ISOLATION OF BACTERIA FROM THE SURFACE OF CANNED DRINKS

IN BENIN-CITY, EDO STATE.

BY

OBOSHI IFEANYI KIZITO

BMS1601840

DEPARTMENT OF MEDICAL LABORTORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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CERTIFICATION

This is to certify that this project was carried out by **OBOSHI IFEANYI KIZITO** with matriculation number **BMS1601840** under the supervision of **DR (MRS.) A.N. OLISE** and submitted to the Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, in partial fulfilment of the requirement for the award of Bachelor of Medical Laboratory Science (BMLS) degree.

__________________ _________________

DR (MRS.) A.N. OLISE. **DATE**

Supervisor

__________________. _________________

PROF.(MRS) E.O. OSIME. **DATE**

Head of the Department

DEDICATION

I dedicate this project work to God Almighty for His love and infinite mercies and to my family for their unconditional love and selfless support towards me especially during the period of this work.

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ABSTRACT

Sales outlets that have poor sanitary conditions can present health risks to consumers. The direct consumption of drinks in their container can expose consumers to serious foodborne diseases, thus increasing the risk of toxi-infection and its transmission constituting a serious public health problem. This present study is aimed at evaluating the possible contamination and determination of the prevalence of Bacteria found on canned drinks and their antimicrobial susceptibility in some selected distributing and retail points in Benin-city, Edo State. The microbiological analysis was performed from the collection of beverage cans sold in distributing and retail points Benin city. The cans were stored in sterile plastic bags and introduced into individual thermal bags for temperature maintenance. Samples were taken from the surface of beverage cans with a sterile swab and transferred to vials containing 10mL of BHI broth. After 24 hours, the same swabs were used for seeding on selective culture media. The results showed that out of the hundred (100) samples examined, only 34 (34%) was positive for bacterial contamination, showing a general prevalence of 34%. Of the 39 sample collected from retailers, 15.38% (6/39) showed contamination while 54.09% (28/61) of samples collected from distributors showed contamination. The highest bacterial count was 7.0×10^5 while the lowest bacterial count was found to be 1.9×10^2.*Escherichia coli* has the most prevalence with 12 (35.3%) different isolates from the 34 positive samples followed by *Klebsiella spp.* and *Enterococcus faecalis* with 8 (23.5%) different isolates each. Four (11.8%) different isolates of *Staphylococcus aureus* were gotten while just 2 (5.9%) isolates of *Streptococcus spp*. were observed in this study. Most of the isolates were sensitive to the three different antibiotics used in this study. Five isolates were resistant to Piperacillin, while one isolate was resistant to both Gentamycin and Cefoxitin. The implications of antibiotic resistance on healthcare systems are enormous as resistance leads to the limitation of treatment options. Considering a significant p-value of ≤ 0.05, statistical analysis shows significant relationship between refrigeration and prevalence of bacterial contamination (p=0.015). Moreso, this research shows a significant relationship between purchase site and prevalence of bacterial contamination. More contamination was observed among canned drinks gotten from retailers when compared to distributors. This was found to be statistically significant (p=0.048). However, no statistically significant relationship existed between location from which canned drinks were gotten and the prevalence of bacterial contamination (p-value= 0.068).

CHAPTER ONE

INTRODUCTION

1.0. BACKGROUND OF STUDY

Drinks that can be consumed as food or refreshment are known as canned beverages since they are stored in cans for human use. The term "drink can" refers to a metal can that is made to hold a fixed amount of liquid, such as carbonated soft drinks, alcoholic beverages, fruit juices, teas, herbal teas, energy drinks, etc. (Kigighai and Jonathan, 2012). Aluminum or steel with a tin plating makes up drink cans. Approximately 370 billion beverage cans are produced annually in the world.

Aluminum beverage cans were first introduced in 1965 and currently account for about 75% of the beverage can market. The aluminum can manufacturers association estimates that the US produces about 100 billion aluminum beverage cans annually. Beverages are packaged in a variety of container types and materials, including plastic, aluminum, and glass bottles, all of which have the potential to become contaminated. Cans are widely used to hold beverages due to the popularity of beer and soft drinks. These cans are frequently moved and kept in boxed packaging. Can tops are frequently left off while packaging and displaying cans. Cans may become contaminated by bacteria while being stored and transported. Therefore, drinking from a can puts one's mouth in close proximity to the can lid, potentially facilitating the spread of microbes.

Numerous studies have demonstrated that bacteria can colonize inanimate objects such canned beverages and other items used to consume canned beverages (Dantas et al., 2006); mobile phones used by medical personnel (Kilic et al., 2009); and domestic surfaces as well as food surfaces (Othman, 2015). Microbes are, to put it mildly, not only abundant in nature, but a variety of their environmental activities can have either favorable or unfavorable effects on people, animals, and plants. Few studies have been conducted on the potential health risks connected with soft drink consumption in cans or bottles due to microbial contamination of the surfaces and orifices, particularly in underdeveloped and Third World nations. In their 2012 study, Kigighai and Jonathan found that non-alcoholic carbonated drinks contain germs that are harmful to human health, including Staphylococcus, Bacillus, Enterococcus, Micrococcus Proteus, and Pseudomonas species. A study on the quality of the components used in the soft drink industries was conducted by Griffiths et al. in 1997 (quoted in Kigigha and Jonathan, 2012). Amusa et al. (2005) conducted nutritional and microbiological studies on the quality of hawker sorrel drinks, commonly referred to as zobo, in the City of Ibadan. The report revealed a number of microbial contaminants of public health importance, including Bacillus cereus, B. subtilis, Staphylococcus, Streptococcus, and Escherichia coli. There have also been reports of pollutants that are fungi, specifically Aspergillus, Penicillium, Fusarium, and Rhizopus species. In a technical report published in 1994, Oranusi et al. studied the microbial contaminants of commercially packaged non-alcoholic beverages available in Nigeria at the time. They hypothesized that 50% of the 90 samples examined were contaminated with saprophytic and non-pathogenic bacterial species like Bacillus, Lactobacillus, Pediococcus, Staphylococcus epidermidis, and Micrococcus. Ezemba et al. (2021) conducted another study to isolate and characterize microorganisms from the surfaces of canned and bottled drinks. 28 of the 64 sample cans that had not been washed were found to contain Leptospira spp. thanks to the development of the Dinger's ring, a circular haze or disc formation. E. coli (71.8%), Klebsiella (43.6%), Enterobacter (30.9%), Salmonella (39.4%), and Shigella (22.3%) were among the other pathogenic bacteria that were isolated and identified (Ezemba et al., 2021).

1.1. JUSTIFICATION OF STUDY

There is a lack of published studies on the bacterial presence, survival, and transfer to can lids, as well as the accompanying health risk to users of canned or bottled soft beverages, especially in developing nations. Scarcity of information in this regard, therefore, necessitates this study.

Taking into account the lack of research of this nature in Benin City, the probability of contamination by microorganisms on the surface of cans and the risk to the health of the consumer population, the present research is justified.

1.2. AIM OF STUDY

This study is aimed at determining the prevalence of Bacteria found on canned drinks and their antimicrobial susceptibility in some selected Distributing and Retail points in Benin-city, Edo state.

1.3. SPECIFIC OBJECTIVES

1. To isolate and biochemically characterize bacterial species from surfaces of canned drinks sold in Benin city.

2. To carry out antimicrobial susceptibility tests on the Bacteria isolated.

3. Determine the number of colony forming units (cfu) bacteria on the top surface of beverage cans and determine the type of bacteria found.

1.4 RESEARCH HYPOTHESIS

HA. There is low prevalence / frequency of bacterial contamination of canned drinks in Benin city.

CHAPTER TWO

LITERATURE REVIEW

2.1 Drink Cans

A can, or drink box, or metal box, designates since the 1980s by the formation of the English word Can, a metal box (made of aluminum and/or tinplate) which contains a drink that can be taken away then drink without tools or cups.

Numerous studies have been conducted throughout the years to assess any potential associations between soft drink intake and related health issues. The results are still hotly debated, though. However, both the industry and the public are placing more and more emphasis on the health benefits of soft drinks. Additionally, there are currently laws in existence to guarantee that producers of soft drinks adhere to recognized national and international standards. The majority of customers think soft drinks are safe to drink in any form and that their quality is guaranteed, but they don't realize that there is another cause to be concerned about our public health when it comes to canned drinks or food. One area of science that receives little attention from the general public is hygiene. Nevertheless, everything is a potential infection vehicle according to hygiene research, including the opening or surfaces of canned drinks (Nassif *et al.,* 2010; Sousa *et al.,* 2012; Abralatas, 2016).

Packaging emerged from a need to store, transport and preserve food and drinks. Aluminum cans are a type of packaging for of great importance, which are intended to protect products from actions that may deteriorate it, whether of a chemical, physical or microbiological nature, as well as they must not represent a danger to the consumer, they must ensure their integrity, safety and health. Products in aluminum cans represent a successful market in the country, in 2014 Brazil produced 24.7 billion units for the beverage industry (Nassif *et al.,* 2010; Sousa *et al.,* 2012; Abralatas, 2016).

The packages can be considered fomites, since they can be microorganism reservoirs. Although studies reveal low concentrations of these microorganisms (MOs) in aluminum cans it is important to highlight the risk of their presence. (Mata *et al.,* 2010; Vale & Dinis, 2011).

It is common for people to consume drinks directly from the can without any type of prior cleaning, whether acquired in a restaurant, in concerts, cafeterias, supermarket or on the street by a street vendor. This habit can cause risks to the health of consumers, who should not rule out the chance of contracting diseases by ingesting microorganisms present in the packaging surface (Mata *et al.,* 2010).

In this sense, there are some studies that analyzed microbiologically the outer surfaces of various canned drinks, as well as the conditions in which those packages were in the sales establishment and storage (Dantas *et al.,* 2006; Prado *et al.,* 2009; Pro Teste, 2009; Mata *et al.,* 2010; Melo *et al.,* 2012). The results of some studies carried out on the surface of cans of aluminum revealed the presence of microorganisms: *Staphylococcus aureus*, Staphylococcus saprohyticus, *Klebsiella pneumoniae, Klebsiella sp., Proteus vulgaris, Escherichia coli,* molds and yeasts, as well as total and fecal coliforms. Regardless of the low concentrations of microorganisms found, we must take into account their presence, and the risk of diseases that they can cause consumers (Prado *et al.,* 2009; Dantas *et al.,* 2009; Bittencourt *et al.,* 2009; Mata *et al.,* 2010). The diseases that can commonly be associated with these microorganisms are food poisoning, meningitis, pneumonia and urinary tract infection (Franco and Landgraf, 2008; Bittencourt *et al.,* 2009; Melo *et al.,* 2012).

There are still no microbiological standards for the surface of these packaging, which makes it difficult to quantitatively assess which are the indices of microorganisms that, if ingested, may cause disease.

It is believed that the presence of pathogenic microorganisms in these packaging is caused by lack of information about care necessary in its handling, the deficient hygiene of its handlers, as well as inappropriate storage locations.

Modern forms of presentation and marketing of food have encouraged the habit of consumption in the packages themselves, which, although visually attractive and protective of the internal content, are exposed to superficial or of its contents resulting from environmental exposure and handling. The security of these packages, with regard to direct contact with the hands and, mainly, with the mouth, has not been evaluated, although it represents an important area of investigation, given its importance as a persuasive tool for the adoption of preventive care for the occurrence of diseases of this type or nature.

And when it comes to contamination by drinking in aluminum cans, a great discussion is raised, there has always been a need for asepsis in cans, even if they leave the factories clean, the environment where they are disposed, whether in transport or in storage, where they are exposed to any type of microorganisms, it is at this stage where the cans are vulnerable to being contaminated, subject to harm to health when coming into contact with our body.

One of the major problems found in canned drinks is that when opening the external part, which is exposed, it comes into contact with the preserved liquid, and another factor is the high level of contamination on the surfaces of cans sold by street vendors, which have the highest rate of contamination, as they sell beverages cooled by immersion in ice, there are other sales outlets that are also at risk for this problem, supermarkets and other types of retail.

2.1.1 Different Types of Drink can Packaging

Traffic jam

Drink bottling can be done in glass bottles. The Glass containers are always recommended for a variety of reasons. You will choose glass bottles for your packaging if you want communicate the high quality of your drink, since glass is the material traditional from which drink bottles have always been made. The glass is hygienic, aesthetically pleasing and natural.

Disadvantages of bottled beer packaging include glass weight, its cost and the increased difficulty in transporting the bottles, because they take up more space to store than other containers Carvalho *et al.,* (2010).

Canning

Canning is a very common solution for packaging beer. Many large brewing industries have chosen the canning to package their beers, sometimes also paired with canning in a bottle. Cans don't have the same traditional aura as cans with bottles, but, on the other hand, they appear more innovative, intelligent and easy: the perfect choice if you want to package a beer aimed at a young audience Cerqueeiro *et a*l., (2015).

Casking

Casking is the least common process for conditioning beers on the market, but it is the most used when a brewery wants to sell its beer to another industry or business in large quantities. The reasons are well known and are on the need to save space and time in large-scale packaging and transport. Casking is much faster than bottling and canning; it is less expensive since, for example, you need to disinfect one keg instead of several bottles Cerqueeiro *et a*l., (2015).

Plastic Bag

The plastic bag with a thermo-sealed closure after filling is possible. It is generally used for small volumes because once opened, the pocket must be consumed.

2.2. DRINK CAN MANUFACTURING PROCESS

2.2.1 Cutting the Blank

The blank is created by cutting an aluminum sheet into a tiny circular as the initial stage in making soda. This will create the soda can's bottom and sides. From the sheet, 12% to 14% of the aluminum is wasted. The blank is then drawn to create a cup..

2.2.2 Redrawing the Cup

In order to heighten, thin the walls, and reinforce the base of the soda can, the second process involves taking the cup that has already been created and drawing it again in a different machine. Drawing and ironing is the name given to this procedure. The entire process just needs one-fifth of a second to complete.

2.2.3 Trimming the Ears

Trimming the top of the can is the third step. The second stage causes the can's top to develop tiny ripples known as ears. The effect of the created ears cannot be avoided. To make the can level and straight, the surplus aluminum is removed from the top by about a quarter inch.

2.2.4 Cleaning and Decorating

The can is prepared for cleaning and labeling in the fourth phase. A neck is created for the drink can after it has been painted.

2.2.5 The Lid

The lid is eventually created in the sixth phase. Due to the unique design of the lid, more magnesium and less manganese in the aluminum are used. The can's lid is cut and scored before being attached to the rest of the can's body.

The cans are also examined for flaws at this stage. 50,000 parts may be discovered to be flawed throughout this process.

2.2.6 Filling and Seaming

The cans are ultimately filled with the soda beverage in the sixth and final phase. They can then be purchased.

Figure 1: Filling and steaming, the inal step of drink can manufacturing process.

2.2.7 Waste

Reviewing the processes involved in making drink cans reveals that some aluminum is being wasted. There is always a method to recycle and use this aluminum. Producers can reduce the amount of energy required during production and save money if they keep this in mind. The amount of energy used during production represents a significant cost.

2.3 DRINK PRODUCTION

The production process for the soda product is simple.

2.3.1 Syrup Production

The beverage company must locate a syrup producer before it can begin manufacturing. Finding the ideal syrup or concentrate producer will enable you to create the ideal drink flavor, therefore this process is crucial. The soda company has to locate a syrup manufacturer in order to begin manufacturing. Finding the ideal syrup or concentrate producer will enable you to create the ideal drink flavor, therefore this process is crucial.

2.3.2 Bottler

It's time to add the remaining elements to make soda after locating a syrup manufacturer. Once a soda beverage is created, a bottle manufacturer must be located. Once the bottler has been identified, the search for the distributor can begin.

2.3.3 Producer

Soda producers have a variety of distribution options from which to select when selling their goods to consumers. The greatest distribution route is a supermarket, with eating establishments and bars coming in second. The biggest and most dependable markets for soda sales are Wal-Mart and Target.

2.4 ADVANTAGES AND DISADVANTAGES OF DRINK CANS

Indeed, canned drinks have several advantages over its friend the bottle: transport: its weight and its flexibility allow the can to better travel; the preservation of freshness: hops in beer do not like sunshine. To preserve the freshness of the hops, the can is really ideal. And that's important for your hoppy beers; practicality: here again, when it's about getting rid of empty cans, it's a real deliverance.

Space saving by compacting it, no breakage; storage: The can of par its shape saves more space than the bottle, especially since you can usually stack them; express cooling: If your beer was not cool, the can of beer will cool faster than the bottle of beer Bittencourt et al., 2009.

Cans are cheaper than glass bottles, although less impact and bump resistant. They take up less storage space and transportation, resulting in considerable savings in terms of logistics Carvalho et al., 2010.

2.5 Disadvantages of drink cans

Finally, on the side of the major drawbacks of the can, we note: the fragility bobbin; the impossibility of carrying out re-fermentation in the can for a natural gasification; contraindications for aging; the express warming: temperature changes are more frequent and mistreats the product Bittencourt et al., 2009.

2.6 BACTERIA LIKELY TO CONTAMINATE THE LID OF CANS OF DRINKS

Some beverage companies have sought alternative means to try to minimize this problem, using aluminum seal that surrounds the top of the can, but there is still a lot of controversy about the protective seal, if it really solves the problem in question.

Among the main pathogenic microorganisms responsible for the transmission of foodborne diseases, *Staphylococcus aureus* (Figure 1) and Esc*herichia coli* stand out. These bacteria have a high proliferation rate and a short incubation period, causing the infected individual to have a gastrointestinal clinical picture manifested by diarrhea, nausea, vomiting and abdominal pain with or without fever. Normally, these diseases are short-lived, with the patient making full recovery. However, in very young, elderly and immunosupressed individuals, they can cause serious diseases such as skin abscess and bronchop pneumonia, even leading to death.

2.6.1 Leptospiral Species

Leptospira interrogans is one of the most widespread and virulent leptospires which is the cause of a disease called leptospirosis. This disease affects almost 600 people each year in France. These main reservoirs are the rodents, especially rats, which secrete the bacteria in their urine Anne-Sophie, (2019).

The disease is transmitted to humans by contact with a skin lesion or mucous membranes with urine from infected animals.

It can also be contracted through an infected moist environment (water soft, muddy soils) Patrick Hocherez and Curie (2015).

Rodents are the main reservoir of the bacteria, although many other domestic or wild species can also carry the bacteria, such as dogs, cattle, cattle, hedgehogs Patrick Hocherez et al., (2015).

The disease, after an incubation of 4 to 14 days, appears suddenly, marked by many clinical forms: high fever with chills (symptom flu), headaches, muscle and joint pain, and sometimes conjunctivitis, skin rash and digestive disorders. Untreated, the disease can progress to renal, hepatic, pulmonary, meningeal damage, up to lead to death.

Diagnosis can be confirmed by identification of the bacteria by culture or by serological tests from the second week of illness Patrick Hocherez et al., 2015.

2.6.2 Enterobacteriaceae

Enterobacteriaceae are the most isolated bacteria in pathologies infectious. They are the largest family of bacteria. These are the bacteria in the digestive tract of humans and animals. They are Gram bacteria negative, immobile or mobile. These bacteria are cultured on the usual media or ordinary, reduces nitrates to nitrites, does not produce cytochrome oxidase and optional aero-anaerobic. They ferment glucose with or without production of gas.

2.6.3 Escherichia coli

Escherichia coli is a bacterium commonly found in the digestive tract of humans and warm-blooded animals. Also called coli bacilli.

Escherichia coli is quantitatively the most represented aerobic species. Escherichia coli in the environment is evidence of fecal contamination.

2.6.4 Staphylococcus

Staphylococci are bacteria that are widespread in nature. They are ubiquitous commensal bacteria of human skin and mucous membranes. This are spherical, Gram-positive, non-spore-forming, immobile bacteria.

2.6.5 Shigella

Shigella is a genus of bacteria that is Gram-negative, facultative anaerobic, non-spore-forming, non-motile bacteria genetically closely related to *Escherichia coli*. Shigella has a worldwide distribution and is endemic in developing countries. Shigella flourishes in the human intestine and is frequently passed from person to person as well as through food.

2.6.6 Salmonella

Salmonella species are facultative anaerobic bacteria that can survive under low oxygen tension such as in mature slurry pits. They are known to survive for very long periods in soil and in water. Salmonella thrives better under warm weather conditions.

2.6.7 Bacillus cereus

A Gram-positive rod-shaped bacteria called Bacillus cereus is frequently discovered in food, sponges from the ocean, and soil. It thrives at room temperature.

Table 1. *Main causes of food poisoning*

Agent	Incubation Period	Main symptoms	Diagnosis	Treatment
Escherichia coli 0157: H7	3-4days	Diarrhea with blood pain and fever	Isolate microorganisms in faeces or toxin in food and faeces	Antibiotic use
Staphylococcus aureus	1 to 5 days	Watery diarrhea, pain	Isolation of Staph in stool, food or vomitus	Antibiotic use
Shigella	1 to 3 days	Fever, bloody diarrhea, dehydration, stomach cramping	Isolation of shigella in faeces or rapid diagnostic test that detects its genetic material.	Antibiotic use
Salmonella	6 - 72hours	Chills, Abdominal pain, fever, diarrhea	Isolate microogranisms in faeces or toxin in food and faeces	Antibiotic use
Bacillus cereus	4 – 16 hours	Nausea, vomiting, diarrhea	Isolation of the microorganism in food, stool or vomit	Antibiotic use

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA AND POPULATION

This project was conducted in University of Benin teaching Hospital, located in Egor Local Government Area (L.G.A.) of Edo state. Egor Local Government Area has it's headquarter in Uselu town, Benin City. It is a very small LGA and has an area of 93km^2, located close to the southern axis of Edo state. It is surrounded by Ovia North East, Oredo and Ikpoba-Okha LGAs. The population of Egor LGA is estimated at 544,479 and 359.802 km² and lies within the Latitudes 6°261 and 6°341 North of Equator and Longitude 5°351 and 5°41 East of Greenwich Meridian (National population census, 2006). TThe local government area of Egor has an estimated humidity level of 68%. The Bini, Owan, and Esan languages are spoken in the region, which is also home to Christians, Muslims, and adherents of traditional religions. The people of Egor LGA are mostly marketers and these markets provide veritable platforms for a wide variety of goods to be sold. The main occupation of the people in this LGA includes trading, farming and private transport system.

3.2 Materials Used

The chemical used for this study include Ringers solution, Nystatin (for prevention of fungal growth) and other chemicals such as Gram's stain, Kovacs reagent, Oxidase Reagent, Citrate reagent, Indole reagent and Urease medium, are of analytical or equivalent grade of high purity, other materials used include Nutrient agar and MacConkey agar, Glasswares such as Glass slides, cover slips, and test tubes, pippete The materials, including the glassware, were all produced in England by Pyrex®.

3.3 Sample size

The minimum sampling size was determined using the statistical formula:

N = (Z2pq)/ D2 where,

q = (1-p)

N = Sample Size

p = Prevalence Rate in %

Z= Confidence interval of 95% which is equivalent to Confidence of 1.96

D = Desired level of Size Significance (5%)

The prevalence of bacterial contamination of canned drinks in selected areas in Benin city, Edo state was 15 % (Osuntokun *et al*., 2015).

Substituting values:

$N = 1.96^2 \times 0.86 \times (1-0.90) / 0.05^2$

$N = 3.8416 \times 0.86 \times 0.05 / 0.0025 = 66$

A total sample size of 100 was collected.

3.4 Sources of Canned Drinks from Wholesale(Distributors) and Retail Shops

The Canned Drinks used in this project were obtained from distributors and retail shops in Uselu, Akpakpava, G.R.A. and Oluku, Edo State, Nigeria. With a geographical location of 5° 28’ 0” North, 7° 44’ 0 East.

3.5 Collection of Canned Drinks Test Sample

For this study, 100 canned drinks swab samples were obtained using the full aseptic precautions.

The 61 canned drinks swabs were obtained from retailers, 39 from distributors.

The American Public Health Association's swab-rinse procedure was used to collect the samples.

The swabs of the surfaces of the Canned drinks were collected .

3.6 Isolation of Microorganisms from Test Samples

To obtain microorganisms from canned drinks and wine swabs: The swab stick were inserted into a small test tube containing 9ml of sterile water for a serial dilution procedure.

Serial dilution of test sample

For each sample, 9 ml of Ringers solution were poured into a total of 6 sterile test tubes. A millimeter of the produced inoculum was placed in a test tube with 9ml of a diluted version of Ringers solution [10-1]. Then, 1ml of the resulting dilution was transferred into a second test tube containing 9ml of distilled water using a different micro pipette [10-2]. The process was repeated for additional dilutions up to a 10-6 dilution, with 1ml of the inoculum being discarded in the final dilution.

3.7 Spread plates techniques for isolation of isolates

The method used for isolation was pour plates techniques. Sterile Petri dishes were arranged on a working bench for each samples collected and also for the type of organism to be cultivated, which is bacteria. A 0.5ml of each dilution was poured into the Petri dishes that have already been arranged and properly labeled, about 20ml of nutrient agar were poured into each Petri dish. The plates were inverted and incubated at 37^0C for 24hours to allow bacterial growth on the nutrient agar.

3.8 Identification of isolates [Macroscopic Examination]

The pure isolates were transferred to agar slants and stored in the refrigerator. The organisms were sub cultured again and identified based on their cultural and morphological examination [Macroscopic examination] Colonial characteristics of all the various isolates were carried out by recording their characteristics growth patterns on the plates which were incubated at 37^{o}C for 24hours.

3.9 Colonial Morphology

The isolated microorganisms appeared as various forms of colonies on the agar surface, which were later recognized morphologically by the following observations:

Texture: characterize the colony's surface, including its smoothness, shimmering quality, mucoidity, sliminess, dryness, powderiness, flakiness, etc.

Transparency: colonies may be transparent (can see through them), translucent (light passes through them), or opaque (solid-appearing).

Colour or Pigmentation: Numerous bacteria produce intracellular pigments that give their colonies a distinctive color, including red, yellow, pink, or purple. Many bacteria are colorless and have a gray or white appearance.

Shape: the shape of the organism may include any of the following; circular, dotlike, irregular etc.

Colony elevation – the colony will have any of the following elevation; thin film, raised, convex etc.

3.10 Gram Staining of Isolates [Microscopic examination]

According to the manufacturer's instructions, the working solution of the reagents used for the Gram staining procedure was made. The process of staining involved emulsifying a single, isolated colony that was 18 to 24 hours old in a drop of water until a thin smear was formed in the center of a clean, grease-free slide. By running the slide through a Bunsen burner flame, the smear was air heated fixed, and it was then air dried. Crystal violet, a fundamental aniline dye, was applied to the heat-fixed smear for 60 seconds. Iodine from Lugol's was poured over it and let to sit for 60 seconds. After that, it was washed off with running water. To prevent complete decolorization, the smear was promptly washed after being decolored with 70% ethanol. The stain was applied on the counter for 60 seconds, removed with running water, then blot dried. Next, a microscope with an oil immersion objective was used to view the slide. Gram-positive organisms were those that retained the purple hue of the crystal violet-iodine complex (CV-1 complex), while Gram-negative organisms had a pink appearance.

3.11 BIOCHEMICAL TESTS

3.11.1 Indole Test

By using the intermediary molecule indolepyruvic acid, tryptophan is converted to indole through reductive deamination. Before completing the test, pure bacterial culture must be grown for 24–48 hours in sterile tryptophan or peptone broth. After incubation, the culture broth was treated with five drops of Kovac's reagent, which consists of isoamyl alcohol, para-Dimethylaminobenzaldehyde, and strong hydrochloric acid.

Method

- One colony from a pure culture of the bacterium was suspended in a suitable medium (tryptophan medium).
- The medium was incubated at 37°C during 20-28 hrs.
- A few drops of Kovác's reagent were added.

Indole reagent changes to a cerise red color as a result of a successful test.

The indole reagent is still light yellow, indicating a negative test result.

3.11.2 Citrate Test

The citrate test determines whether the tested bacterium has the ability to use citrate as the only carbon source, which some bacteria can do.

Method

A little bit of bacteria was injected into a tube containing citrate media. Another option is to streak or deep-inoculate into a "Simmons citrate tube."

It was incubated for 24-48 hours at 30-37°C.

Growth in citrate medium or growth with a blue color change in Simmon's citrate tube indicate a positive test result.

Negative test results include no growth in the citrate medium or growth but no color change (the Simmon's citrate tube remained green).

3.11.3 Oxidase test

Cytochrome C and a cytochrome c oxidase are frequently found in bacteria species that respire aerobically. These elements can be used for typing in conjunction with other techniques. It is common practice to employ a commercial test that uses an artificial electron acceptor (N, N, N', N'-tetramethyl-p-phenylenediamine). Based on its redox state, this artificial electron acceptor changes color. The material can be oxidized by the oxidized form of cytochrome c and is also known as a redox indicator. The final enzyme in the electron transport chain, cytochrome c oxidase typically converts oxygen to water.

Method

Two drops of the oxidase reagent was added onto a piece of filter paper.

The bacteria colony was transferred on the filter paper with a plastic or platinum loop onto the spot with the oxidase reagent. For 18 to 24 hours, the colonies will be incubated at the proper temperature.

Positive test: A 10- to 30-second change in color from dark blue to purple.

Negative results include no color change or a color change that takes longer than 30 seconds.

3.11.4 Urease test

Some bacteria possess the enzyme urease, which when present with water transforms urea (= carbamide) to NH3 (ammonia) and CO2 (carbon dioxide), resulting in the formation of ammonium carbonate.

Method

Since the bacteria were grown in a urease medium that also contained a pH indicator, it was possible to verify whether they expressed urease. The medium will become alkaline and urea will be transformed to ammonium carbonate if the bacteria have urease. As a result, the color will turn to red.

3.11.5 Coagulase test

Coagulase, an enzyme that turns fibrinogen into fibrin and can coagulate plasma, is produced by several bacteria. The ability of staphylococci to manufacture coagulase is thought to be connected to their pathogenicity. The test is used to distinguish between staphylococci that produce coagulase and those that do not.

Method

One colony from the suspected pure culture was placed in 0.5 ml of plasma on a glass slide.

The slide was rocked and observed for the presence of clumping.

Positive response: stable plasma that coagulates. It shouldn't dissolve when stirred.

Negative outcome: if the plasma does not coagulate or if, after stirring, the coagulate dissolves once more.

3.11.6 Catalase test

The enzyme catalase is produced by the majority of facultatively anaerobic bacteria as well as many aerobic bacteria. This enzyme's job is to detoxify hydrogen peroxide (H2O2), which is produced by superoxide dismutase from the superoxide radical. Instead of catalase, peroxidase, which is not the same enzyme as cytochrome c oxidase, is present in many aerotolerant anaerobic bacteria. Catalase and superoxide dismutase are absent in obligatory anaerobic microorganisms.

Method

A sterile inoculating loop made of plastic or platinum was used to capture bacteria from one colony and apply the microorganisms to a microscope slide.

The bacteria suspension was then seen after a drop of 3% H_2O_2 was introduced.

A catalase-producing bacterium will produce gas (O_2) in the form of bubbles as a positive test result.

Negative results: No gas formation.

3.11.7 Motility test

Nutrient agar, a semisolid agar medium, was made and sterilized in a test tube at 121° C for 15 minutes.

The samples were obtained and inoculated into the agar by a single straight stab down to the tube's center, or around half the depth of the medium, using a straight wire. The tubes were plugged and covered with the wire still in place.

Overnight, the media were incubated at 37° C.

The agar samples were incubated before being checked for growth within or outside the test tube.

Motile organisms were indicated by the spread of growth away from the line of the stab (Motility positive). A negative result for motility is shown by growth only along the stab line (non-motile organisms).

3.11.8 Antimicrobial Susceptibility Testing

To achieve turbidity corresponding to 0.5 McFarland standards, isolates were cultivated overnight on nutrient agar and suspended in sterile physiological saline. The whole surface of Nutrient agar plates was covered with the standardized inoculum using a sterile, non-toxic cotton swab (NCCLS, 2002). The agar plates were covered with antibiotic discs and all of the plates were incubated at 37°C for 24 hours.

3.12 INCLUSION CRITERIA

Retail outlets, shops and other outlets in Benin City.

3.13. EXCLUSION CRITERIA

Retail outlets, shops and other outlets outside Benin City.

3.14. Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 20.0. Data obtained was analysed using descriptive statistics and reported as the mean and standard deviation (SD). Chi square test was used to check for relationship between variables. Differences were considered significant at $P<0.05$.

CHAPTER FOUR

RESULTS

The results showed that out of the hundred (100) samples examined, only 34 (34%) was positive for bacterial contamination i.e., there was visible growth seen after various forms of analysis done in this study. The remaining 66 was free from bacterial contamination.

Table 4.1 shows the total bacterial count of all the positive samples. The highest bacterial count was 7.0×10^5 while the lowest bacterial count was found to be 1.9×10^2.

Table 4.2a and **4.2b** shows the colonial morphologic of isolates on both Nutrient and MacConkey agar.

Gram's staining and various biochemical tests showed *Escherichia coli* having the most prevalence with 12 (35.3%) different isolates from the 20 positive samples followed by *Klebsiella spp.* and *Enterococcus faecalis* with 8 (23.5%) different isolates each (Table 4.3). Four (11.8%) different isolates of *Staphylococcus aureus* were gotten while just 2 (5.9%) isolates of *Streptococcus spp*. were observed in this study.

Table 4.4 shows the prevalence of the different organisms isolated in percentage.

Table 4.5 shows the relationship between Location and prevalence of Bacterial contamination in canned drinks. There was no statistically significant relationship between location from which canned drinks was gotten and the prevalence of bacterial contamination (p=0.068).

Table 4.6 shows a statistically significant association between Refrigeration and prevalence of bacterial contamination (p=0.015).

Table 4.7 shows a significant relationship between purchase site and prevalence of bacterial contamination. More contamination was observed among canned drinks gotten from retailers when compared to distributors. This was found to be statistically significant (p=0.048).

Table 4.8 shows the antimicrobial susceptibility patterns of organisms isolated. It was observed that most of the isolates were sensitive to the three different antibiotics used in this study. Five isolates were resistant to Piperacillin, while one isolate was resistant to both Gentamycin and Cefoxitin.

Table 4.1: Total bacterial count of Canned drinks samples positive for bacterial contamination

S/N	Sample code	Bacteria count (CFU/ml)
1	D4	5.7×10^2
2	D14	2.1×10^2
3	D20	8.0×10^2
4	D24	4.5×10^2
5	D31	3.0×10^2
6	D32	3.7×10^2
7	D35	7.1×10^2
8	D37	4.0×10^4
9	D40	2.0×10^2
10	D41	3.8×10^3
11	D59	3.9×10^2
12	D60	2.3×10^2
13	D61	6.4×10^2
14	D66	6.1×10^2
15	D80	1.8×10^3

16	D84	3.4×10^4
17	D85	2.7×10^5
18	D86	2.6×10^2
19	D101	2.4×10^5
20	D112	8.0×10^3
21	D115	7.0×10^5
22	D142	5.5×10^2
23	D144	5.7×10^2
24	D148	3.9×10^3
25	D155	2.1×10^2
26	D156	3.1×10^2
27	D170	9.0×10^2
28	D171	1.8×10^2
29	D173	2.3×10^2
30	D189	1.9×10^2
31	D191	3.2×10^3
32	D194	3.8×10^4
33	D195	1.1×10^4
34	D198	2.6×10^2

Key: D=Drink can, CFU=Colony forming unit

Table 4.2a: Colonial Morphology of Bacteria Isolated from surface of canned drinks on Nutrient Agar

Sample code	Size(mm)	Shape	Edge	Elevation	Colour	Surface
D4	2-3	Round	Entire	Convex	Gray	Mucoid
D14	1-3	Round	Entire	Convex	Greyish white	Smooth
D20	1-3	Round	Entire	Convex	Greyish white	Smooth
D24	1-3	Sphere	Entire	Raised	Cream	Smooth
D31	1-3	Round	Entire	Convex	Greyish white	Smooth
D32	1-3	Sphere	Entire	Raised	Cream	Smooth
D35	2-3	Round	Entire	Convex	Gray	Mucoid
D37	1-2	Round	Entire	Raised	Gray	Glistening
D40	1-3	Round	Entire	Convex	Greyish white	Smooth
D41	1-3	Sphere	Entire	Raised	Cream	Smooth
D59	2-4	Round	Entire	Convex	Yellow	Smooth
D60	2-3	Round	Entire	Convex	Gray	Mucoid
D61	2-4	Round	Entire	Convex	Yellow	Smooth
D66	2-3	Round	Entire	Convex	Gray	Mucoid
D80	1-3	Round	Entire	Convex	Greyish white	Smooth

D84	1-3	Sphere	Entire	Raised	Cream	Smooth
D85	2-4	Round	Entire	Convex	Yellow	Smooth
D86	2-3	Round	Entire	Convex	Gray	Mucoid
D101	1-3	Sphere	Entire	Raised	Cream	Smooth
D112	1-3	Round	Entire	Convex	Greyish white	Smooth
D115	1-2	Round	Entire	Raised	Gray	Glistening
D142	1-3	Round	Entire	Convex	Greyish white	Smooth
D144	2-3	Round	Entire	Convex	Gray	Mucoid
D148	1-3	Round	Entire	Convex	Greyish white	Smooth
D155	2-3	Round	Entire	Convex	Gray	Mucoid
D156	1-3	Sphere	Entire	Raised	Cream	Smooth
D170	1-3	Round	Entire	Convex	Greyish white	Smooth
D171	1-3	Round	Entire	Convex	Greyish white	Smooth
D173	1-3	Sphere	Entire	Raised	Cream	Smooth
D189	1-3	Round	Entire	Convex	Greyish white	Smooth
D191	2-4	Round	Entire	Convex	Yellow	Smooth
D194	2-3	Round	Entire	Convex	Gray	Mucoid
D195	1-3	Sphere	Entire	Raised	Cream	Smooth
D198	1-3	Round	Entire	Convex	Greyish white	Smooth

Key: D=Drink can

Table 4.2b: Colonial Morphology of Bacteria Isolated from surface of canned drinks on MacConkey Agar

Sample code	Size(mm)	Shape	Edge	Elevation	Colour	Surface
D4	3-4	Round	Entire	Raised	Pink	Mucoid
D14	2-3	Round	Entire	Flat	Pink	Dry
D20	2-3	Round	Entire	Flat	Pink	Dry
D24	-	-	-	-	-	-
D31	2-3	Round	Entire	Flat	Pink	Dry
D32	-	-	-	-	-	-
D35	3-4	Round	Entire	Raised	Pink	Mucoid
D37	-	-	-	-	-	-
D40	2-3	Round	Entire	Flat	Pink	Dry
D41	-	-	-	-	-	-

D59	-	-	-	-	-	-
D60	3-4	Round	Entire	Raised	Pink	Mucoid
D61	-	-	-	-	-	-
D66	3-4	Round	Entire	Raised	Pink	Mucoid
D80	2-3	Round	Entire	Flat	Pink	Dry
D84	-	-	-	-	-	-
D85	-	-	-	-	-	-
D86	3-4	Round	Entire	Raised	Pink	Mucoid
D101	-	-	-	-	-	-
D112	2-3	Round	Entire	Flat	Pink	Dry
D115	-	-	-	-	-	-
D142	2-3	Round	Entire	Flat	Pink	Dry
D144	3-4	Round	Entire	Raised	Pink	Mucoid
D148	2-3	Round	Entire	Flat	Pink	Dry
D155	3-4	Round	Entire	Raised	Pink	Mucoid
D156	-	-	-	-	-	-
D170	2-3	Round	Entire	Flat	Pink	Dry
D171	2-3	Round	Entire	Flat	Pink	Dry
D173	-	-	-	-	-	-
D189	2-3	Round	Entire	Flat	Pink	Dry
D191	-	-	-	-	-	-
D194	3-4	Round	Entire	Raised	Pink	Mucoid
D195	-	-	-	-	-	-
D198	2-3	Round	Entire	Flat	Pink	Dry

Key: D=Drink can

Table 4.3: Gram's staining, Morphological and Biochemical Identification of organisms isolated from the surface of Canned drinks.

Predominant Bacteria Isolated	Identification Parameters									
	GR	MM	CA	CO	LA	CI	IN	UR	OX	MO
Escherichia coli	−	R	ND	ND	+	−	+	−	−	+
Klebsiella spp.	−	R	ND	ND	+	+	−	+	−	−
Enterococcus faecalis	+	C	−	−	+	−	−	−	−	−
Staphylococcus aureus	+	C	+	+	−	ND	ND	ND	ND	−
Streptococcus spp.	+	C	−	ND	−	ND	ND	ND	ND	−

Key: GR=Gram reaction, MM=Microscopic morphology, CA=Catalase, CO=Coagulase, LA=Lactose, CI=Citrate, IN=Indole, UR=Urease, OX=Oxidase, MO=Motility. ND=Not Done

Table 4.4. Prevalence of bacteria isolates on the surfaces of canned drinks

Isolate	Frequency	Percent (%)
Escherichia coli	12	35.3
Klebsiella spp.	8	23.5
Enterococcus faecalis	8	23.5
Staphylococcus aureus	4	11.8
Streptococcus spp.	2	5.9

Total	34	100

Table 4.5: Prevalence of Bacterial contamination on surface of canned drinks in different Locations used in the Study

Location	No. Examined	No. Contaminated (%)	P-Value
Akpakpava	27	4 (11.8)	0.068
GRA	21	7 (20.6)	
Oluku	22	11 (32.4)	
Uselu	30	12 (35.3)	

Total	100	34 (100)

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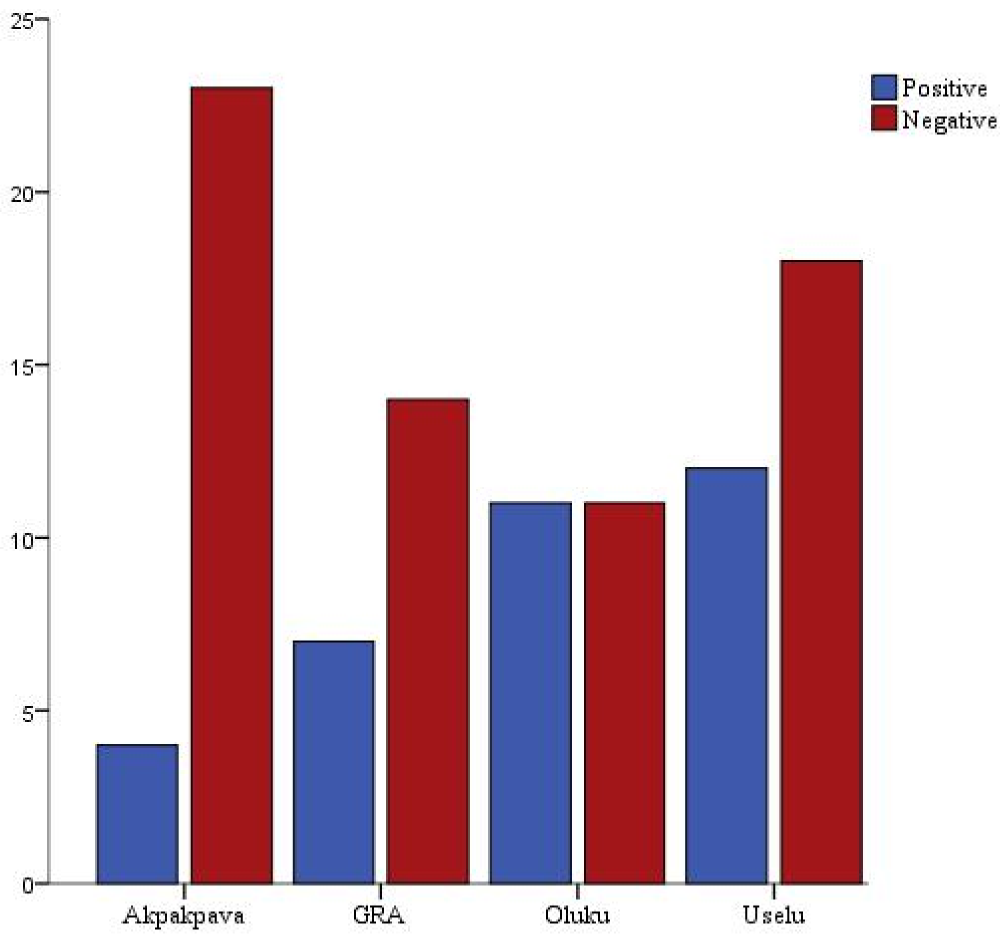

Fig 4.1. Chart showing the distribution of bacterial contamination on the surface of canned drinks in the different location used in this study

Table 4.6: Prevalence of Bacterial contamination on the surface of canned drinks in relation to Refrigeration

Variable	No. Examined	No. Contaminated (%)	P-Value
Refrigerated	56	10 (29.4)	0.015
Unrefrigerated	44	24 (70.6)	
Total	100	34 (100)	

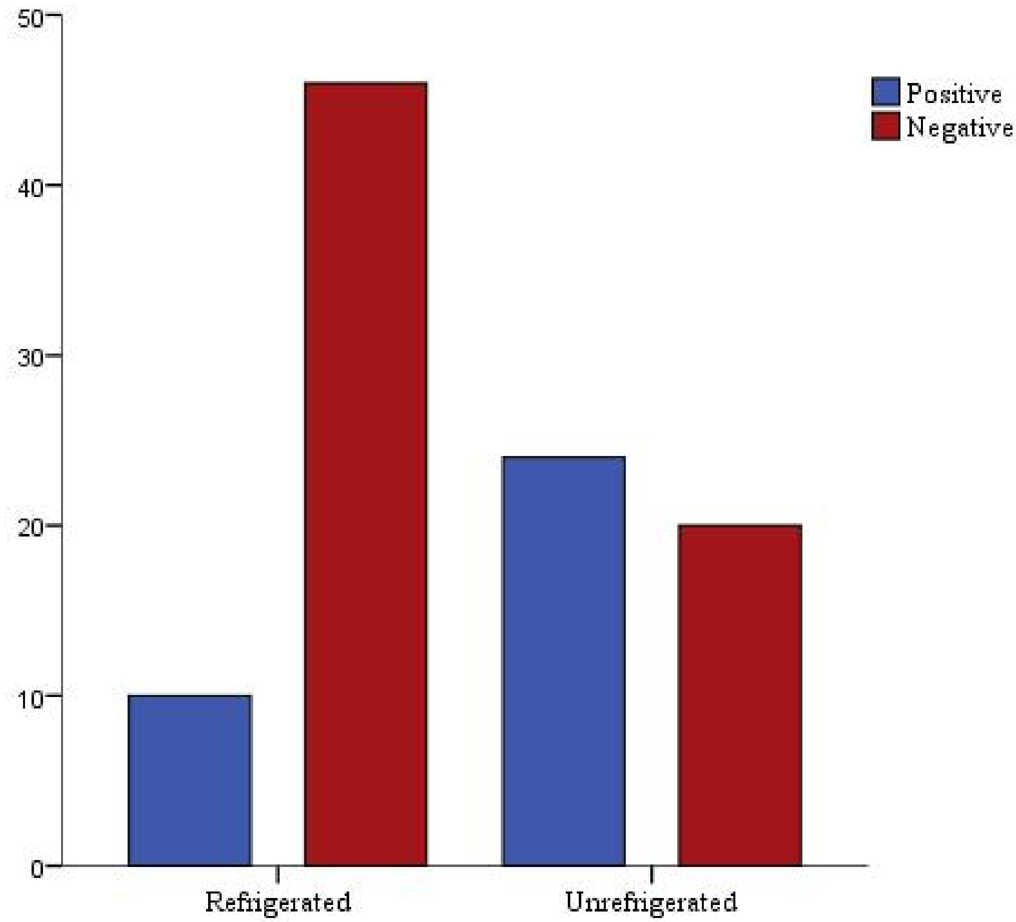

Fig 4.2. Chart showing the distribution of bacterial contamination on the surface of canned drinks in the relation to refrigeration

Table 4.7: Prevalence of Bacterial contamination on the surface of canned drinks among Distributors and Retailers

Source	No. Examined	Contaminated (%)	Non Contaminated (%)	Total (%)	P-Value
Distributors	39	6(15.38)	33(84.61)	100	0.048
Retailers	61	28(45.90)	33(54.09)	100	
Total	100	34 (100)	66(100)		

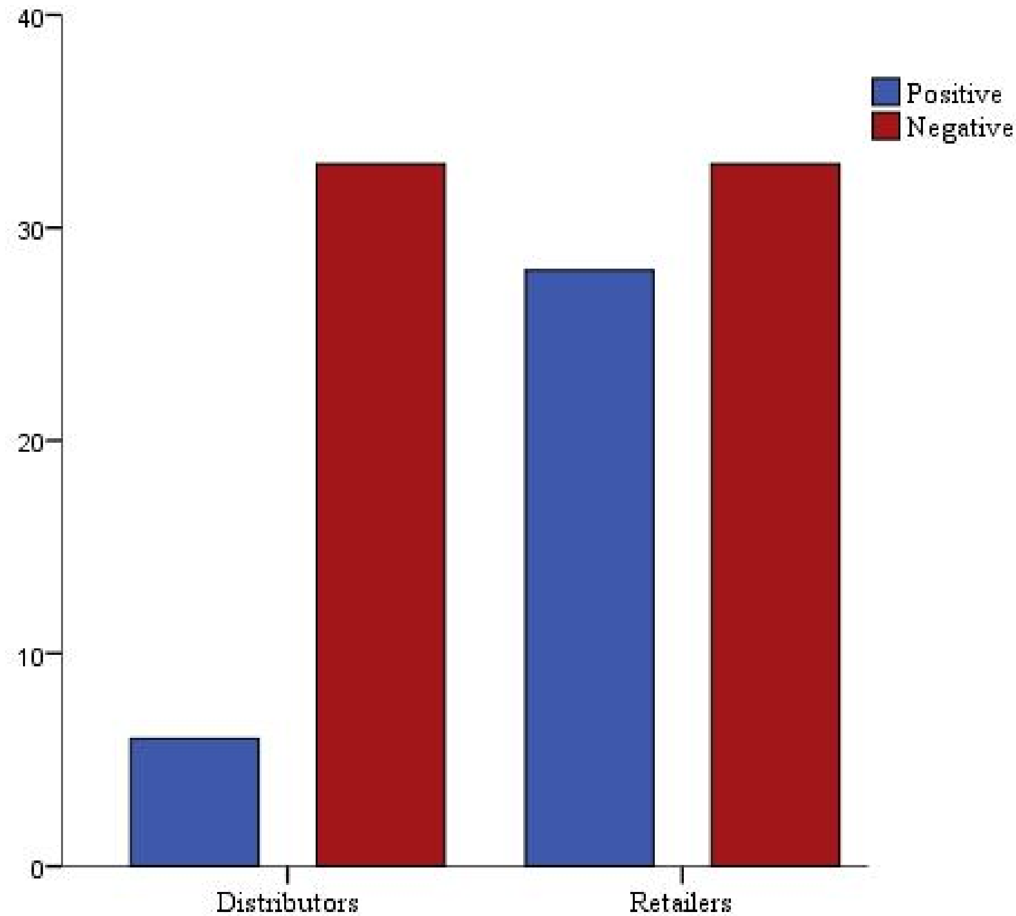

Fig 4.3. Chart showing the distribution of bacterial contamination on the surface of canned drinks among distributors and retailers

Table 4.8: Antimicrobial Susceptibility patterns of Bacteria Isolated from the surface of Canned drinks

Sample Code	Predominant Bacteria Isolated	Zones of Inhibition		
		CN	FOX	PRL
D4	*Klebsiella spp.*	25/S	21/S	18/S
D14	*Escherichia coli*	20/S	21/S	21/S
D20	*Escherichia coli*	20/S	18/S	24/S
D24	*Enterococcus faecalis*	21/S	17/S	19/S
D31	*Escherichia coli*	19/S	18/S	20/S
D32	*Enterococcus faecalis*	19/S	19/S	17/S
D35	*Klebsiella spp.*	21/S	12/R	18/S
D37	*Streptococcus spp.*	21/S	20/S	17/S
D40	*Escherichia coli*	19/S	22/S	12/R
D41	*Enterococcus faecalis.*	21/S	23/S	18/S
D59	*Staphylococcus aureus*	22/R	16/S	21/R
D60	*Klebsiella spp.*	25/S	24/S	19/S
D61	*Staphylococcus aureus*	21/S	21/S	21/S
D66	*Klebsiella spp.*	20/S	15/S	26/S
D80	*Escherichia coli*	21/S	20/S	16/S
D84	*Enterococcus faecalis*	23/S	21/S	24/S
D85	*Staphylococcus aureus*	17/S	24/S	19/S
D86	*Klebsiella spp.*	24/S	21/S	18/R
D101	*Enterococcus faecalis*	19/S	19/S	18/S
D112	*Escherichia coli.*	21/S	18/S	09/R
D115	*Streptococcus spp*	20/S	19/S	21/S

D142	*Escherichia coli*	21/S	25/S	17/S
D144	*Klebsiella spp.*	22/S	16/S	20/S
D148	*Escherichia coli*	21/S	19/S	17/S
D155	*Klebsiella spp.*	22/S	20/S	18/S
D156	*Enterococcus faecalis*	17/S	21/S	20/S
D170	*Escherichia coli*	24/S	21/S	16/S
D171	*Escherichia coli*	20/S	22/S	09/R
D173	*Enterococcus faecalis*	21/S	19/S	24/S
D189	*Escherichia coli*	22/S	22/S	19/S
D191	*Staphylococcus aureus*	24/S	22/S	17/S
D194	*Klebsiella spp.*	16/S	22/S	18/S
D195	*Enterococcus faecalis*	21/S	19/S	17/S
D198	*Escherichia coli*	19/S	25/S	18/S

Key: D= Drink can, FOX=Cefoxitin, CN=Gentamycin, PRL=Piperacillin, MEM=Meropenem, S=Susceptible, R=Resistant.

CHAPTER FIVE

DISCUSSION

In this study, the total prevalence of bacterial contamination observed across four different locations in Benin-city, Edo state was 34%. This finding is comparable to reports from Zannou (2020) (40%) and Owerri (37%) Ezelote *et al.,* 2022. However, this study is lower than was reported in Plateau (100%) Dasat et., (2022). A possible explanation for the corroboration of the work by Zannou (2020) and Ezelote *et al* is the similarity in the number of samples analyzed, as well as the method used. However the disparity between this study and Plateau (100%) Dasat *et al.,* 2022 could be owing to regional differences and geographical variations.

The findings of this study showed a statistically significant association between Refrigeration and prevalence of bacterial contamination (p=0.015). The results showed that out of the fifty-six (56) refrigerated samples examined, only 10 (17.9%) was positive for bacterial contamination whereas only 24 (54.5%) were positive for contamination out 44 unrefrigerated samples. The findings of this study showed that although surfaces of canned drinks can be readily contaminated irrespective of whether they are kept in the refrigerator or in packs, low contamination was mainly observed in refrigerated samples. It showed that the cans from the refrigerator and the non-redrigerated had viable bacteria on them. This was consistent with the findings of Ogofure *et al.,* (2018), which noted that there was low contamination in refrigerated samples. This result is also consistent with FDA (2015), which equally reported the isolation of certain microorganisms from refrigerators.

This study shows there is a statistically significant relationship between purchase site and prevalence of bacterial contamination. More contamination was observed among canned drinks gotten from retailers when compared to distributors. It is likely that the probable cause for Retailers having greater bacterial contamination is due to the storage method of the cans, which usually are not properly sanitized, and another important factor is the questionable origin of the water used to produce the ice used to cool the cans and other food Items they are stored with.

Escherichia coli had the highest bacterial occurrence (35.3%) followed by *Klebsiella species* (23.5%). *Escherichia coli* was isolated from all the surfaces of refrigerated and non-refrigerated samples of the canned drinks. This is comparable to Melo *et al.* (2012), who identified the presence of Escherichia coli and Coagulase positive Staphylococcus in their analyses. The results found for *Escherichia coli* in the present study show a percentage of 35.3% of the total (12/34), corroborating the research by *Melo et al*. (2012). The genus *Escherichia* is one of the most studied, given the importance of Escherichia coli as a fecal indicator in food. It is responsible for gastroenteritis, diarrhea, vomiting and infections (Jay *et al.,* 2005). Possibly, the difference in results may be related to the causes environmental conditions, climatic differences and the way the cans are stored in each study site.

The Gram-positive bacteria isolated were; *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus species*. The isolated Gram-negative bacteria were; *Klebsiella species* and *Escherichia coli*. Similar bacterial isolates were reported by Dasat *et al.,* (2022) from surfaces of canned drinks from retail shops sold at Plateau State Polytechnic Barkin Ladi Heipang campus. In their findings, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Bacillus species, and Enterococcus species were reported to have been isolated. The researchers found that the isolated bacteria were important for public health and were multidrug resistant. These bacteria species may have gotten onto the canned drinks from the vendor's unhygienic personal hygiene habits or from food items kept in the refrigerator.

Inadequately packaged food supplies could be the cause of the occurrence of Klebsiella species (raw milk). This organism has been observed to thrive inside refrigerators as a result of frequent power outages and unstable power supply (Ogofure et al., 2015). Additionally, the majority of these freezers were not properly sanitized. The surface contamination that has been noticed is clearly a result of the vendors' poor hygiene habits as well as the environmental factors that encourage the survival and spread of bacterial diseases. Generally speaking, Klebsiella species are thought to be a sign of fecal contamination. The nose and feces are two places where this bacteria can be found. Through nasal drilling with the hands of the sellers and subsequent touching of the cans' surfaces, it may have entered the surfaces of the canned drinks.

By assessing the isolates' susceptibility to common antibiotics, the potential health effects of the bacteria were assessed. Some of the isolates showed signs of some degree of antibiotic resistance. Antibiotic-resistant microorganisms are important for public health. Antibiotic resistance, according to Chitanand et al. (2010), demonstrates the pathogen's significance as a hazard to public health. These isolates' demonstrated resistance is consistent with other researchers' findings that these organisms exhibit multi-antibiotic resistance (Oyetayo, 2008). The transfer of drug resistance plasmids among the isolates may be the cause of the high level of antibiotic resistance found in this investigation for some of the isolates. There have been reports of the intergeneric transfer of drug resistance between various genera, with Staphylococci spp. serving as a significant donor (Odimegwu *et al*., 2011).

5.1 CONCLUSION

From this study, results obtained suggest that surfaces of canned drinks sold in selected areas in Benin-city have been found to harbour multiple drug resistant bacteria pathogens which could be of public health risk to consumers once consumed without cleaning the orifice The findings revealed that the canned drinks taken from the refrigerator(s) had higher bacterial isolates and microbial plate counts. One or more antibiotics were discovered to be resistant in some of the microorganisms isolated from the can beverage surfaces. However, some were susceptible to some antibiotics.

Microorganisms are ubiquitous, but what we either drink or eat must be void of pathogenic organism for good health welfare. Therefore, it is strongly advised to thoroughly wash and rinse the surfaces of canned drinks with either sterile wipes or potable water, whether they are from the refrigerator or not, to lower the risk of illness from bacterial infections.

5.2 RECOMMENDATION

According to the result obtained by the microbiological analysis of canned drinks in this study, the surfaces of canned drinks can pose a risk to health of consumers. Given the findings of this study:

- Canned drink consumers should be more vigilant and always clean the top surface of cans drink before drinking the contents.
- Proper awareness should be created by the producers (manufacturers) of these drinks as to the potential hazards that might be asscociated with the improper storage and distribution of their products by the distributors and especially the retailers.

- Proper education on personal and food hygiene to canned drink handlers and the entire populace at large.

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APPENDIX

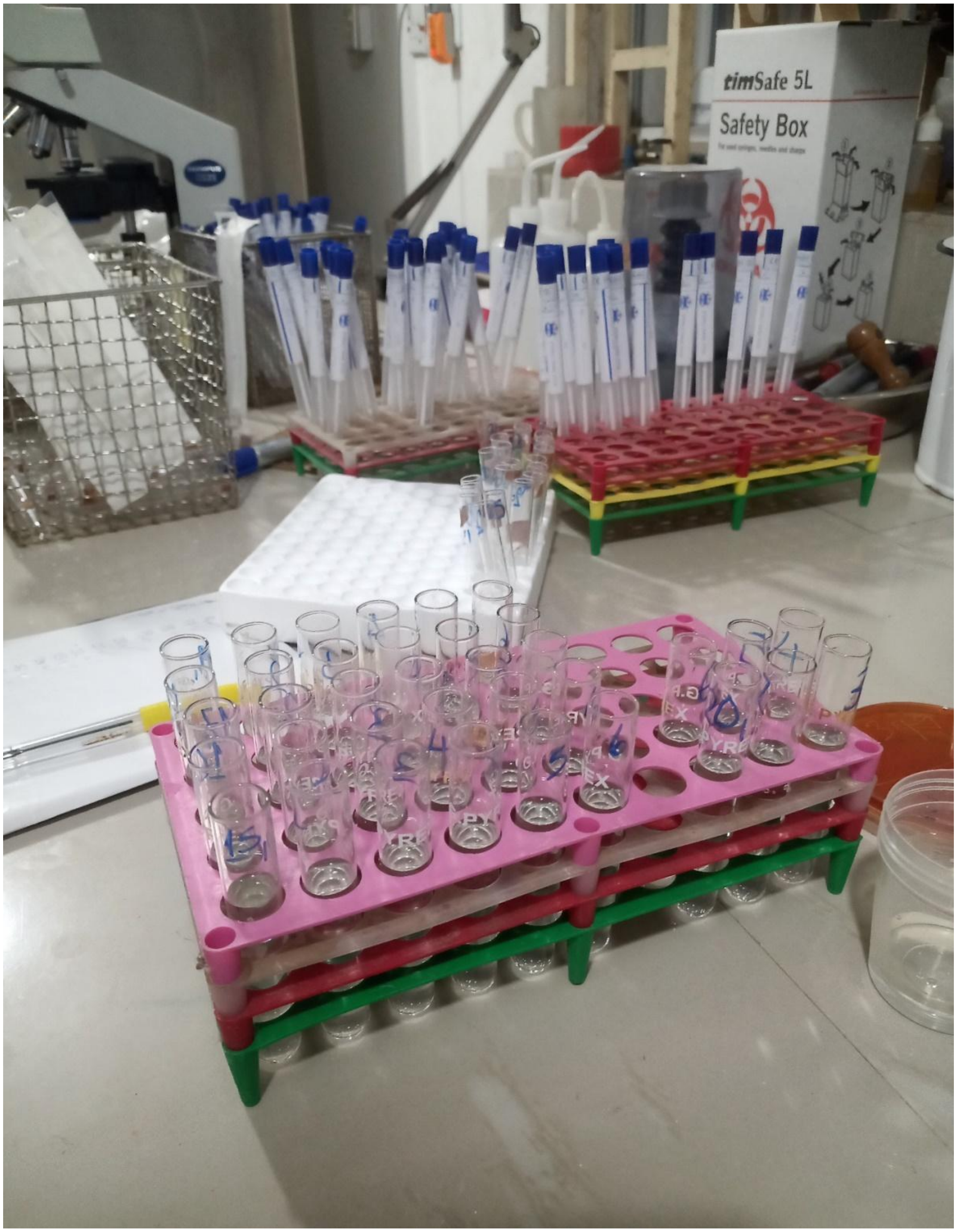

Figure 2: Serial double dilution using Ringer's solution

Figure 3: Biochemical tests being carried out on isolates.