatures of 30C˚ to 37C˚, and of pH 7.0 to 7.5. The organism can survive in sodium chloride concentrations of up to 15%. A wide variety of foods that require manipulation during processing, including fermented food products can support the growth of these bacteria (Cruickshark *et al*., 1973). Distinguishing streptococci (catalase-negative) from staphylococci, the catalase test is important. Catalase-positive organism produce O_2 and bubble once the test is performed by adding 3% hydrogen peroxide on an agar plate. Blood itself contains catalase so the test should not be done on blood agar (Schneewind *et al*., 1995).

More than 50 species and subspecies of staphylococci have been described according to their potential to produce coagulase. Designation between coagulase positive staphylococci (CPS) and non-coagulase-producing strains, called coagulase-negative staphylococci (CNS) has been classified (Becker *et al*., 2001). Some of CNS plays a role in the fermentation of meat and milk-based products. The CNS enterotoxigenic potential has been a subject of controversy. In most investigations cases, enterotoxin production or enterotoxin-like have failed to detect gene in CNS (John, 2016). Production of enterotoxins, in some studies showed that CNS strains could lead to food poisoning (Zell *et al*., 2008). Even *et al*., (2010) demonstrated that, among 129 CNS strains isolated from fermented foodstuffs, only one carried SE genes. The main causative agent described in staphylococcal food poisoning outbreaks is *S. aureus. S. intermedius* has been described as a potential enterotoxigenic CPS (Becker *et al*., 2001). Enterotoxigenic potential particularly for SEC of this species has been isolated from dogs. Such strains producing toxins raises possible health hazard, especially when carried by animals such as dogs, which come in close contact with humans (John, 2016). In the United States *S. intermedius* was involved in one outbreak caused by blended margarine and butter. Other enterotoxigenic potentials are *S. delphini* and *S. pseudintermedius*, which is the main species, isolated from dogs.

The bacteria *S. aureus* can be found as a normal flora of skin and mucous membranes of mammals and birds. This bacterium when disseminates in the environment survives for long periods in the host (Schmitt *et al*., 1990). Close adaptation of this bacterium to its host has been demonstrated and identified based on four biochemical tests namely *staphylokinase, ß-haemolysin* production, coagulation of bovine plasma and growth on crystal violet agar following the simplified biotyping

scheme described by Devriese, (1997). Based on this, human, poultry, cattle and sheep/goat were described. Many strains could not be assigned to these host-specific biotypes and belong to non hostspecific (NHS) biotypes, particularly those associated with several hosts (Schmitt et al., 1990). Protein A production, and phage typing are additional biochemical test that allowed researchers to differentiate the poultry biotype from the new biotype. Protein-A test is no longer commercially available, and as phage typing cannot be routinely used, therefore these two biotypes test cannot be easily distinguished (John, 2016). Bio typing has been useful in tracing or estimating the origin of *S. aureus* in various food products despite these drawbacks (Devriese *et al*., 1997), and for epidemiological investigations and food poisoning outbreaks in the food industry (Kérouanton *et al*., 2007).

Intoxication is caused by the ingestion of enterotoxins within foods, usually because the food has been left at room temperature (Walls and Scott, 1997). There are five major classical types of *staphylococcal enterotoxins* (SEs): SEA, SEB, SEC, SED and SEE, as well as new SEs or SE-like super antigens (Sags) such as SEG to SEU (Chiang *et al*., 2008). Foods requiring considerable handling during preparation and kept without refrigeration are usually involved in staphylococcal poisoning. This bacterium is able to grow in a wide temperature range (7-48 C˚), with an optimal growth at 35-37 C˚, a frequent value in warm climates (Baeza *et al*., 2007).

When food or its ingredients are contaminated with enterotoxigenic strains of *Staphylococcus sp*., it will result to *Staphylococcal* food poisoning (SFP). To induce SFP five conditions for staphylococci growth and enterotoxin production is required. First, there must be a source containing enterotoxin producing staphylococci such as raw materials, healthy or infected carrier. Second is the transfer of staphylococci from source to food, like unclean food preparation tools because of poor hygiene practices. Third is that food should compose of favourable physicochemical characteristics for *S. aureus* growth and toxinogenesis (Asao *et al*.,2003). Temperature should be favourable and sufficient time for bacterial growth and toxin production. Lastly, food containing sufficient amounts of toxin to provoke symptoms should be ingested. Most SFPOs arise because of poor hygiene practices during processing, cooking or distributing the food product (Pereira *et al*, 2009). Inadequate cooling of foods after contamination can induce *Staphylococcus* growth that can stimulate toxin production, resulting in food poisoning (John, 2016). Transfer of staphylococci found in mammals and birds to food has two main sources that include human carriage during food processing and dairy

animals in cases of mastitis. The human strains are mainly involved in SFPOs but animals are known to be potential source of primary contamination. *Staphylococcus aureus* can be carried over from the udder into the milk in cases of *Staphylococcal mastitis* of ruminants such as cows, goats or ewes. A study on 178 *S. aureus* strains associated with 31 SFPOs isolates, animal strains were demonstrated for the first time to be responsible for two outbreaks (Kérouanton *et al*., 2007).

Worldwide *Staphylococcal* food disease is considered as one of the most common Foodborne Diseases, which are of major concern to the public (Le Loir *et al*., 2003 and Hennekinne *et al*., 2012). In United States of America, this organism is one of the most common causes of reported food borne diseases (Balaban and Rasooly, 2000 and Murray, 2005). Vaughan and Sternberg in Michigan, USA, investigated the first documented event of SFD due to the consumption of contaminated cheese in 1884 (Hennekinne *et al*., 2012). *Staphylococcus aureus* foodborne poisoning has a rapid onset usually 3–5 hours following ingestion of contaminated food with this bacterium. During growth at permissive temperatures, these bacteria produce one or more enterotoxins (Le Loir *el al*., 2003). *Staphylococcal* food poisoning has an abrupt onset to cause symptoms which includes nausea, vomiting, hyper salivation, abdominal cramping, with or without diarrhea. Dehydration and hypotension may occur if significant fluid is lost (Argudin *et al.*, 2010). Within 24–48 hours of onset, although self-limiting SFD can be severe, especially in infants, elderly, and immune compromised patients (Argudin *et al*., 2010 and Hennekinne *et al*., 2012). Reported foodborne outbreaks in 2008 by European Food Safety Authority from 27 European Union Member States, showed that *S. aureus* was the fourth most common causative agent (EFSA, 2010). An extensive *staphylococcal* food poisoning outbreak occurred in Kansai district in Japan affecting as many as 13,420 people (Asao *et al*., 2003). Dairy products from a factory in Hokkaido, which experienced a transient shortage of power supply during the manufacturing process, were incriminated. In 2009 Ministry of Health, Labour and Welfare recorded 7.6% incidents caused by *S. aureus*, affecting 690 persons (Asao *et al*., 2003).

2.8 Global controls for fresh food products

Fresh produce is consumed and cultivated globally, and as such, agricultural practices can vary widely. It is therefore important to note that different countries and/or retailers have different criteria or legal requirements concerning produce quality, and the quality of water used for its production and preparation (Uyttendaele *et al*., 2015).

The European Commission (EC) Regulation 852/2004 on the hygiene of foodstuffs outlines the implementation of good agricultural practices (GAPs), providing general requirements to be implemented from primary production to the later stages of the food production chain (EC,2004). More specifically, the EC Regulation 2073/2005 on microbiological criteria for foodstuffs sets limits for microbiological contamination of different food categories, designating analytical reference methods to be used for each test (EC, 2005), and the EC Regulation 98/83/EC on the quality of water intended for human consumption determines that water that is in contact with food should be of good microbiological quality (EC, 1998). The microbiological quality parameters of this regulation are outlined in Part A of Annex I of the document, referring to the absence of *E. coli* and *Enterococci* in 100 mL of water as the quality criteria. Similarly, the United States Food and Drug Administration provides industry-oriented guidelines concerning microbiological quality of produce, namely, the "Guide to Minimize the Microbial Food Safety Hazards for Fresh Cut Fruits and Vegetables" (U.S. Food and Drug Administration [USFDA], 2008).

Chapter 3
3. Materials and Methods

3.1 The study area

This study was to evaluate the bacteriological contamination of natural juices sold in the city of Benghazi. The samples are intended to be collected from local points from four different areas including (North, East, South and city Center). Microbiological examination was performed immediately after sample collection.

3.2 Samples Collection

A total of 128 the fresh fruit juice, samples were collected from different areas in the city of Benghazi, the first isolation 64 samples and second isolation 64 samples, in all isolation (32 strawberry juice_ 32 mango juice) samples. The local points of collection into the city were divided into four areas (North, East, South, city Center), from cafes and restaurants, two types of juices, four samples each (for every café in all isolation). The collection of the samples were carried out in two different seasons. The study was conducted in the end of summer period (September, October 2020 “the first isolation”) and (September, October 2021 “the second isolation”). According to the initial screening about the juices that representing the most consumable rate by customers, strawberry juice and mango juice were the most popular fresh juices, therefore they were chosen to be our experimental target to make the microbial evaluation of contamination.

During the samples collection it was cheated the integrity of the packaging of the juices containers, after collection the samples were transferred in cool conditions (in an ice pack) to the laboratory for microbial investigation.

3.3 Media used for the isolation of bacteria from samples

3.3.1 Nutrient agar

Nutrient agar is a basic culture medium commonly used for the culture of non-fastidious microorganisms, and for quality control and checking purity prior to biochemical or serological testing. This media has a relatively simple formula that has been retained and is still widely used in the microbiological examination of a variety of samples and is also recommended by standard methods. It is a general purpose media that is mostly used for routine culture or to ensure prolonged survival of microorganisms. It is one of the most important and commonly used non-selective

media for the routine cultivation of microorganisms. Nutrient agar has been used for the cultivation and enumeration of many bacteria that are not particularly fastidious.

3.3.2 MacConkey agar

Is a selective and differential medium designed to isolate and differentiate *Enterobacteriaceae* from (different type of samples including juices), based on their ability to ferment lactose. Bile salts and crystal violet inhibit the growth of Gram positive organisms. Lactose provides a source of fermentable carbohydrate, allowing for differentiation. Neutral red is a pH indicator that turns red at a pH below 6.8 and is colorless at any pH greater than 6.8. Organisms that ferment lactose and thereby produce an acidic environment will appear pink because of the neutral red turning red. Bile salts may also precipitate out of the media surrounding the growth of fermenters because of the change in pH. Non-fermenters will produce normally-colored or colorless colonies.

3.3.3 Selenite Broth

Is used as a selective medium for the isolation of *Salmonella* and *Shigella* species, reduced growth of fecal coliforms. Selenite broth is used as a selective enrichment medium and must not be autoclaved.

3.3.4 Mueller-Hinton agar

Is a microbiological growth medium that is commonly used for antibiotic susceptibility testing, specifically disk diffusion tests. It has a few properties that make it excellent for antibiotic use. It is a nonselective, no differential medium, this means that almost all organisms plated on it will grow, additionally it contains starch. Starch is known to absorb toxins released from bacteria, so that they cannot interfere with the antibiotics. Also, it is a loose agar, this allows for better diffusion of the antibiotics than most other plates. A better diffusion leads to a truer zone of inhibition.

3.3.5 Blood agar

Are enriched medium used to culture those bacteria or microbes that do not grow easily. Such bacteria are called “fastidious” as they demand a special, enriched nutritional environment as compared to the routine bacteria. Blood Agar is used to grow a wide range of pathogens particularly those that are more difficult to grow. It is also required to detect and differentiate hemolytic bacteria, especially Streptococcus species. It is also a differential media in allowing the detection of hemolysis (destroying the RBC) by cytolysis toxins secreted by some bacteria.

3.3.6 Eosin methylene blue (EMB)

Is a selective stain for Gram negative bacteria. Is contains dyes that are toxic to Gram positive bacteria. EMB is the selective and differential medium for coliforms. It is a blend of two stains, eosin and methylene blue in the ratio of 6:1, which slightly inhibits the growth of Gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment lactose (*e.g., E. coli*) and those that do not (*e.g., Salmonella, Shigella*). This medium is important in medical laboratories by distinguishing pathogenic microbes in a short period of time.

3.3.7 Triple sugar iron agar

Is a microbiological test named for its ability to test a microorganism's ability to ferment sugars and to produce hydrogen sulfide. An agar slant of a special medium with multiple sugars constituting a pH-sensitive dye (phenol red), 1% lactose, 1% sucrose, 0.1% glucose, as well as sodium thiosulfate and ferrous sulfate or ferrous ammonium sulfate is used for carrying out the test. All of these ingredients when mixed together and allowed solidification at an angle result in a agar test tube at a slanted angle. The slanted shape of this medium provides an array of surfaces that are either exposed to oxygen-containing air in varying degrees (an aerobic environment) or not exposed to air (an anaerobic environment) under which fermentation patterns of organisms are determined. To determine the ability of an organism to ferment glucose, lactose, and sucrose, and their ability to produce hydrogen sulfide.

The triple sugar iron agar test employing Triple Sugar Iron Agar is designed to differentiate among organisms based on the differences in carbohydrate fermentation patterns and hydrogen sulfide production. Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow.

3.3.8 Simmons citrate agar

Is an agar medium used for the differentiation of *Enterobacteriaceae* based on the utilization of citrate as the sole source of carbon. bacteria that can grow on this medium produce an enzyme, citrate-perm ease, capable of converting citrate to pyruvate. Pyruvate can then enter the organism's metabolic cycle for the production of energy. Growth is indicative of utilization of citrate, an intermediate metabolite in the Krebs cycle. When the bacteria metabolize citrate, the ammonium salts are broken down to ammonia, which increases alkalinity. The shift in pH turns the bromthymol blue indicator in the medium from green to blue above pH 7.6.

3.3.9 Mannitol Salt Agar (MSA)

This type of medium is both selective and differential. The MSA will select for organisms such as Staphylococcus species which can live in areas of high salt concentration. The differential ingredient in MSA is the sugar mannitol. Organisms capable of using mannitol as a food source will produce acidic byproducts of fermentation that will lower the pH of the media. The acidity of the media will cause the pH indicator, phenol red, to turn yellow. *Staphylococcus aureus* is capable of fermenting mannitol.

3.3.10 Lactose broth

Presumptively identify coliform organisms in milk, water and foods as specified by the American Public Health Association. Beef extracts and peptone in this liquid medium supply essential nutrients while lactose is a fermentable carbohydrate for coliforms. Coliforms ferment lactose in the medium resulting in gas formation which is indicative of positive results.

3.4 Sample preparation:

One ml sample was transferred into a test tube containing nine ml of sterile normal saline to make 10^{-1} dilution and shaken with vortex mixture. A serial dilution up to 10^{-5} were also made in same procedure. From each dilution, one ml was spreads on agar plate.

3.4.1 Bacterial count

Colonies were formed on streak series. The colonies were counted, the plate were screened for the presence of discrete colony forming units per ml cfu/ml. (Tortora *et al*., 2013).

$$\text{CFU/ml} = \text{Colony counted} \times 1/\text{df} \times V(\text{ml})$$

Where df: dilution factor and V(ml): volume of solution transferred between serial dilution.

3.5 Morphological and biochemical characteristics of foodborne pathogens isolated for natural juices: (McCance *et al*,. 1998).

3.5.1 Morphological of characteristics

3.5.1Gram staining

3.5.2 Biochemical characteristics

Conventional biochemical test used for the identification of the isolates mentioned below: (Kaniz *et al*,. 2016).

3.5.2.1 Indole test

In case of positive reaction tryptophan is oxidize by the microbial enzyme tryptophanase, yielding indole and pyruvic acid. Indole reacts with paradimethylminobenzaldehyde producing a visible colorful dye. The tryptophan broth medium was prepared the pH was adjusted. Dispensed in to the test tube at the rate of ten ml per tubes. The medium was then sterilized at 121C˚ temperatures and 15 lb pressure for thirty minutes in the autoclave and cooled. The tubes of tryptophan broth in duplicate or inoculated with 48 hours old nutrient broth culture. The tubes were then incubated at 37 C˚ for three days. After incubation few drops of kavac's solution or were added to the tubes. The tubes were shaken vigorously for one minute and observed for the pink color formation in the tubes.

3.5.2.2 Citrate utilization

The test was used to differentiate among enteric organism on the basis of their ability to ferment citrate as a soul source of carbon.

3.5.2.3 Catalase test

This test was used to differentiate those bacteria that produce the enzyme catalase from non-catalase producing one. Aerobic, facultative aerobes, microaerophiles can produce catalase while the anaerobes unable to produce this enzyme. The production of bubble indicates the positive catalase test results and the absence of bubble production indicate negative results.

3.5.2.4 Oxidase test

Place a piece of filter paper. Then I added three drops of oxidase reagent. Then move the living object onto the filter paper. Blue indicates a positive result and color does not indicate negative results.

3.5.2.5 Lactose fermentation

Weight and dissolve triptycase nutrient broth and phenol red in 100 ml distilled water at 0.5 ml lactose insert Durham's tube in to all test tubes should be fully filled with lactose broth. Sterilizing at 121 C˚ for 15 psi. Inoculation culture organism by loop. Incubation for 18- 24 hours at 37 C˚. Blanks Durham's tubes indicates gas production and color change indicate produce acid. Not color change indicates alkaline.

3.5.2.6 Glucose fermentation

Weight and dissolve triptycase nutrient broth and phenol red in 100 ml distilled water at 0.5 ml Glucose insert Durham's tube in to all test tubes should be fully filled

with Glucose broth. Sterilizing at 121 C˚ for 15 psi. Inoculation culture organism by loop. Incubation for 18-24 hours at 37 C˚. Blanks Durham's tubes indicates gas production and color Change indicate produce acid. Not color change indicates it alkaline.

3.5.2.7 Sucrose fermentation

Weight and dissolve triptycase nutrient broth and phenol red in 100 ml distilled water at 0.5 ml Sucrose insert Durham's tube in to all test tubes should be fully filled with Sucrose broth. Sterilizing at 121 C˚ for 15 psi. Inoculation culture organism by loop. Incubation for 18-24 hours at 37 C˚. Blanks Durham's tubes indicates gas production and color change indicate produce acid. Not color change indicates it alkaline.

3.5.2.8 Coagulase test

Coagulase is an enzyme that clots blood plasma. This test was performed on Gram-positive, catalase positive species to identify the coagulase positive *Staphylococcus aureus.* Coagulase is a virulence factor of *S. aureus.* The formation of clot around an infection caused by this bacteria likely protects it from phagocytosis. This test differentiates *Staphylococcus aureus* from other coagulase negative *Staphylococcus* species.

3.5.2.9 Urease test

This test was used to identify bacteria capable of hydrolyzing urea using the enzyme urease. It is commonly used to distinguish the genus Proteus from other enteric bacteria. The hydrolysis of urea forms the weak base, ammonia, as one of its products. This weak base raises the pH of the media above 8.4 and the pH indicator, phenol red, turns from yellow to pink.

3.5.2.10 DNase test

DNase agar was a differential medium that tests the ability of an organism to produce an exoenzyme, called deoxyribonuclease or DNase, that hydrolyzes DNA. DNase agar contains nutrients for the bacteria, DNA, and methyl green as an indicator. Methyl green is a cation which binds to the negatively-charged DNA Deoxyribonuclease allows the organisms that produce it to break down DNA into smaller fragments.When the DNA is broken down, it no longer binds to the methyl green, and a clear halo will appear around the areas where the DNase-producing organism has grown.

3.6 Determination the most predominant pathogens

This investigation aimed to determine the most frequent pathogen isolated from the juice samples. This was done by examining the samples at different time from the same collection point. This was according to a study was carried out by (Tambekar *et al*., 2009).

3.7 Bacterial identification Using Phoenix automated system 100X

The BD Phoenix™ automated identification and susceptibility testing system provides rapid, accurate and reliable detection of known and emerging antimicrobial resistance. It also enables workflow efficiency by utilizing automated nephelometry, which results in a standardized isolate inoculum and a reduction in potential technologist error. The BD Phoenix™ system offers identification-only panels and combination ID/AST panels, using 51 wells for identification substrates. The instrument can provide rapid identification of most clinically significant Gram-negative and Gram-positive bacteria, as well as yeast. Phoenix Test Method, There are two basic solutions for the test, AST and the other is ID and panel. ID is solution and function of bacteria identification, The AST is antimicrobial susceptibly testing. All materials before use are at room temperature, We take a part of the colony to be tested (using a cotton swab) and put it in the ID tube and then put it on the shaker until it mixes well, then we measure the optical density in the ID tube, it should be 0.5 We take by pipet 25 microliters (one drop) of the suspension following the ID and add it to the AST tube. The last step, which is to pour the content of the two tubes ID and AST in their respective places on the kit. The kits are numbered with the colony number and placed in the phoenix. It usually takes 10 to 16 hours to finish reading.

3.8 Antimicrobial sensitivity test

The Kirby-Bauer test or called disk-diffusion test is a standard that has been used for years. McFarland turbidity standards are used as a reference to the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial test. The bacteria is swabbed on the Mueller-Hinton agar and the antibiotic discs are placed on top. The antibiotic diffuses from the disc into the agar in decreasing amounts the further is away from the disc, the plates are read after 18 hours and incubation at 37C˚, if the organism is killed or inhibited by the concentration of the antibiotic there will be no growth in the immediate area around the disc this is called the Zone of inhibition, the zone sizes are looked up on a standardized chart to give a result

of sensitive, resistant or intermediate. Modified Kirby-Baure method recommended by National Committee on clinical Laboratory Services (NCCLS), antimicrobial that that used in this study shown as in table (3.1).

3.9 Statistical analysis

Statistical analysis used in this study was frequencies, average, percentages, using Microsoft excel, as well as SPSS (Static Package for Social Science).

Table (3.1) : Antibiotics used for susceptibility test.

NO	Antibiotics	Symbol	Disk potency
1	Ciprofloxacin	Cip	5 µg
2	Clarithomycin	CLR	15 µg
3	Imipenem	IPM	10 µg
4	Amikacin	AK	30 µg
5	Amoxicillin	AMC	30 µg
6	Oxacillin	OX	5 µg
7	Cefixime	CFM	5 µg
8	Cephalexin	CL	30 µg
9	Sulfamethoxazole-Trimethoprim	SXT	25 µg
10	Doxycycline	DO	30 µg
11	Cefuroxime	CXM	30 µg

Chapter 4
4. Results

4.1 Bacterial identification

4.1.1 Gram negative pathogen

Types of Gram-negative bacteria that isolated from the fresh juices were including f*ecal Escherichia coli* and *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonia, Pseudomonas aeruginosa*. Each type of bacteria was identified by traditional biochemical tests shown in the table (4.1). Confirmed of *fecal E. coli* was carried out by grown on EMB agar at 44.5C˚ for 24 h. Produced colonies with a greenish metallic luster. Subsequently, the microbial species were confirmed at species level using Phoenix 100 automated system.

4.1.2 Gram positive pathogen

One type of Gram-positive bacteria was recognized in the culture media, the identification using biochemical tests shown in table (4.1) represented *Staphylococcus sp. o*nly genus type of pathogen contaminated the juice, to determine and confirm the identification of the species level, automated Phoenix 100 multi system was used, the results showed two types of pathogen including *Staphylococcus aureus* and *Staphylococcus schleiferi.*

Table (4.1): Traditional biochemical test used for the identification of bacteria isolated from fresh juice.

Biochemical test	*fecal E. coli*	*E. coli*	*E. aerogenes*	*K. pneumoniae*	*P. aeruginosa*	*S. schleiferi*	*S. aureus*
Gram stain	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**+ve**	**+ve**
Urease test	+	-	-	+/-	-	+	+
Oxidase test	-	-	-	-	+	-	-
Indole test	+	-	-	-	-	-	-
Catalase test	+	+	+	/	+	+	+
Citrate test	-	-	+	+	+	-	-
Dnase	/	/	/	/	/	+	+
Coagulase test	/	/	/	/	/	+	+
TSA -H2S	-	-	-	-	-	/	/
Gas	+	-/+	+	+	-	/	/
A/A	**A/A**	**A/A**	**A/A**	**K/A**	**K/A**	/	/
Mannitol	/	/	/	/	/	+	+

-ve Gram negative, +ve Gram positive, + Test is positive, - Test is negative, / Not determined, A acid, K alkaline.

4.2 Assessment of bacterial contamination (Gram negative and Gram positive) in fresh juices

The assessment was carried out according to increase the sources and the risk factors, that contaminate the fresh juices with bacterial pathogens leading to health problems. The microbiological screening for strawberry and mango juices was carried out in two season, collected from 64 samples from cafes and restaurants, North, South, East, city Center of Benghazi city, with total samples was 128. The sampling rate for each cafe and restaurant was eight samples (four samples of fresh strawberry juice, four samples of fresh mango juice).

The results showed that 91.41% samples, were contaminated with bacteria table (4.2), figure (Appendix1). According to the initially morphological characterization most of the samples showed one type or more of pathogen. The bacterial determination revealed that Gram-negative pathogens representing the most predominant 94% out of 117 selected pathogen, table (4.3), figure (Appendix2). The distribution of isolated bacteria according to the season of isolation was showing at first isolation 90.62% out of 64 samples microbial growth and 96.55% out of 58 samples, were showing Gram negative pathogens and 3.45% Gram positive. During the second season the results of microbial screening showed 92.19% out of 59 samples were contaminated with pathogens, as well as 91.53% of isolated pathogens were belong to Gram negative bacteria and 8.47% Gram positive, table (4.3).

The statistical analysis by using t test for two independent sample there is no any significant different between first isolation and second isolation at significant level 0.05 (t=-3.313 with p-value 0 .755). The statistical analysis for frequency of Gram negative and Gram positive in fresh juices by using t test for two independent sample there is no any significant different between first isolation and second isolation at significant level 0.05 (t=-1.143 with p-value 0.256).

Table (4.2): Samples Growth and NO .Growth.

Samples	Growth		NO .Growth	
	No.	%	No.	%
Frist isolation	58	90.62%	6	9.37%
Second isolation	59	92.19%	5	7.81%
Total	117	91.41%	11	8.59%

Table (4.3): Frequency of Gram negative and Gram positive in fresh juices.

Bacteria Isolated	Gram negative		Gram positive	
	No.	%	No.	%
Frist isolation	56	96.55%	2	3.45%
Second isolation	54	91.53%	5	8.47%
Total	110	94.00%	7	5.98%

4.3 Frequency of bacterial contamination according to the area

In order to investigate the bacterial contamination of fresh juices according to area of collection, Benghazi city was divided into four areas (North, South, East, city Center) form cafes and restaurants, within two different time season. From each geographic area four points of collection where chosen to be our target for samples collection (four samples from each point of collections).

During the first and second isolation most of samples showed bacterial growth higher than (75%), however, only one point of collection in south of the city represented (25%) rate of infection, table (4.4), figure (Appendix3). Bacterial contamination appeared in all cafes and restaurants, and every cafe and restaurant has isolated one or more types of bacteria. The percentage of contamination in the first isolation was (90.62%) for all cafes in all areas, while the second isolation was (92.18%). Where it was in the first isolation for the areas South and North (93.75%), East, Center (87.5%). As for the second isolation of the areas North and East (100%), South (81.25%) and Center (87.5%).

The statistical analysis by using F (ANOVA Analysis) test for there is no any significant different between four isolation areas at significant level 0.05 (f=0.636 with p-value 0.598).

Table (4.4): Frequency of Bacterial contamination according to the areas in two season.

Isolation areas	No. of Cafes	Frist isolation				Second isolation			
		Growth		No. Growth		Growth		No. Growth	
		No.	%	No.	%	No.	%	No.	%
North	N1	4	100.00%	0	0.00%	4	100.00%	0	0.00%
	N2	4	100.00%	0	0.00%	4	100.00%	0	0.00%
	N3	3	75.00%	1	25.00%	4	100.00%	0	0.00%
	N4	4	100.00%	0	0.00%	4	100.00%	0	0.00%
Total	**4**	**15**	**93.75%**	**1**	**6.25%**	**16**	**100.00%**	**0**	**0.00%**
South	S1	3	75.00%	1	25.00%	4	100.00%	0	0.00%
	S2	4	100.00%	0	0.00%	1	25.00%	3	75.00%
	S3	4	100.00%	0	0.00%	4	100.00%	0	0.00%
	S4	4	100.00%	0	0.00%	4	100.00%	0	0.00%
Total	**4**	**15**	**93.75%**	**1**	**6.25%**	**13**	**81.25%**	**3**	**18.75%**
East	E1	4	100.00%	0	0.00%	4	100.00%	0	0.00%
	E2	4	100.00%	0	0.00%	4	100.00%	0	0.00%
	E3	3	75.00%	1	25.00%	4	100.00%	0	0.00%
	E4	3	75.00%	1	25.00%	4	100.00%	0	0.00%
Total	**4**	**14**	**87.50%**	**2**	**12.50%**	**16**	**100.00%**	**0**	**0.00%**
Center	C1	4	100.00%	0	0.00%	4	100.00%	0%	0.00%
	C2	4	100.00%	0	0.00%	3	75.00%	1	25.00%
	C3	3	75.00%	1	25.00%	4	100.00%	0	0.00%
	C4	3	75.00%	1	25.00%	3	75.00%	1	25.00%
Total	**4**	**14**	**87.50%**	**2**	**12.50%**	**14**	**87.50%**	**2**	**12.50%**
Sum	**16**	**58**	**90.62%**	**6**	**9.37%**	**59**	**92.19%**	**5**	**7.81%**

4.4 Distribution of bacterial varieties isolated according to the area and season

The types of pathogens were counted and identified in each cafe and restaurant separately. The results are annotated according to the type of pathogen versus the area. In the first isolate, the highest bacterial growth rate of all bacterial isolates was *fecal Escherichia coli*, which is also the most predominant bacteria that was isolated from all species about 44.83% and found in fourteen cafés table (4.5). The results showed that the highest bacterial growth rate of *fecal E. coli* was in North and East 26.92% and then South and Center 23.8%. The second type of isolated bacteria is *Klebsiella pneumoniae* 32.8%, it was found in thirteen cafés as follows, and the highest bacterial growth rate was in South 38.09%, then center 28.57%, East 19.05% followed by North 14.28%. *Escherichia coli* was also isolated at an average rate among the bacteria that isolated 14% and found in nine cafes. The results showed that the highest percentage was in the following cafes North 44.4%, East 33.3%, and the lowest growth rate of bacteria was in the South and Center 11.1%. The next type of bacteria isolated from fresh juices is *Staphylococcus schleiferi*. The lowest growth rate among the isolated bacteria was 3% and appeared in the North and center cafes. The results investigated in the second isolation extra types of pathogens including *Enterobacter aerogenes* and *Pseudomonas aeruginosa*, *Staphylococcus aureus*, as shown in table (4.5), figure (Appendix4).

The statistical analysis by using F (tow way ANOVA Analysis) test it is clear that there are significant different between the bacterial types at significant level 0.05 (f=20.363 with p-value 0.000), while there are no a significant different between the isolation areas at significant level 0.05 (f=0.032 with p-value 0.992).

In the second isolate, the highest bacterial growth rate of all bacterial isolates was *Klebsiella pneumoniae*, which represented the most predominant bacterial isolate among all species with about 37.29% in eleven cafes table (4.6), figure (Appendix5). The results revealed the highest bacterial growth rate for *Klebsiella pneumoniae* was in center 68.75%, then North and East 31.25%. The second type of bacteria isolated is *Pseudomonas aeruginosa* 25.42%, found in seven cafes as follows, and the highest bacterial growth rate was in South 62.5%, then East and Center 12.5%, North 6.25%. *fecal Escherichia coli* was also isolated with an average of isolates 16.95% and found in six cafes. The results showed that the highest percentage was in the following cafes North and East 31.25% and South and Center 0%. The next type of bacteria isolated from fresh juices is *Enterobacter aerogenes* the growth rate among isolates was 11.86%

and appeared in the north, south and east cafés in equal proportions 12.5% and in Center 6.25%. While *Staphylococcus aureus* had the lowest bacterial growth rate in fresh juices 8.47%, in north was 18.75% and in east 12.5%. *Escherichia coli* and *Staphylococcus schleiferi,* did not grow in any type of fresh juices in the second isolate as shown in table (4.6).

The statistical analysis by using F (tow way ANOVA Analysis) test it is clear that there are significant different between the bacterial types at significant level 0.05 (f=9.357 with p-value0.000), as well as there are significant different between the isolation areas at significant level 0.05 (f=0.032 with p-value 0.029).

Table (4.5): Distribution of bacterial types at different areas in Benghazi city(first isolation).

Isolation areas	No. of cafes	First isolation													
		fecal E. coli		***E. coli***		***E. aerogenes***		***K. pneumonia***		***P. aeruginosa***		***S. schleiferi***		***S. aureus***	
		No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
North	N1	2	50.00%	1	25.00%	0	0.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%
	N2	1	25.00%	2	50.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
	N3	2	50.00%	0	0.00%	0	0.00%	2	50.00%	0	0.00%	0	0.00%	0	0.00%
	N4	2	50.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%	1	25.00%	0	0.00%
Total	**4**	**7**	**43.75%**	**4**	**25.00%**	**0**	**0.00%**	**3**	**18.75%**	**0**	**0.00%**	**1**	**6.25%**	**0**	**0.00%**
South	S1	2	50.00%	0	0.00%	0	0.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%
	S2	3	75.00%	0	0.00%	0	0.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%
	S3	1	25.00%	0	0.00%	0	0.00%	3	75.00%	0	0.00%	0	0.00%	0	0.00%
	S4	0	0.00%	1	25.00%	0	0.00%	3	75.00%	0	0.00%	0	0.00%	0	0.00%
Total	**4**	**6**	**37.50%**	**1**	**6.25%**	**0**	**0.00%**	**8**	**50.00%**	**0**	**0.00%**	**0**	**0.00%**	**0**	**0.00%**
East	E1	2	50.00%	1	25.00%	0	0.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%
	E2	2	50.00%	0	0.00%	0	0.00%	2	50.00%	0	0.00%	0	0.00%	0	0.00%
	E3	1	25.00%	2	50.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
	E4	2	50.00%	0	0.00%	0	0.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%
Total	**4**	**7**	**43.75%**	**3**	**18.75%**	**0**	**0.00%**	**4**	**25.00%**	**0**	**0.00%**	**0**	**0.00%**	**0**	**0.00%**
Center	C1	2	50.00%	1	25.00%	0	0.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%
	C2	3	75.00%	0	0.00%	0	0.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%
	C3	1	25.00%	0	0.00%	0	0.00%	1	25.00%	0	0.00%	1	25.00%	0	0.00%
	C4	0	0.00%	0	0.00%	0	0.00%	3	75.00%	0	0.00%	0	0.00%	0	0.00%
Total	**4**	**6**	**37.50%**	**1**	**6.25%**	**0**	**0.00%**	**6**	**37.50%**	**0**	**0.00%**	**1**	**6.25%**	**0**	**0.00%**
Sum	**16**	**26**	**44.83%**	**9**	**15.52%**	**0**	**0.00%**	**21**	**36.21%**	**0**	**0.00%**	**2**	**3.45%**	**0**	**0.00%**

Table (4.6): Distribution of bacterial types at different areas in Benghazi city(second isolation).

Isolation areas	No. of cafes	Second isolation													
		Fecal E. coli		*E. coli*		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*S. schleiferi*		*S. aureus*	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
North	N1	2	50.00%	0	0.00%	0	0.00%	1	25.00%	1	25.00%	0	0.00%	0	0.00%
	N2	1	25.00%	0	0.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%	2	50.00%
	N3	1	25.00%	0	0.00%	0	0.00%	3	75.00%	0	0.00%	0	0.00%	0	0.00%
	N4	1	25.00%	0	0.00%	1	25.00%	1	25.00%	0	0.00%	0	0.00%	1	25.00%
Total	**4**	**5**	**31.25%**	**0**	**0.00%**	**2**	**12.50%**	**5**	**31.25%**	**1**	**6.25%**	**0**	**0.00%**	**3**	**18.75%**
South	S1	0	0.00%	0	0.00%	0	0.00%	1	25.00%	3	75.00%	0	0.00%	0	0.00%
	S2	0	0.00%	0	0.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
	S3	0	0.00%	0	0.00%	1	25.00%	0	0.00%	3	75.00%	0	0.00%	0	0.00%
	S4	0	0.00%	0	0.00%	0	0.00%	0	0.00%	4	100.00%	0	0.00%	0	0.00%
Total	**4**	**0**	**0.00%**	**0**	**0.00%**	**2**	**12.50%**	**1**	**6.25%**	**10**	**62.50%**	**0**	**0.00%**	**0**	**0.00%**
East	E1	3	75.00%	0	0.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
	E2	0	0.00%	0	0.00%	0	0.00%	2	50.00%	1	25.00%	0	0.00%	1	25.00%
	E3	2	50.00%	0	0.00%	1	25.00%	1	25.00%	0	0.00%	0	0.00%	0	0.00%
	E4	0	0.00%	0	0.00%	0	0.00%	2	50.00%	1	25.00%	0	0.00%	1	25.00%
Total	**4**	**5**	**31.25%**	**0**	**0.00%**	**2**	**12.50%**	**5**	**31.25%**	**2**	**12.50%**	**0**	**0.00%**	**2**	**12.50%**
Center	C1	0	0.00%	0	0.00%	0	0.00%	2	50.00%	2	50.00%	0	0.00%	0	0.00%
	C2	0	0.00%	0	0.00%	0	0.00%	3	75.00%	0	0.00%	0	0.00%	0	0.00%
	C3	0	0.00%	0	0.00%	0	0.00%	4	100.00%	0	0.00%	0	0.00%	0	0.00%
	C4	0	0.00%	0	0.00%	1	25.00%	2	50.00%	0	0.00%	0	0.00%	0	0.00%
Total	**4**	**0**	**0.00%**	**0**	**0.00%**	**1**	**6.25%**	**11**	**68.75%**	**2**	**12.50%**	**0**	**0.00%**	**0**	**0.00%**
Sum Total	**16**	**10**	**16.95%**	**0**	**0.00%**	**7**	**11.86%**	**22**	**37.29%**	**15**	**25.42%**	**0**	**0.00%**	**5**	**8.47%**

4.5 Diversity of the bacterial pathogens at different of the four study areas for two season

In order to determine the most predominant pathogen in each geographic studied area, Benghazi city was divided into four main places, from each isolated sample the bacterial diversity on each plate was recognized, using the growth bacterial selectivity on agar media, lactose fermentation, shape and size of the colonies, as well as using the traditional biochemical tests for bacterial identification. The results are annotated by pathogen type versus region. In total isolates, the highest bacterial growth rate of all bacterial isolates was *Klebsiella pneumoniae*, which is also the most predominant pathogen isolated among all species with about 35.89%, table (4.7). The results showed that the highest bacterial growth rate of *Klebsiella pneumoniae* was in the Center 50%, South and East 28.13%, then North 25%. The second type of isolated bacteria is *fecal Escherichia coli* 30.76%, found in fourteen cafés as follows, and the highest growth rate of bacteria was in North and East 37.5%, then South and Center 18.75 %. *Pseudomonas aeruginosa* was isolated with an average of isolates 12.82% and found in seven cafes. Where the results showed that the highest percentage was in the following cafes South 31.25%, East and Center 6.25%, and the lowest growth rate of bacteria was in the North 3.13%. The bacteria isolated from fresh juices is *Escherichia coli* 7.69%, and it is found in seven cafés. Where the results showed that the highest percentage was in the following cafes North 12.5%, East 9.38%, and the lowest bacterial growth rate was in South and Center 3.13%. Then it was followed by *Enterobacter aerogenes* with a bacterial growth rate of 6.84% and the proportion was equal in all areas 6.25%.

The samples were having limited contamination of Gram positive pathogen the results showed, *Staphylococcus aureus* 4.27% was found in North Cafe 9.37% and East 6.25%, while *Staphylococcus schleiferi sp.*, It was 1.71% and it was seen in the North and Center cafes 3.13%, as shown in table (4.7), figure (Appendix6).

The statistical analysis by using F (tow way ANOVA Analysis) test it is clear that there are significant different between the bacterial types at significant level 0.05 (f=17.599 with p-value0.000), while there are no a significant different between the isolation areas at significant level 0.05 (f=0.090 with p-value 0.965).

Table (4.7): Diversity of the bacterial pathogens at different of the four study areas for two season in Benghazi city.

Area	*Fecal E. coli*		*E. coli*		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*S. schleiferi*		*S. aureus*	
	NO.	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**
North1	4	50.00%	1	12.50%	0	0.00%	2	25.00%	1	12.50%	0	0.00%	0	0.00%
North2	2	25.00%	2	25.00%	1	12.50%	0	0.00%	0	0.00%	0	0.00%	2	25.00%
North3	3	37.50%	0	0.00%	0	0.00%	5	62.50%	0	0.00%	0	0.00%	0	0.00%
North4	3	37.50%	1	12.50%	1	12.50%	1	12.50%	0	0.00%	1	12.50%	1	12.50%
Total	**12**	**37.50%**	**4**	**12.50%**	**2**	**6.25%**	**8**	**25.00%**	**1**	**3.13%**	**1**	**3.13%**	**3**	**9.37%**
South1	2	25.00%	0	0.00%	0	0.00%	2	25.00%	3	37.50%	0	0.00%	0	0.00%
South2	3	37.50%	0	0.00%	1	12.50%	1	12.50%	0	0.00%	0	0.00%	0	0.00%
South3	1	12.50%	0	0.00%	1	12.50%	3	37.50%	3	37.50%	0	0.00%	0	0.00%
South4	0	0.00%	1	12.50%	0	0.00%	3	37.50%	4	50.00%	0	0.00%	0	0.00%
Total	**6**	**18.75%**	**1**	**3.13%**	**2**	**6.25%**	**9**	**28.13%**	**10**	**31.25%**	**0**	**0.00%**	**0**	**0.00%**
East1	5	62.50%	1	12.50%	1	12.50%	1	12.50%	0	0.00%	0	0.00%	0	0.00%
East2	2	25.00%	0	0.00%	0	0.00%	4	50.00%	1	12.50%	0	0.00%	1	12.50%
East3	3	37.50%	2	25.00%	1	12.50%	1	12.50%	0	0.00%	0	0.00%	0	0.00%
East4	2	25.00%	0	0.00%	0	0.00%	3	37.50%	1	12.50%	0	0.00%	1	12.50%
Total	**12**	**37.50%**	**3**	**9.38%**	**2**	**6.25%**	**9**	**28.13%**	**2**	**6.25%**	**0**	**0.00%**	**2**	**6.25%**
Center1	2	25.00%	1	12.50%	0	0.00%	3	37.50%	2	25.00%	0	0.00%	0	0.00%
Center2	3	37.50%	0	0.00%	1	12.50%	3	37.50%	0	0.00%	0	0.00%	0	0.00%
Center3	1	12.50%	0	0.00%	0	0.00%	5	62.50%	0	0.00%	1	12.50%	0	0.00%
Center4	0	0.00%	0	0.00%	1	12.50%	5	62.50%	0	0.00%	0	0.00%	0	0.00%
Total	**6**	**18.75%**	**1**	**3.15%**	**2**	**6.25%**	**16**	**50.00%**	**2**	**6.25%**	**1**	**3.13%**	**0**	**0.00%**
Sum	**36**	**30.76%**	**9**	**7.69%**	**8**	**6.84%**	**42**	**35.89%**	**15**	**12.82%**	**2**	**1.71%**	**5**	**4.27%**

4.6 Distribution of bacterial species according to the type of juice in two season

In order to investigate the most affected fresh juices by bacterial contamination. The types of pathogens were counted and identified in each café and restaurant separately for two types of fresh juices, the best sellers for both adults and children (strawberry and mango). The highest bacterial growth rate for all bacterial isolates was recorded for *Klebsiella pneumoniae* 54.76% case of contamination representing the most predominant pathogen that contaminate mango juice, whereas 45.24% container of strawberry juice showed contamination with the same pathogen. The second highest rate of contamination was recorded *fecal Escherichia coli,* the results showed 61.11% of mango juice samples are contaminated with *fecal E .coli*, strawberry juice showed 38.89% contaminated samples. *Pseudomonas aeruginosa* was also isolated and its bacterial contamination number was higher in strawberry juice 53.33% and mango juice 46.67%. Then *Escherichia coli* where the number of bacterial contamination was higher in strawberry juice 66.67% and mango juice showed a lower number of bacterial contamination 33.33%. The samples were also contaminated with *Enterobacter aerogenes* where the number of bacterial contamination in mango juice 87.5% was higher than that of strawberry juice 12.5%.

The fresh juices contaminated with limited varieties of gram-positive pathogen including *Staphylococcus*. The bacterial contamination with *Staphylococcus aureus* was higher in strawberry juice 80% than mango juice that showed a lower number of bacterial contamination 20%. *Staphylococcus schleiferi* which has the lowest growth rate among the isolates bacteria which is 1.72% and was in the first isolate only in strawberry juice 100% table (4.8), figure (Appendix7). Table (Appendix8) also shows the diversity of bacterial species at different times of isolation for two types of juice (strawberry_ mango).

The statistical analysis there are no a significant different between the type of juices at significant level 0.05 (t=-0.738 with p-value 0.488). There are significant relationship between strawberries and mango juices (R2 =0.904 with p-value 0.005).

Table (4.8): Distribution of bacterial species according to the type of juice in two season.

Type of juice	*Fecal E. coli*		*E. coli*		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*S. schleiferi*		*S. aureus*	
	N	***%***	***N***	***%***	***N***	***%***	***N***	***%***	***N***	***%***	***N***	***%***	***N***	***%***
Strawberries	14	38.89%	6	66.67%	1	12.50%	19	45.24%	8	53.33%	2	100.00%	4	80.00%
Mango	22	61.11%	3	33.33%	7	87.50%	23	54.76%	7	46.67%	0	0.00%	1	20.00%
Total	**36**	**30.76%**	**9**	**7.69%**	**8**	**6.84%**	**42**	**35.89%**	**15**	**12.82%**	**2**	**1.71%**	**5**	**4.27%**

4.7 Bacterial count (*cfu/ml*) in fresh juices tested

The most likely number method was used to directly measure the number of microbial contamination of fresh juices. Serial dilutions were prepared using 1ml of samples and mixed with sterile 9ml normal saline. From each dilution, 100 µl was spread onto agar plates and incubated at 37C° for 24h. The plates that showed the number of cfu/ml modified microbial colonies were recorded. Microbial count of different freshly prepared fruit juices were shown in the table (4.10), figure (Appendix9). From the results it is clear that all the juices contain a significant amount of microorganisms. The mean total viable count (microbial load) showed the presence of bacteria in all the freshly prepared fruit juices in the range of $0.1x10^2$ to $1.48x10^3$ cfu/ml. Maximum samples contained higher load of microbes than the Gulf standard (Gulf Standards. 2000) for foods described in table (4.9).

The samples 110 of 128 contain coliforms, ranged from $0.1x10^1$cfu/ml to $0.1x10^5$cfu/ml. Fecal *coliform* was present (in 36 of 110 samples), the rate of $0.1x10^1$cfu/ml to $0.1x10^5$ cfu/ ml with an average of $1.228x10^3$cfu/ml. In the case of total coliforms it exceeds Gulf standard for most of the cases. It was found that fecal contamination accompanied by presence of *Escherichia coli* ranged from $0.3x10^1$ to $0.2x10^4$ with an average of $3.7x10^2$ cfu/ml and *Klebsiella pneumoniae* (in 42 of 110 samples) rate $0.5x10^1$ to $0.8x10^4$ with an average of $9.70x10^2$ cfu/ml. Whereas *Pseudomonas aeruginosa* represented total bacterial count (in 15 out of 117 samples) ranged from $2.8x10^1$ to $0.5x10^4$ with an average of $1.484x10^3$ cfu/ml. Only seven samples of juices (7 of 117samples) (5.46%) showed the presence of coagulase positive *Staphylococcus, staphylococcus schleiferi sp.* was ranged $0.7x10^2$ with an average of $0.7x10^2$ cfu/ml, while *staphylococcus aureus* ranged from $0.7x10^1$ to $0.1x10^2$ with an average of $0.1x10^2$ cfu/ml, the presence of coagulase positive *Staphylococcus aureus* in juices indicate severe contamination through handling.

Table (4.9): Recommended Gulf Standard. Asghar *et al*,. (2018).

Limits	**Total viable count/l**	***Coliform*/ml**	***Fecal Coliform*/ ml**	***Staphylococcal* /ml**
Maximum bacterial load anticipated	$5.0X10^3$	10	0	10
Maximum bacterial load permitted	$1.0X10^4$	100	0	$10X10^3$

Table (4.10): Total bacterial counts (*cfu/ml*) in evaluation the fresh juices.

Bacteria	***Count (cfu/ml)***		
	Min	***Max***	***Average***
Fecal E. coli	$0.1x10^1$	$0.1x10^5$	$1.228x10^3$
E. coli	$0.3x10^1$	$0.2x10^4$	$3.7x10^2$
E. aerogenes	$3.5x10^1$	$3.5x10^3$	$0.574x10^3$
K. pneumoniae	$0.5x10^1$	$0.8x10^4$	$9.7x10^2$
P. aeruginosa	$2.8x10^1$	$0.5x10^4$	$1.484x10^3$
S. schleiferi	0	$0.7x10^2$	$0.7x10^2$
S. aureus	$0.7x10^1$	$0.1x10^2$	$0.1x10^2$

4.8 Antibiotic susceptibility of bacteria contaminating fresh juices

Antibiotic susceptibility testing was performed according to the Kirby Power disc diffusion method which is based on bacteria swabbed on Mueller-Hinton agar and antibiotic tablets are placed on top. All isolated bacteria were tested on the most commonly used antibiotics table (3.1). The results showed that all the results of the effect of antibiotics were recorded as sensitive or resistant. Antibiotic sensitivity on Gram-negative isolates showed that the highest bacterial resistance rate of the most common antibiotic was by *Fecal Escherichia coli* that recorded resistance to Clarithromycin (100%), while it was less resistant to Sulfamethoxazole-Trimethoprim (3.85%). However, the isolates showed greater sensitivity to other antibiotics including Ciprofloxacin, Imipenem and oxacillin, Cefixime and Doxycycline and Cefuroxime with percentage of sensitivity reached to (100%), whereas Sulfamethoxazole trimethoprim (96.15%), followed by Amikacin (84.6%) and Amoxicillin, Cephalexin (76.9%).

The rate of bacterial resistance *Escherichia coli* and *Enterobacter aerogenes* to Clarithromycin and Amoxicillin was (100%), while they were less resistant to Amikacin (89%). The isolates showed greater sensitivity to other antibiotics including Ciprofloxacin, Imipenem and Oxacillin, Cefixime and Doxycycline, Cefuroxime, Sulfamethoxazole trimethoprim, and Cephalexin with percentage of sensitivity reached to (100%), table (4.11).

Klebsiella pneumoniae was resistant to Clarithromycin and Amoxicillin, Sulfamethoxazole trimethoprim (95%). The isolates showed greater sensitivity to other antibiotics including Ciprofloxacin, Cefixime and Doxycycline, Cefuroxime, and Cephalexin (100%), Amikacin (95%), Imipenem and oxacillin (90%).

Pseudomonas aeruginosa has recorded resistance to Imipenem, Amikacin and Amoxicillin (100%). The isolates showed greater sensitivity to other antibiotics including Ciprofloxacin, Cefixime and Doxycycline, Clarithromycin, Cefuroxime, and Cephalexin, Sulfamethoxazole trimethoprim (100%).

Antibiotic sensitivity on Gram-positive isolates showed the highest bacterial resistance rate to the most common antibiotic focused on *Staphylococcus schleiferi sp.* That was resistant to in Ciprofloxacin and Clarithomycin, Amikacin, Amoxicillin, Oxacillin ,Cefixime and Cephalexin (100%), while it were less sensitivity to Sulfamethoxazole trimethoprim and Imipenem (50%). However, the isolates showed

greater sensitivity to other antibiotics including Doxycycline, Cefuroxime (100%), while the other antibiotics were less sensitivity to Sulfamethoxazole trimethoprim and Imipenem (50%) table (4.11). However *Staphylococcus aureus* showed full resistance to the rest of the antibiotics (100%) with the exception of Imipenem and Sulfamethoxazole Trimethoprim, Doxycycline, Cefuroxime table (4.11).

The statistical analysis by using F test (ANOVA analysis) there are significant different between all antibiotic at significant level 0.05 (F=5.044with p-value 0.000).

Table (4.11): Antibiotic Susceptibility to the isolated pathogens.

Antibiotics *vs.* Bacteria	**fecal *E. coli***		***E. coli***		***E. aerogenes***		***K. pneumoniae***		***P. aeruginosa***		***S. schleiferi***		***S. aureus***	
	R%(N)	**S%(N)**	**R%(N)**	**S%(N)**	**R%(N)**	**S%(N)**	**R%(N)**	**S%(N)**	**R%(N)**	**S%(N)**	**R%(N)**	**S%(N)**	**R%(N)**	**S%(N)**
Ciprofloxacin	0(0)	100	0(0)	100(9)	0(0)	100(8)	0(0)	100(42)	0(0)	100(15)	100(2)	0(0)	100(5)	0(0)
Clarithromycin	100(36)	0(0)	100(9)	0(0)	100(8)	0(0)	95.2 (40)	4.8(2)	0(0)	100(15)	100(2)	0(0)	100(5)	0(0)
Imipenem	0(0)	100	0(0)	100(9)	0(0)	100(8)	9.5(4)	90.5(38)	100(15)	0(0)	50(1)	50(1)	0(0)	100(5)
Amikacin	15.4(5)	84.6(31)	88.8(8)	11.2(1)	100(8)	0(0)	4.8(2)	95.2 (40)	100(15)	0(0)	100(2)	0(0)	100(5)	0(0)
Amoxicillin	23.1(8)	76.9(28)	100(9)	0(0)	100(8)	0(0)	95.2(40)	4.8(2)	100(15)	0(0)	100(2)	0(0)	100(5)	0(0)
Oxacillin	0(0)	100(36)	0(0)	100(9)	-	-	0(0)	100(42)	-	-	100(2)	0(0)	100(5)	0(0)
Cefixime	0(0)	100(36)	0(0)	100(9)	0(0)	100(8)	9.5(4)	90.5(38)	0(0)	100(15)	100(2)	0(0)	100(5)	0(0)
Cephalexin	23.1(8)	76.9(28)	0(0)	100(9)	0(0)	100(8)	0(0)	100(42)	0(0)	100(15)	100(2)	0(0)	100(5)	0(0)
Sulfamethoxazole Trimethoprim	3.9(1)	96.1 (35)	0(0)	100(9)	0(0)	100(8)	95.2(40)	4.8(2)	0(0)	100(15)	50(1)	50(1)	0(0)	100(5)
Doxycycline	0(0)	100(36)	0(0)	100(9)	0(0)	100(8)	0(0)	100(42)	0(0)	100(15)	0(0)	100(2)	0(0)	100(5)
Cefuroxime	00)	100(36)	0(0)	100(9)	0(0)	100(8)	0(0)	100(42)	0(0)	100(15)	0(0)	100(2)	0(0)	100(5)

Chapter 5
Discussion

Drinking fresh juices are popular with people all times especially in summer. They often find juice street sellers. People drink these types of juices that overlook microbiology as well as the health standard, therefore high load of distributors and lack of hygienic practicing, so people often get sick. In this study, two main types of juice are used from different area in the city of Benghazi. Most juice samples showed high level of bacterial contamination. The resources of the contamination may be due to contaminated water or the use of ice to dilute juices. It could be because contamination by unsterilized container, place, air, and preparing bare, hands. Micro-organisms can spoil or decompose fruits through damaged surfaces, such as holes, cuts and cracks that occur during growth or harvesting (Durgesh *et al*., 2008). Contamination from raw materials and equipment, additional processing conditions, improper handling, and the spread of unsanitary conditions contribute significantly to the entry of bacterial pathogens into juices prepared from these fruits (Nicholas *et al*., 2007; Ogodo *et al*., 2016; Oliviera *et al*., 2006).

In this study, samples of fresh fruit juices were collected from sixteen cafes and restaurants in the city of Benghazi. The collected samples were checked for the free of bacterial contamination. The total number of (128) samples from fresh juices, reached (117) positive samples of the total bacteria isolated. seven types of bacteria were isolated, the most prevalent was *Klebsiella pneumonia* (35.89%), *Fecal Escherichia coli* (30.76%), followed by *Pseudomonas aeruginosa* (12.82%), *Escherichia coli* (7.69%), then *Enterobacter aerogenes* (6.84%), *Staphylococcus aureus* (4.27%), *Staphylococcus schleiferi sp.* (1.71%).

In a study by Ghenghesh *et al*., (2004) on the microbiological quality of fruit juice sold in Tripoli_ Libya, it was reported that 146 samples of fruit juice were examined. *Staphylococcus aureus* was (5.5%), *Streptococcus sp*., (2.7%), *E. coli* (22.6%), *Escherichia coli* (none of the serogroup were O157)(2.1%), *Klebsiella pneumonia* (11.6%), *Aeromonas sp*., (2.1%), *Pseudomonas aeruginosa* (4.1%), *Candida sp*., (74.7%) and in other yeasts. The results were similar to those of Kaniz *et al*., (2016), there study for microbiological quality assessment of hand-made juice in Dhaka city street and found that ten bacteria were finally isolated. of which *Escherichia coli, Klebsiella pneumoniae*, which is similar to our study.

A similar study by Berhanu *et al*., (2020) on the microbial quality spectrum of fresh and packaged fruit juices in supermarkets and cafes in Gondar city, Northwest Ethiopia, they

found six different types of bacterial pathogens including *E. coli, Staphylococcus aureus, Salmonella sp., Shigella sp., Pseudomonas sp.* and *Klebsiella sp*. have been detected in fresh fruit juice samples from pineapple and mango Rani Juice company.

A study by Wedajo and Kadire, (2019), on the assessment of bacterial load of some fresh and packed fruit juices in Arba Minch town, Ethiopia, had investigated that the juice samples represented prevalence of *E. coli, Salmonella* and *Staphylococcus aureus* in to all fresh fruit juices samples. Also appears in a study was done by Nagwa *et al*., (2017), on the microbiological profile and safety of fresh juices sold in the center bus station at Khartoum state. They found that the predominant bacterial isolates were identified as *Staphylococcus aureus* (19.6%), *Bacillus cereus* (13.1%), *Escherichia coli* (8.7%), and as well as other bacterial types.

In study of Asmamaw and Mulugeta, (2012) for bacteriological safety of freshly squeezed mango and pineapple juices served in juice houses of Bahir Dar town, Northwest Ethiopia, The dominant bacterial groups isolated from sample juices were *Citrobacter sp.* (45.7%) followed by *Salmonella sp.* (20%), *E. coli sp.* (14.3%), *Enterobacter sp.* (11.4%), *Klebsiella sp*. (5.7%), and *Pseudomonas sp.* (2.9%) species. What is observed in this study is similar to that reported by Mesfin, (2011) Bacteriological profile of locally prepared fresh juices in Hawassa town. The presence of *Salmonella* and *E. coli* in juices indicates possible risks of gastrointestinal infections from their consumption. Thus consumption of freshly squeezed and unpasteurized juice requires special attention.

Bacterial count mean unit used to estimate the number of viable bacterial cells in a sample cfu/ml, the results showed that *Fecal Escherichia coli* ranged from $0.1x10^1$ to $0.1x10^5$ cfu/ml with an average of $1.228x10^3$ cfu/ml, *Escherichia coli* ranged from $0.3x10^1$ to $0.2x10^4$ cfu/ml with an average of $3.7x10^2$ cfu/ml, while *Enterobacter aerogenes* ranged from $3.5x10^1$ to $3.5x10^3$ with an average of $0.574x10^3$ cfu/ml. *Klebsiella pneumoniae* ranged from 0.5×10^1 to 0.8×10^4 with an average of $9.70x10^2$ cfu/ml, as for *Pseudomonas aeruginosa* ranged from 2.8×10^1 to $0.5x10^4$ with an average of $1.484x10^3$ cfu/ml.

Staphylococcus schleiferi sp., ranged $0.7x10^2$ with an average of $0.7x10^2$ cfu/ml, while *Staphylococcus aureus* ranged from $0.7x10^1$ to $0.1x10^2$ with an average of $0.1x10^2$ cfu/ml. Compared to the study by Reddy *et al*., (2009) that studies have shown the presence of four different pathogenic coliforms in all four foods and water samples. They are *Klebsiella pneumonia, Citrobacter freundii, Enterobacter aerogens*, and *E. coli*, MPN results showed

that the first sample was the most contaminated with 1, 40,000 coliforms per 100ml of juice sample. Second highest contamination was seen in juice sample 3 with count From 1, 10,000 coliforms per 100 ml. Third highest contamination was observed in juice sample 2 with 1500 coliform per 100 ml. The least contamination was observed in the juice sample-4 with A 400 coli per 100 ml. Comparing the acceptable limit of the Gulf Standard (2000). The percentage of contamination is high, so the samples are not acceptable for consumption. A similar study by Babiye, (2017). The study to isolation and identification of bacteria from fresh fruit juice prepared in cafeterias and restaurants, Axum town, Ethiopia. The most results showed that sample 10^{-1} was the most contaminated in the mango and avocado sample with 150 and 120 coliforms per 100 ml of the juice sample, respectively. The second highest contamination was seen in the juice sample 10^{-2} with the number of 100 and 100 coliforms per 100 ml of mango and avocado. Also study by Nagwa *et al*., (2017), study showed the microbiological parameters studied were total viable bacterial count (TVBC), coliforms. The TVBCs of all investigated samples exhibited counts in the range of $1.8x10^3$ to $0.3x10^6$ cfu/ml. The count of total coliforms ranged from 3.4 to 1300 MPN/ml. According to Andres *et al*., (2004). reported that presence of coliform in fruit juice is not permitted by safe food consumption standard. This result is also in agreement with some previous research works Ahmed et al., (2010) and Durgesh *et al*., (2008), also fruit juices were heavily contaminated by *E. coli* (Bagde and Tumane, (2011).

A few reports have shown the prevalence of *staphylococci* in fruit juice samples (Ahmed *et al*., (2010); Tambekar *et al*., (2009). In this study, *staphylococci* were found in seven samples. In study carried out by Asmamaw and Mulugeta, (2012) was to assess bacteriological quality and safety of freshly squeezed mango and pineapple juices in Bahir Dar town, Ethiopia. The mean aerobic mesophilic count of mango juice (4.76 log cfu/ml) was relatively higher than pineapple juice (4.21 log cfu/ml) across each juice house. The mean *Staphylococcus aureus* counts were 3.84 log cfu/ml in mango and 3.74 log cfu/ml in pineapple juices. Total coliform counts were in the range of 9.2 to > 1100 MPN/ml in mango and from < 3 to > 1100 MPN/ml in pineapple juices. Total coliform counts in water samples were in the range of < 0.018 to > 16 MPN/ml.

Report by Mengistu *et al*., (2022) on the bacteriological quality of locally prepared fresh fruit juice sold in juice houses in eastern Ethiopia. Shows the 78 juice samples analyzed, 85.9% of the samples had a total viable bacterial count, 64.1% a total coliform count, 60.3%

fecal coliform, and 33.3% of the samples had *Escherichia coli* higher than the maximum permitted level of Gulf standard 2000.

Counts above the permissible limits coincide with Iqbal *et al*., (2015) and Mohd Nawawee *et al*., (2019), who reported counts of coliforms of 5,45 ± 1,06 Log cfu/ ml and 4.75 ± 0.79 Log cfu/ml in fruit juices, respectively. The total counts indicate that samples had *fecal coliform,* with a highest average reading of 4.64 ± 0.04 Log cfu/ml. According to Reda *et al*., (2017), the presence of thermotolerant *fecal coliform* can be attributed to fecal contamination of the water used to wash utensils, fruits, or transferred directly from the vendors, as well as the environment in which the juice is prepared, and leaving food at room temperature, at this case of these juices, and these can multiply to reach high concentrations. Consequently confirmed the presence of *Escherichia coli* and *Salmonella sp*. (Winn *and* Koneman, 2008), indicative of recent fecal contamination and unsanitary processing (Food Drug Administration, (2021).

In study carried out by Rahman *et al*., (2011) an assessment of microbiological quality of some commercially packed and fresh fruit juices available in Dhaka city, reported that the total viable bacterial count in most of the fresh juice samples was higher than the commercially packed juice samples, so the highest counts they obtained for fresh and packed juice samples were 2.4×10^4 cfu/ml and 3.2×10^3 cfu/ml, respectively. Shakir *et al*., (2009) in microbiological quality of local market Vended freshly squeezed fruit juices in Dhaka city, also reported that the total aerobic bacteria count of 8.00×10^3 to 8.05×10^8 cfu/ml for mango juices and the mean total viable count (microbial load) showed the presence of bacteria in all the freshly prepared fruit juices in the range from 3.00×10^2 to 9.60×10^8 cfu/ml.

Study for microbiological safety of fruit juices served in cafes/restaurants, Jimma town, southwest Ethiopia, for Tsige *et al*., (2008) also reported that the mean aerobic mesophilic bacteria counts (cfu/ml) of avocado, papaya and pine-apples were 8.0×10^6, 3.1×10^7, and 7.9×10^6 cfu/ml, respectively, and the difference in colonial count between studies may be attribute to different factors such as geographical variation, PH, seasonal variation, hygiene, incubation time, sample transportation time, handling and processing, and storage.

Geta *et al*., (2019) study to microbiological safety of fruit juices served in cafes and restaurants of Debre Markos town, north western Ethiopia, the overall mean total Staphylococcal count was $0.14\pm0.03\times10^5$ cfu/ml with the maximum and minimum mean

counts being $0.27\pm0.07\times10^5$ cfu/ml (from avocado) and 0.004×10^5 cfu/ml (from mango), respectively. Among the types of juice, avocado was show high number of *staphylococcal* species. According to study conducted in Nigeria, the highest number of *Staphylococcus* species (3.5×10^4 cfu/ml) was observed in avocado juices (Bello *et al*., (2014). Even though the types of juices to show high number of *Staphylococcus* species were similar in both study, the magnitude of *Staphylococcus* species was relatively less in this study ($0.1\pm0.7\times10^2$).

The mean total Enterobacteriaceae, coliform and fecal coliform counts were $7.85\pm2.8\times10^4$ cfu/ml, $6.08\pm2.5\times10^4$ cfu/ml and $0.13\pm0.06\times10^4$ cfu/ml, respectively. The mean total Enterobacteriaceae, coliform and fecal coliform counts were $12.15\pm4.8\times10^4$, $6.46\pm3.7\times10^4$ and $0.2\pm0.1\times10^4$ cfu/ml for avocado juice and $3.56\pm2.7\times10^4$, $5.7\pm3.73\times10^4$ and $0.06\pm0.04\times10^4$ cfu/ml for mango juice, respectively. Most of the fruit juices in this study were found to be unfavorable for consumption because many of them showed the presence of Enterobacteriaceae, coliform and fecal coliforms. The presence of coliform in fruit juice is not allowed by safe food consumption standard (Andres *et al*., (2004). Whereas Enterobacteriaceae, *Staphylococci* and *mold* count were significantly different. Several food safety reports published to highlight the safety status of street vended fruits, vegetables and their juices associate consumer health threats with unhygienic environment, poor juice extraction and handling practices, extremely low grade raw material and the general health of the vendors themselves (Lewis *et al*., (2006); Tambekar *et al*., (2009); Titarmare *et al*., (2009). Similarly, compared to these studies large numbers of coliforms were found in this study.

The results of the antimicrobial susceptibility test were as follows, Gram-negative, the highest resistance to Clarithromycin was the bacteria *Escherichia coli* and *Fecal Escherichia coli* (100%), *Enterobacter aerogenes* (100%) and *Klebsiella pnuomuniea* (95%) followed by Amoxicillin for *Escherichia coli* and *Enterobacter aerogenes*, *Pseudomonas aeruginosa* (100%), *Klebsiella pnuomuniea* (95%). *Enterobacter aerogenes* and *Pseudomonas aeruginosa* resistance of Amikacin (100%), *E. coli* (89%). *Klebsiella Pnuomuniea* resistance of Sulfamethoxazole-Trimethoprim (95%). While the most sensitive results were Ciprofloxacin, Imipenem, Oxacillin and Cefixime, Cephalexin, Doxycycline and Cefuroxime. followed by Amikacin *Fecal E. coli* (85%), *K. pnuomuniae* (95%). What about Gram-positive bacteria *Staphylococcus sp.* showed the highest rate of resistance, the results were as follows: Ciprofloxacin, Clarithomycin, Amikacin, Amoxicillin, Oxacillin, Cefixime,

Cephalexin (100%), while other antibiotics were sensitive to Doxycycline and Cefuroxime. It has the same effect, followed by Imipenem, Azithromycin, Tetracycline and Sulfamethoxazole. Some antibiotics have shown an intermediate effect, including Imipenem, Sulfamethoxazole-trimethoprim.

Wedajo and Kadire, (2019) at the assessment of bacterial load of some fresh and packed fruit juices in Arba Minch town, Ethiopia, the study was conducted on antibacterial sensitivity of three species of pathogenic bacteria isolates including *E. coli, Salmonella* and *Staphylococcus aureus,* on different antibiotics (Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Amoxicillin, Vancomycine, Norfloxacin, Tetracycline, Erythromycin, Sulphonamides) and the results were interpreted as resistance, intermediate and susceptible according to drug resistance chart. all *E. coli, Salmonella* isolates and *Staphylococcus aureus* were completely resistant (100%) to Vancomycine. Also the current drug sensitivity pattern indicates that all the isolates of *E. coli, Salmonella* isolates and *Staphylococcus aureus* were 100% susceptible to gentamicin, likewise 100% susceptibility pattern of all *Salmonella* isolates and *Staphylococcus aureus* was observed against chloramphenicol and Ciprofloxacin, this study agrees with Meher *et al*., (2011) for prevalence of multi drug resistant bacteria on raw salad vegetables sold in major markets of Chittagong city, Bangladesh, that reported on susceptibility of *Salmonella* and *Staphylococcus aureus* against Ciprofloxacin. Resistance of *E. coli, Salmonella* isolates and *Staphylococcus aureus* isolates to specific antibiotics could possibly be due to spreading of drug resistance microbes in the environment arising from the misuse of antibiotics among the general population. In this study, the result was sensitive to *E. coli* of gentamicin, intermediate to *Staphylococcus* as per the Phoenix results.

Uddin *et al*., (2017) in report microbial safety of street vended fruit juices in Dhaka city of Bangladesh, results from antibiogram test of the isolated microorganisms were *Klebsiella sp*. found to be less sensitive against Sulfomethoxazole-trimethoprim and Ciprofloxacin having 10% and 22% sensitivity, respectively whereas highest susceptibility was found against Nalidixic acid that was 90%. Most potent *fecal coliform*, *E. coli* showed moderate level of sensitivity against Sulfomethoxazole trimethoprim (55%). Additionally, Ampicillin was found to be less effective against *E. coli* as it showed about 95% resistance against it. Pathogenic *Staphylococcus sp.* showed highest resistance against Netilmicin (90%) followed by Ampicillin (84%). Antibiotic sensitivity of *Klebsiella pneumoniae* and *Staphylococcus aureus* were found against Ciprofloxacin, Imipenem,

gentamicin, levofloxacin except Amoxicillin (Sultana *et al*., (2019), *Staphylococcus sp.* in this study was very resistant to antibiotics (high level).

Pseudomonas aeruginosa was included in the list of serious threats. In 2013, the US Centers for Disease Control and Prevention (CDC) published a comprehensive report identifying the most resistant antibiotics in the United States.

Sharada *et al*., (2010) reported that although bacteria develop multiple resistance, the degree of resistance varies with different isolates and time. In recent research antibiotic resistance of bacterial isolates against commonly used antibiotics has increased from time to time Vicas, (2010). In addition, the vendors have little knowledge of food safety which can contribute and increase bacterial load during juice preparation in an unhygienic environment contaminated with air borne dust, smoke, insects, swarming flies and left them (juices) in ambient temperature as well as for prolonged.

Conclusion

This study was to investigated the level of bacterial contamination in fresh juices. The results showed that Gram-negative bacteria were the most prevalent bacteria isolated from fresh juices by (94.83%), while Gram-positive bacteria were (6.03%), and the most prevalent Gram-negative bacteria was including *Klebsiella pneumonia* of (36.21%), followed by *fecal Escherichia coli* (31.03%), *Pseudomonas aeruginosa* (12.93%), *Escherichia coli* (7.76%), *Enterobacter aerogenes* (6.89%), *Staphylococcus aureus* (4.31%), *Staphylococcus schleiferi sp.* (1.72%). The results also showed that the number of bacteria (cfu/ml) compared to the acceptable limit of the Gulf Standard (2000). *Fecal Escherichia coli* with an average $1.228x10^3$ cfu/ml, *Escherichia coli* with an average $3.7x10^2$ cfu/ml, while *Enterobacter aerogenes* with an average $0.574x10^3$ cfu/ml. *Klebsiella pneumoniae* an average of $9.70x10^2$ cfu/ml, as for *Pseudomonas aeruginosa* with an average $1.484x10^3$ cfu/ml, were at a dangerous level. The *Staphylococcus schleiferi sp.* an average of $0.7x10^2$ cfu/ml, while *Staphylococcus aureus* with an average of $0.1x10^2$ cfu/ml were as satisfactory (bacterial load permitted). For the susceptibility of the isolated pathogens to the antimicrobial agents the result showed the effectiveness of antibiotics on Gram-negative pathogens were resistant to the following antibiotics including Amoxicillin and Amikacin, Clarithomycin. The antibiotics sensitivity for Gram-positive bacteria showed high resistance to the following Ciprofloxacin, Clarithomycin, Imipenem, Amikacin, Amoxicillin, Oxacillin, Cefixime, Cephalexin.

Recommendation

To control contamination of food and beverages with fresh fruit juices in cafes and restaurants, workers must be in good health, and must have a health certificate, in addition, workers must be educated about proper personal hygiene and sanitation and to wear gloves when handling fruit, and maintain on fingernails and head coverings, washing their hands before and after the toilet, to educate them about sanitation and cleaning equipment, surface, and utensils used to prepare juice. In addition to teach them how to wash the fruits well. Juices should be prepared in a sanitary and suitable environment away from sewage, so workers should also be educated about cleaning with detergents and soaps in cafes and restaurants before and after preparing juices. Store fruit at a cool temperature, use sterile water to dilute juice/cleaning equipment as well as used ice, inform and instruct persons who are involved in the preparation and handling of fruit juices. Government agencies should take measures to educate vendors about food safety and hygiene practices and enforce adequate juice preparation guidelines, Especially fruit juices sold on the streets.

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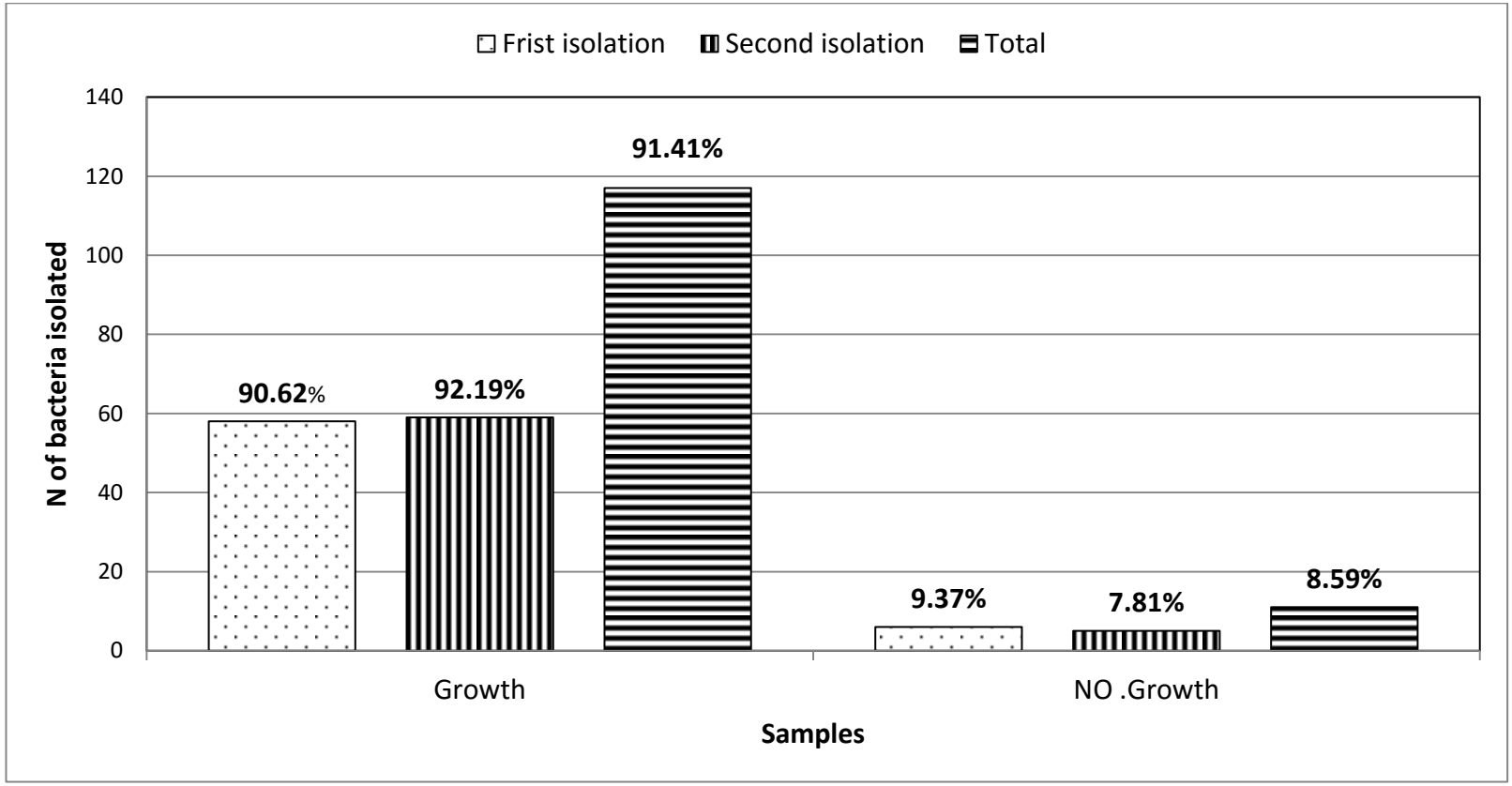
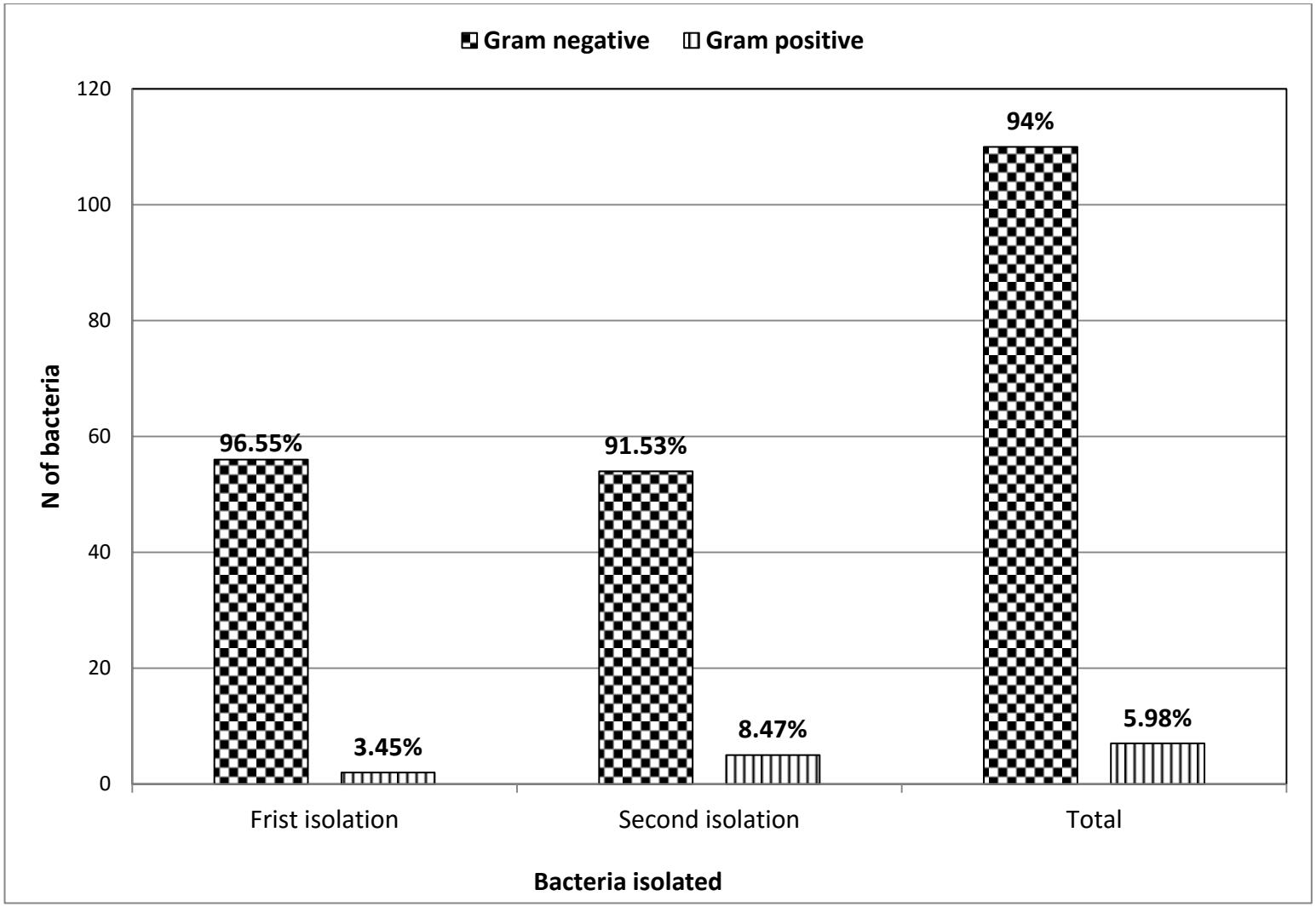
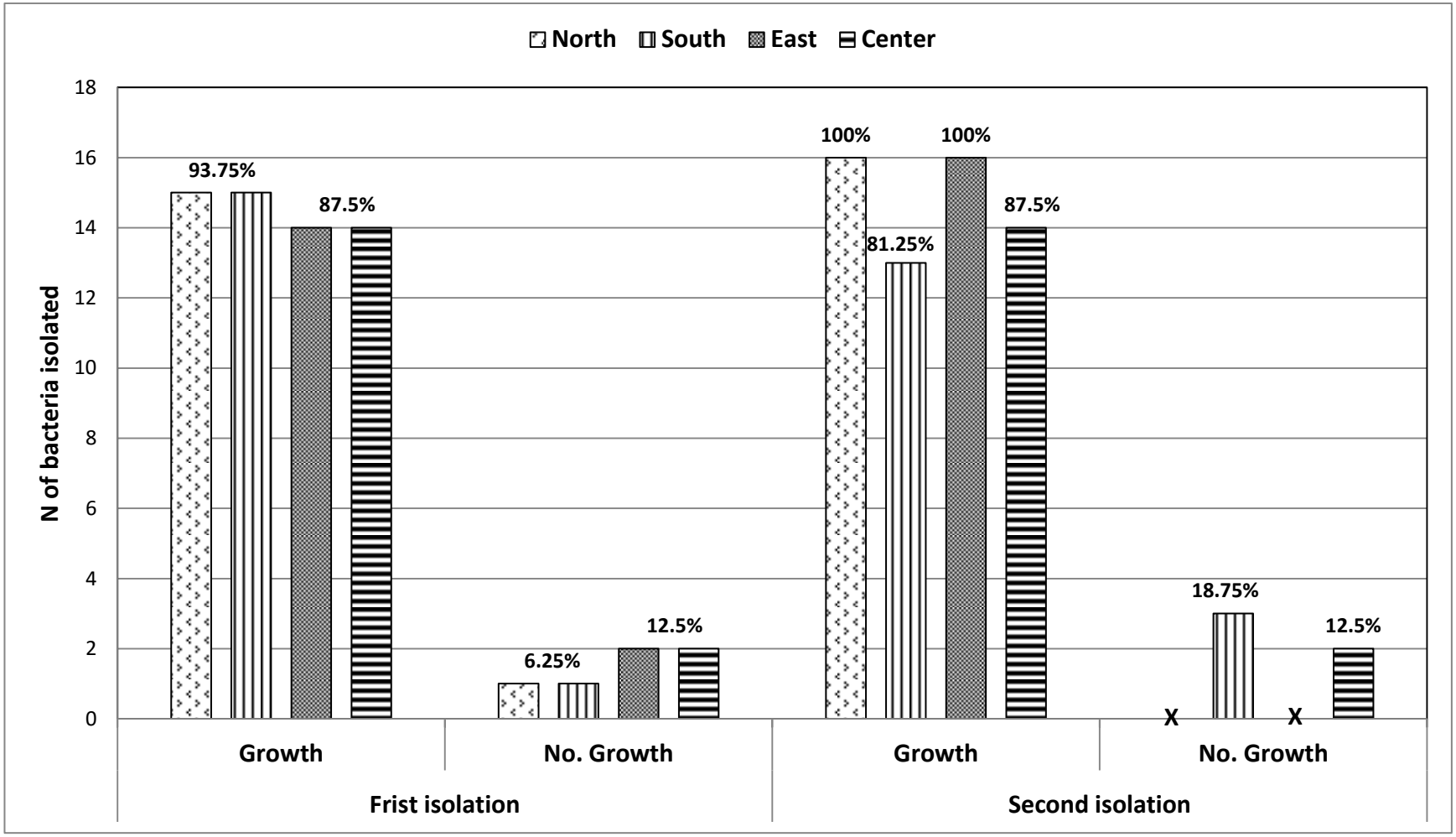
Appendices



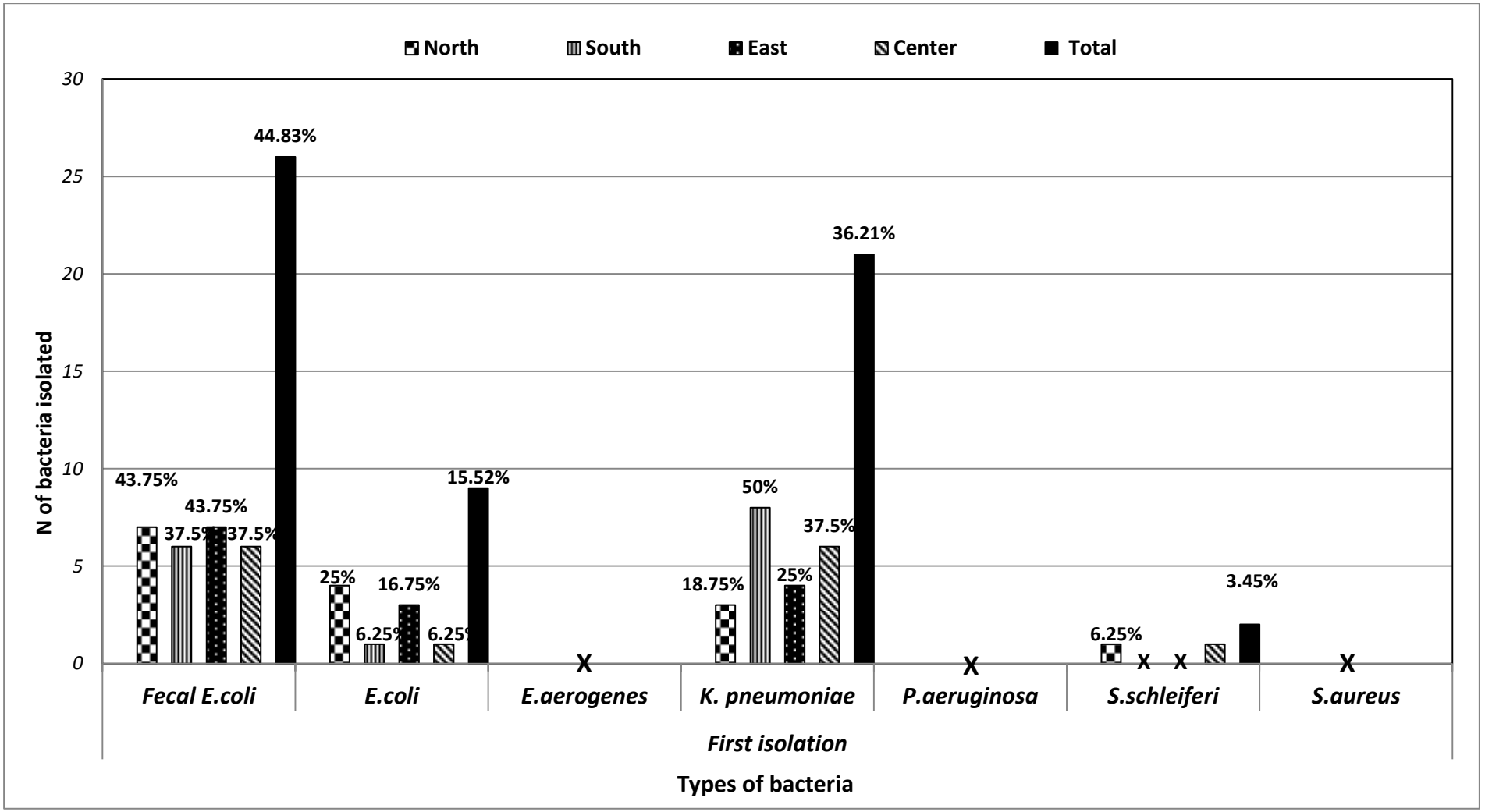
Appendix(1): Samples Growth and NO. Growth.



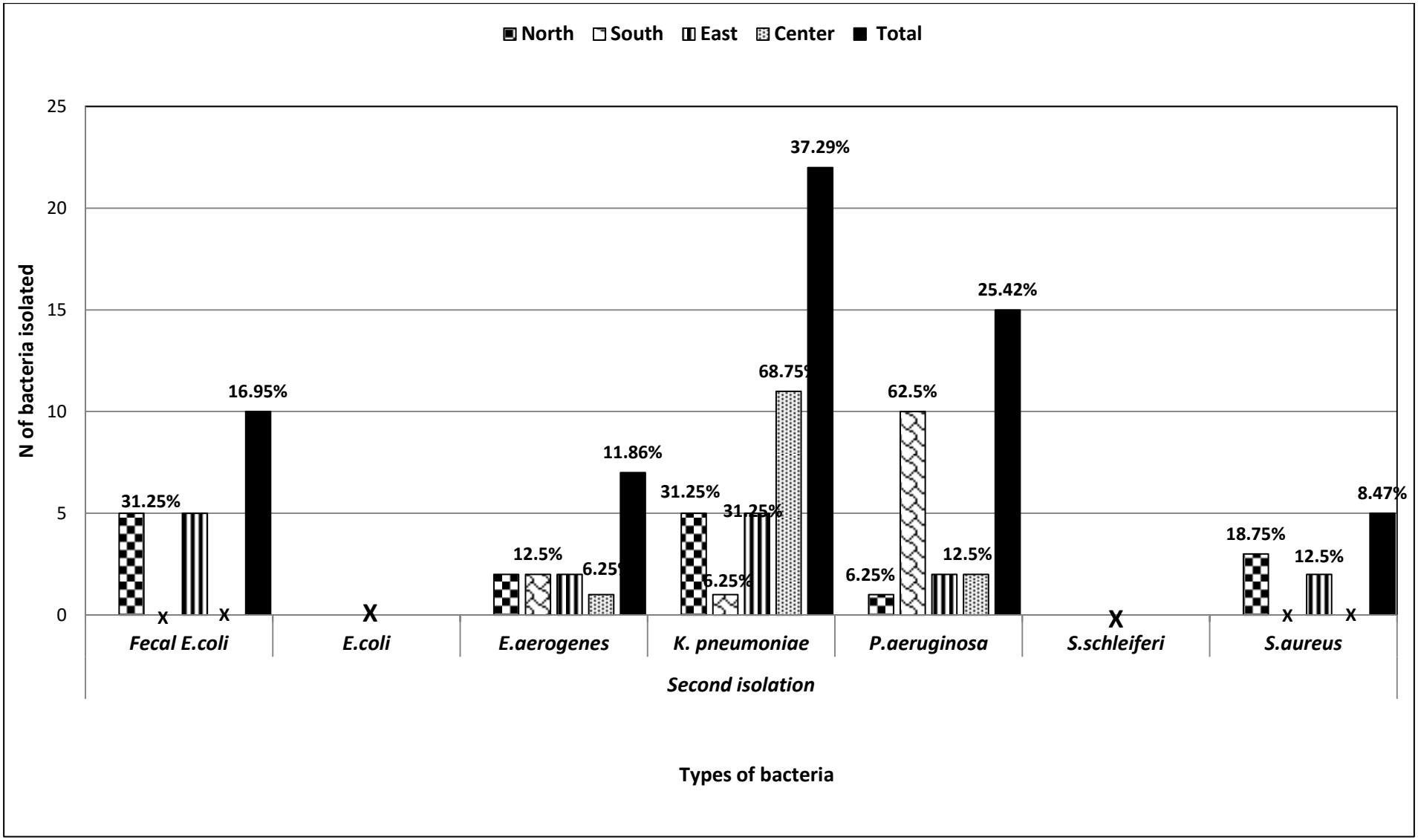
Appendix(2): Frequency of Gram negative and Gram positive bacteria isolated from fresh juices.



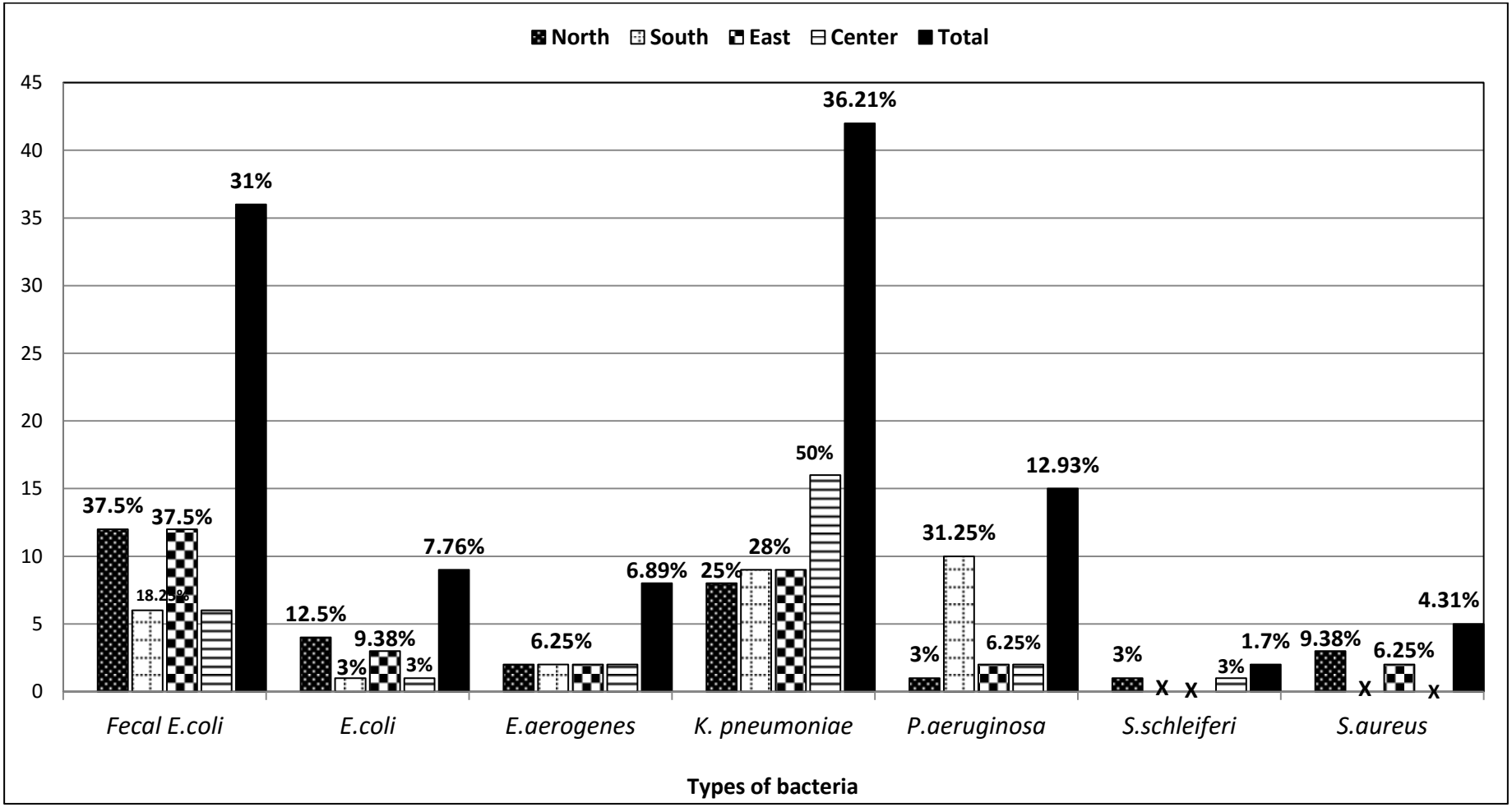
Appendix(3): Frequency of bacterial contamination according to the areas.



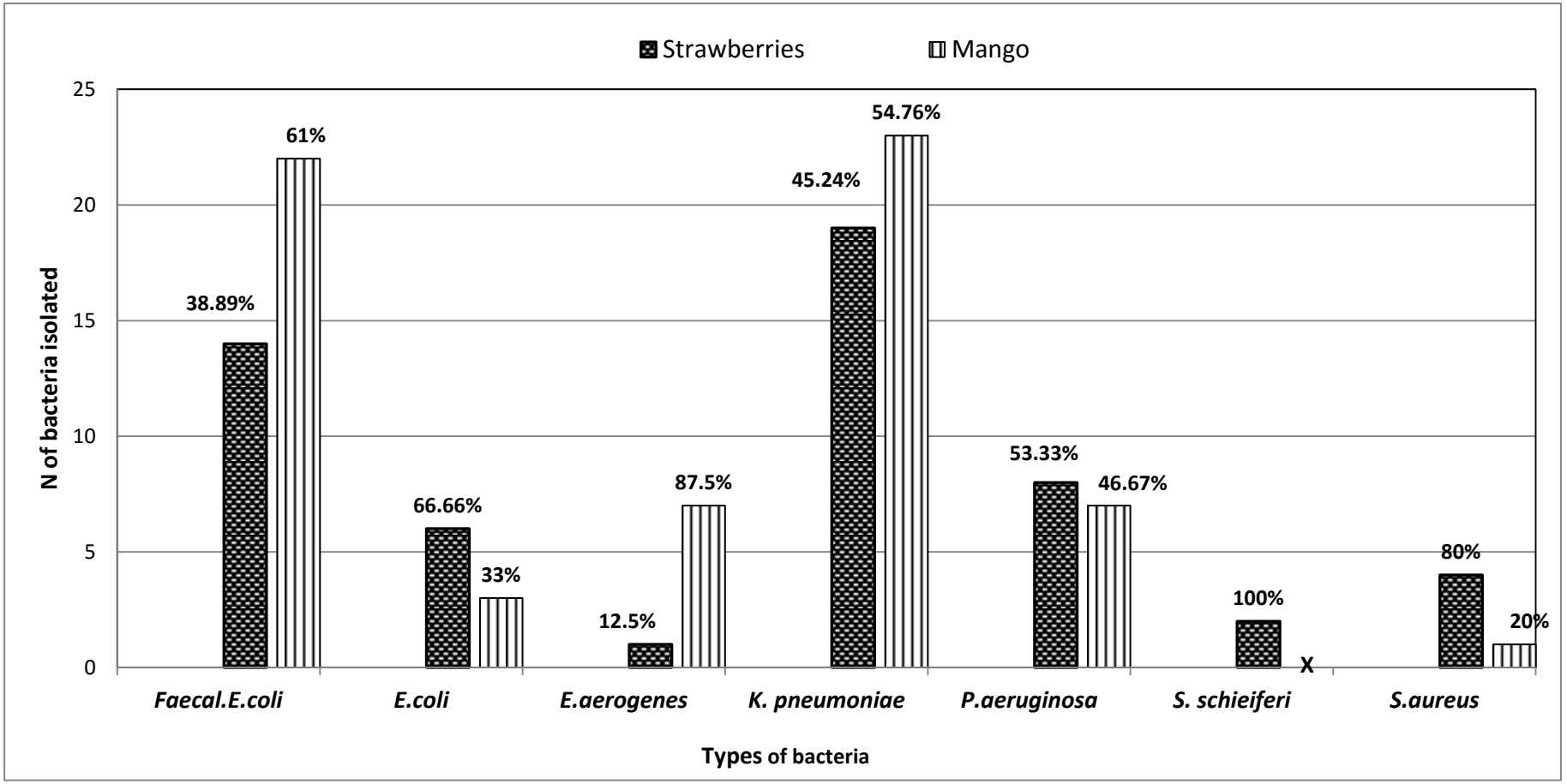
Appendix(4): Distribution of bacterial types at different selected cafes in Benghazi city (first isolation).



Appendix(5): Distribution of bacterial types at different selected cafes in Benghazi city (second isolation).



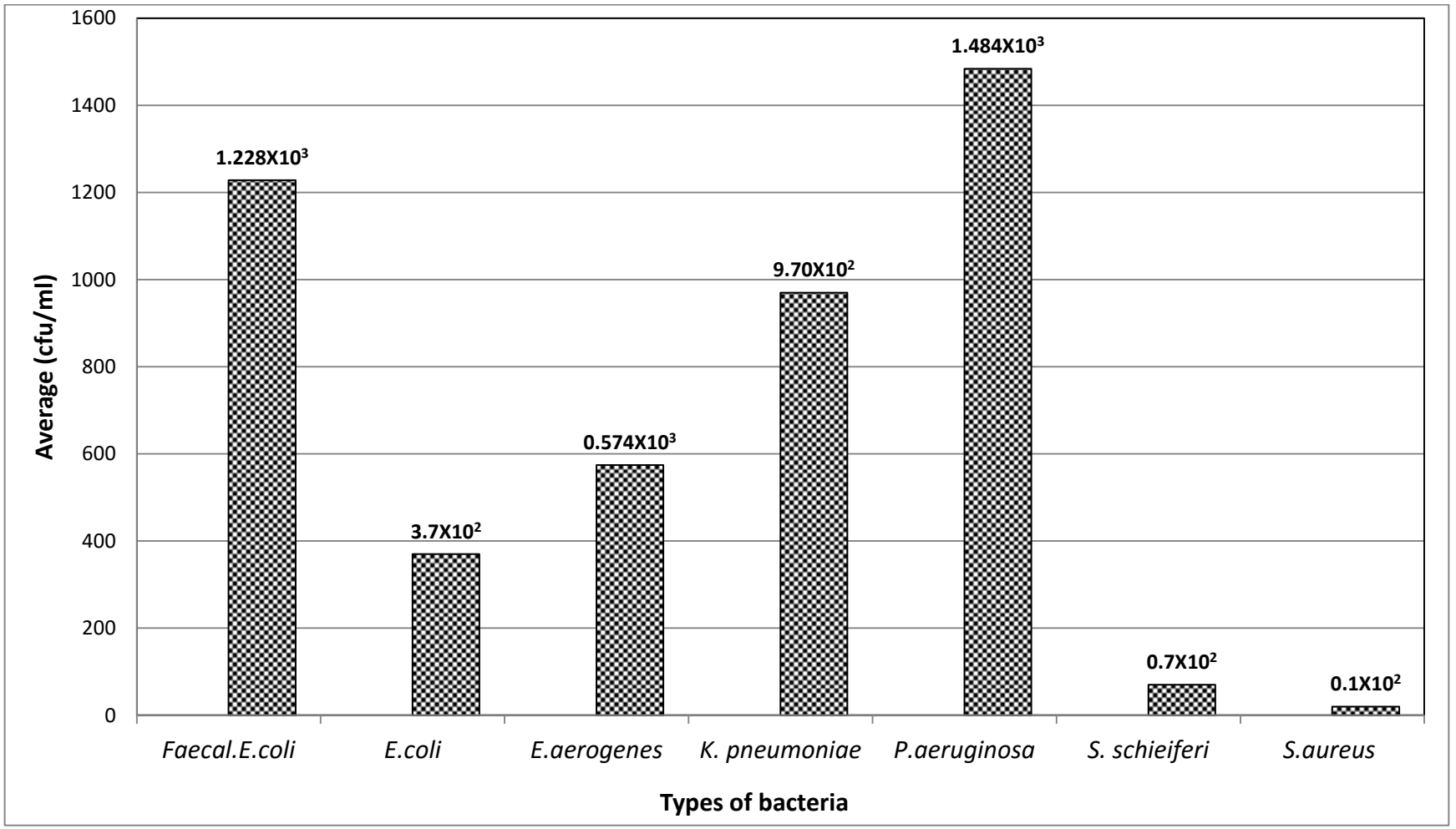
Appendix(6): Diversity of the bacterial pathogens at different selected cafes and restaurants of the four studied areas.



Appendix(7): Distribution of bacterial species according to the type of juice.

Appendix(8): Diversity of bacterial types at different times of isolation for two types of juice (Strawberries_ Mango).

Areas	Cafes	Frist Isolation		Second Isolation	
		Strawberries	**Mango**	**Strawberries**	**Mango**
North	N1	*E. coli _Fecal E. coli*	*Fecal E. coli _K. pnuomuniea*	*Fecal E. coli_ P. aeruginosa*	*Fecal E. coli _ K. pnuomuniea*
	N2	*E. coli*	*Fecal E. coli_ E. coli*	*Fecal E. coli _S. aureus*	*E. aerogenes _ S .aureus*
	N3	*Fecal E. coli _K. pnuomuniea*	*Fecal E. coli_ K. pnuomuniea*	*K. pnuomuniea*	*Fecal E. coli _K. pnuomuniea*
	N4	*Fecal E. coli _S. schleiferi*	*Fecal E. coli_ E. coli*	*K. pnuomuniea_ S. aureus*	*Fecal E. coli_ E. aerogenes*
South	S1	*K. pnuomuniea*	*Fecal E. coli*	*P. aeruginosa*	*K. pnuomuniea_ P. aeruginosa*
	S2	*Fecal E. coli*	*Fecal E. coli _K. pnuomuniea*	_	*E. aerogenes*
	S3	*K. pnuomuniea*	*Fecal E. coli _K. pnuomuniea*	*P. aeruginosa*	*E. aerogenes_ P. aeruginosa*
	S4	*E. coli _K. pnuomuniea*	*2K. pnuomuniea*	*P. aeruginosa*	*P. aeruginosa*
East	E1	*E. coli _Fecal E. coli*	*Fecal E. coli _ K. pnuomuniea*	*Fecal E. coli*	*Fecal E. coli _E. aerogenes*
	E2	*Fecal E. coli _K. pnuomuniea*	*Fecal E. coli _K. pnuomuniea*	*K. pnuomuniea _S. aureus*	*K. pnuomuniea _P. aeruginosa*
	E3	*E. coli*	*Fecal E. coli_ E. coli*	*Fecal E. coli _K. pnuomuniea*	*Fecal E. coli _E. aerogenes*
	E4	*Fecal E. coli*	*Fecal E. coli _K. pnuomuniea*	*K. pnuomuniea_ S. aureus*	*K. pnuomuniea_ P. aeruginosa*
Center	C1	*E. coli _K. pnuomuniea*	*Fecal E. coli*	*K. pnuomuniea _ P. aeruginosa*	*K. pnuomuniea_ P. aeruginosa*
	C2	*Fecal E. coli_ K. pnuomuniea*	*Fecal E. coli*	*K. pnuomuniea*	*E. aerogenes_ K. pnuomuniea*
	C3	*S. schleiferi*	*Fecal E. coli _K. pnuomuniea*	*K. Pnuomuniea*	*K. pnuomuniea*
	C4	*K. pnuomuniea*	*K. pnuomuniea*	*E. aerogenes*	*K. pnuomuniea*



Appendix(9): Bacterial counts (*cfu/ml*) in evaluation the fresh juices.

التقييم الميكروبيولوجي للعصائر الطبيعية في مدينة بنغازي

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الخلاصة

الدراسة كانت لتقييم الجودة البكتريولوجية لنوعين من العصائر الفاكهة الطازجة المتاحة للمستهلكين في مدينة بنغازي. أجريت الدراسة في نهاية فترة الصيف (سبتمبر، أكتوبر 2020 "العزلة الأولى") و (سبتمبر، أكتوبر 2021 "العزلة الثانية") على 128 عينة تم اختيارها عشوائياً من ستة عشر مقهى ومطعم في مدينة بنغازي. هذه الدراسة لعزل والتعرف على البكتيريا المسببة للأمراض التي تلوث العصائر الطازجة لنوعين من العصائر الأكثر شيوعاً بين الأطفال والبالغين (عصير الفراولة وعصير المانجو). تم التعرف على جميع البكتيريا التي تم عزلها في هذه الدراسة من خلال ظهور المستعمرات ومن خلال الاختبارات البيو كيميائية ، وتم التعرف على بعض العزلات بواسطة Phoenix100. وأظهرت النتائج أن معدل النمو البكتيري في العينات المختبرة من العصائر الطازجة كان (91.41٪). أظهرت هذه الدراسة أن أكثر الممرضات السائدة المعزولة للعصائر الطازجة كانت *Klebsiella pneumonia* (36.21٪)، يليها *fecal Escherichia coli* (31.03٪)، *Pseudomonas aeruginosa* (12.93٪)، *Escherichia coli* (7.76%)، بكتيريا *Enterobacter aerogenes* (6.89%)، *Staphylococcus aureus* (4.31%)، *Staphylococcus schleiferi sp.* (1.72%). أظهرت الدراسة وجود تنوع في العزلة البكتيرية بين العزلة الأولى والثانية. بالنسبة لقابلية مسببات الأمراض المعزولة للمضادة

الميكروبات، فقد أظهرت النتيجة فعالية المضادات الحيوية على مسببات الأمراض سالبة الجرام وكانت مقاومة للمضادات الحيوية التالية بما في ذلك Amikacin ،Amoxicillin ،Clarithomycin بينما كانت المضادات الحيوية الأخرى أكثر فعالية. أظهرت حساسية المضادات الحيوية للبكتيريا موجبة الجرام أن العامل الممرض يمثل نسبة مقاومة عالية مقارنة بالعزلات سالبة الجرام، حيث أظهرت العزلات البكتيرية مقاومة عالية لكل من: Ciprofloxacin، Clarithomycin، Imipenem، Amikacin، Amoxicilli ، Oxacillin، Cefixime، Cephalexin.

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قدمت هذه الدراسة استكمالاً لمتطلبات الحصول علي درجة الماجستير

في قسم علم النبات

جامعة بنغازي

كلية العلوم

ربيع 2022