BACTERIOLOGICAL ASSESSMENT OF INDOOR AIR AND SURFACES OF REFRIGERATORS

BY

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MATRICULATION NUMBER

LSC1906995

DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN,

BENIN CITY

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc (HONS) IN MICROBIOLOGY

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CERTIFICATION

This is to certify that the work was carried out by ORISHEDERE OGHENETEGA BENEDICTA in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin city under my supervision.

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(Head of Department)

APPROVAL

This project work is accepted in partial fulfillment for the award of Bachelor of Science, B.sc (Hons) in the Department of Microbiology, University of Benin,Benin city.

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Prof (Mrs) F.I. AKINNIBOSUN **DATE**

(Head of Department)

DEDICATION

This work is dedicated to God for being my solid help. For showing me how good it can get, for being a father to me and loving me despite my shortcomings.

ACKNOWLEDGEMENT

My utmost gratitude goes to God almighty for his guidance,direction, wisdom, knowledge and success of this work. I sincerely appreciate my supervisor MRS F.O. OMOROTIONMWAN for her guiding and supervising during the compilation of the work. I'm grateful to Dr A.G. OGOFURE .I wholeheartedly appreciate my mother MRS BOLA ORISHEDERE and sister OGHENERUKEVWE ORISHEDERE for their support and love. Lastly, I extend my gratitude to my friends and to everybody who has been a vessel to me, God almighty reward you all

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ABSTRACT

Analysis of microorganisms isolated from refrigerator surfaces and interiors is crucial for assessing the cleanliness and potential health risks associated with these appliances. Microorganisms that thrive in low temperatures (psychrophiles) can cause food spoilage and pose a health risk through foodborne diseases such as *Listeriosis, Botulism, Salmonellosis,* and *Diarrhea.* Therefore, it is essential to identify the types and concentrations of microorganisms present in different areas of refrigerators. This study aimed to evaluate the microbial quality of refrigerator exteriors and interiors in homes. Swabs and air samples were collected from 15 refrigerators in Ugbowo city and analyzed in a certified microbiology laboratory. Questionnaires were completed by refrigerator owners. Biochemical tests were used to further characterize the isolates. Antibiotic susceptibility testing was performed to determine the sensitivity or resistance of the identified isolates to various antibiotics. The results showed that the lowest microbial count was observed in refrigerators cleaned weekly (3.01 ± 0.41 CFU/ml), while the highest count was observed in refrigerators cleaned once a month (3.29 ± 0.33 CFU/ml). The lowest microbial count was observed in refrigerators with 5-9 hours of electricity supply (2.74 ± 0.20 CFU/ml), and the highest count was observed in refrigerators with 15-19 hours of electricity supply (3.56 ± 0.00 CFU/ml). The lowest microbial count was observed in refrigerators without external power supply (2.61 ± 0.79 CFU/ml), while the highest count was observed in refrigerators with external power supply (3.25± 0.31 CFU/ml). All identified isolates were

susceptible to gentamicin antibiotics, while they were all resistant to erythromycin, metronidazole, carbenicillin, and cefoperazole antibiotics. All identified microbial isolates except *S. enterica* were susceptible to ciprofloxacin, and all identified microbial isolates except *E. coli* were resistant to tetracycline antibiotics. The Susceptibility Index suggests that *E. coli* is the most susceptible to the antibiotics used, with the lowest Susceptibility Index value of 0.5, while *S. enterica* is the most resistant and least susceptible, with the highest value of 0.75. Both are indicators of high-risk contamination sources, according to Davis and Brown (2016), with a value of ≥0.2 or higher being indicative of a "high-risk" contamination source. These results demonstrate the presence of various types of microorganisms in refrigerators. Domestic refrigerators may be considered as a significant potential source of foodborne illnesses. Therefore, it is imperative to educate households, laboratories, and the general public about proper refrigeration practices. Regular (weekly) and thorough cleaning of refrigerators is essential to reduce the presence of microorganisms/microbial load.

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND OF STUDY

Microbial evaluation of microorganisms confined from surfaces and indoor discuss of fridges is a vital ponder in understanding the cleanliness and potential wellbeing dangers related with these fridges. Amid microbial evaluation tests from diverse surfaces interior fridges such as racks, drawers and entryway handles are collected utilizing sterile swabs or other suitable examining strategies. Microorganisms that can survive extraordinary refrigeration are psychrophiles and cause deterioration of food.

Microorganisms are omnipresent in nature and are more conspicuous in the nearness of supplements, dampness, and temperature favorable for their development and duplication. The conditions that are favorable for the development and duplication of most microorganisms are too favorable to man, so it is inescapable that we live among thousands or indeed millions of microorganisms. Microorganisms are show in the discuss we breathe, the nourishment we expend, on our body surfaces and other near situations. The nearness of microorganisms in an environment has numerous impacts that are either advantageous or destructive to man have different ways of standing up to attack by possibly hurtful microorganisms.

The microbiological quality of discuss is exceptionally vital to guarantee the security and quality of nourishment amid both generation and capacity. Airborne microorganisms can start from different sources which incorporates discuss conditioning frameworks, particular nourishment generation frameworks and indeed crude materials. Airborne microorganisms can be electrostatic and have been appeared to adhere to store on surfaces and development of these microorganisms can cause defilement in the discuss of fridges by airborne formation.

The parts of the fridges cannot be underestimated. A fridge is an awesome resource to any residential home, therapeutic office, research facility or supply room. This is established on the ground of the exercises carried out by fridges such as conservation of nourishment and keeping up the quality of materials in the research facility without deterioration. In spite of the fact that cold capacity is known as one of the most seasoned and commonly utilized nourishment conservation strategies, the clean conditions is greatly vital for open wellbeing all over the world (Ayaz-Topcu *et al.,* 2003).

A fridge is a at risk source of defilement by pathogens which can result to nourishment getting to be spoilt and causing nourishment borne maladies. It shapes a basic connect in wide chain of cross defilement which might inevitably lead to the flare-ups of nourishment maladies.

Refrigerators are one of the most fundamental kitchen apparatuses found in homes utilized for putting away and protecting nourishment items so as to extend the rack life of nourishments. Refrigeration is utilized to protect nourishment that will not be devoured quickly. Generally perishable (nourishment with brief rack life) nourishments are put away for refrigeration to control the microbial defilement at 4-5°C. Domestic fridge is a moo temperature apparatus utilized in domestic for conservation and capacity of nourishment items. Fridges take after the guideline of refrigeration whereby microbial development is controlled by hindrance at a moo temperature to accomplish ideal conservation. The instrument is that it controls the rate of certain chemical and enzymatic responses as well as the rate of development of microorganisms. Decay moderates down as atomic movement moderates, which impedes development of microbes that causes deterioration. Lower temperature in a kept volume brings down the generation rate of microscopic organisms, so the refrigeration decreases the rate of decay. In spite of the fact that moo temperature moderates down decay, but indeed sub solidifying temperature of around 7°C does not block duplication of all microorganisms. Refrigerated nourishments are hence subjected to deterioration by molds, yeasts and microscopic organisms. There are certain considers that have appeared that the perishable nourishments indeed protected at refrigeration temperature moreover experience decay due to a few variables like nearness of microorganisms, chemicals and oxidation, sort of capacity holder or wrapping fabric. The sort of fabric or wrapping fabric they are put away in and term of capacity are imperative components that impact the sort of microbial development, harmfulness and deterioration amid nourishment refrigerated capacity. Moreover, there are other determinants of deterioration such as other components related to the nourishment put away moreover influence the rate of decay. Other determinants of nourishment decay seem be the nearness or nonattendance of supplements, dampness and favorable temperatures. The nearness of supplements and dampness will increment the development and duplication of microorganisms driving to nourishment borne illnesses. Microbes from unwashed crude nourishment, spilling bundles, unclean hands, unclean holder surfaces presented into fridge may maybe cause coordinate defilement of other put away materials and hold on the inside's surfaces. This in turn makes the hazard of roundabout long-term defilement. The inner surfaces of fridges by and large make the chance of circuitous long-term defilement. In any case, insufficient support may make the fridge a breeding put for such bacteria.

Though the suggested normal working temperature of fridges utilized for putting away nourishment in the cold is between 1-4°C, numerous family fridges are erroneously changed and worked over the prescribed temperature. The other critical issue around the refrigerator's temperature is its vacillation behavior (James *et al.,*2016). For the most part, the over-the-top visit and longtime entryway opening cause diminishing the temperature execution of a family fridge.

Notably, the insides surface of family fridges is at chance of getting to be sullied with nourishment borne pathogens, expanding the chances of cross defilement to other nourishment things counting higher hazard prepared to eat nourishment. As a result, the family fridges are a striking component in terms of anticipation of nourishment deterioration, nourishment harming and need of great cleanliness hones (GHPs) in the residential kitchen. Nearness of microorganisms in fridges is a potential wellbeing hazard and can be a source of nourishment borne sicknesses eg *Listeriosis, Botulism, Salmonellosis*, The runs etc. In this way, there is require to know the sorts and plenitude of microorganisms display in distinctive zones of refrigerators.

1.1 Aim of study

This study is aimed at evaluating the bacteriological quality of the surfaces and indoor air of domestic refrigerators.

1.2 Objectives of study

The objectives of this study were;

1. to evaluate the hygienic practices using questionnaire.
2. to evaluate the handling practices of refrigerators.
3. to enumerate bacteria isolates on indoor air and surfaces of refrigerators
4. to determine antibacterial susceptibility.

CHAPTER TWO

LITERATURE REVIEW

2.1 Refrigerator microorganisms

In the realm of food microbiology, the terms "psychrophilic" and "psychrotrophic" refer to microorganisms thriving in food kept at low temperatures, specifically chilling and refrigeration, which generally range between -1°C and 7°C. Domestic refrigerators often maintain a temperature around 4.4°C (40°F), while commercial refrigeration conditions vary based on the perishable item and its expected shelf life. Foods prone to deterioration are chilled or refrigerated using ice. Psychrotrophic microorganisms can flourish in these foods if other growth parameters permit. The definitions of these two terms can be ambiguous, particularly in the context of food microbiology. The terms "psychrophile" and "psychrophilic" are more precise, encompassing microorganisms with optimal growth near 12°C-15°C and a growth temperature range of ≤-5°C to 22°C. These microorganisms thrive in refrigerated and chilled foodstuffs. Conversely, the definition of psychrotrophs is more complex. Initially proposed in 1960, this term aimed to include microorganisms capable of growth at 0°C-5°C, regardless of their optimal growth temperature or range. In practice, they appear to grow most effectively at 25°C-30°C and may struggle to survive above 35°C. Therefore, they seem to represent a subset of mesophiles (optimal growth at 30°C-40°C, range: 5°C-45°C) rather than psychrophiles.

Studies have identified certain mesophilic pathogens (e.g., *Yersinia enterocolitica* and

Listeria monocytogenes) and spoilage-causing bacteria (e.g., L*euconostoc* spp., some *Lactobacillus* spp., and *Serratia* spp.) capable of growth in vacuum or modified atmosphere (MA) packaged foods at 0°C. Spoilage of vacuum-packaged meats by psychrophilic *Clostridium* spp., which can grow between -2°C and 20°C, has also been reported. To avoid confusion, particularly with the designation "psychrotrophic spoilage" for the mesophilic subgroup and "psychrophilic spoilage" for the other group, clear and consistent communication is vital among food production, regulatory bodies, sanitation, academia, research, and related stakeholders. For clarity, it may be appropriate to adopt the term "psychrotrophs" for microorganisms capable of growth (in food) at refrigerated or chilled storage temperatures (≤40°F or 4.4°C). This would encompass both the mesophilic subgroup that thrives at lower temperatures and psychrophiles. Given that both groups contribute to food spoilage and illnesses, and detection and control methods do not distinguish between them, a single terminology can be used for both.

Refrigeration decelerates bacterial growth. Bacteria are widespread in nature, inhabiting soil, air, water, and the food we consume. When they encounter favorable conditions, such as nutrients, moisture, and suitable temperatures, they multiply rapidly, reaching levels that can lead to discomfort. A refrigerator set at 40°F or below safeguards most foods.

2.2 Microorganisms Implicated in Food Storage

Microbes, along with the substances they produce, can reside in and proliferate in edible substances intended for animals and humans. Their presence poses a significant disease threat, endangering the well-being and existence of individuals. A disease-causing agent, referred to as a pathogenic organism, has the ability to induce illnesses within a living host (person). Examples of pathogenic organisms responsible for food-related illnesses or poisoning include bacteria, viruses, parasitic entities, and certain fungi.

2.2.1Viruses

Bacteria lacks the ability to multiply in food, unlike viruses. The primary method of viral spread involves food handlers and the utilization of contaminated utensils, facilitating the transfer of the virus to food, from which humans ingest it.

Viral gastroenteritis is predominantly caused by **Rotaviruses** and **Norwalk** virus. Asymptomatic food handlers are the primary source of **Viral hepatitis A** outbreaks.

2.2. Parasites

Numerous parasites, such as the helminths, have a complex lifecycle including more than one have. The major course of transmission for these parasites to people is by the course of nourishment. The utilization of undercooked pork or hamburger, or the utilization of crude servings of mixed greens washed in sullied water appears to be the trend.

2.2.1 ***Taenia solium*** **and** ***Taenia saginata*****:** Moreover, called pig and hamburger tapeworms. Their sores, display in the muscle of the creature are ingested and the grown-up worm creates in the intestine. The ova may create into hatchlings that may attack other tissues, such as the brain, shaping cysticercosis and serious neurological clutters as a consequence.

2.2.2 ***Trichinella spiralis:*** is found in undercooked pork. The hatchlings can attack tissues and cause a febrile illness.

2.2.3 ***Giardia lambila:*** This disease can be foodborne, waterborne or spread by interpersonal contact. It causes intense or subacute the runs, with malabsorption, greasy stools, and stomach torment and bloating.

2.2.4 ***Entamoeba histolytica***: The transmission is primarily nourishment- or waterborne. The sores posture a major issue since they are exceedingly safe to chemical disinfectants, counting chlorination. The disease is as a rule asymptomatic, but may show up as either a diligent gentle the runs or a fulminant dysentery.

2.3 Foodborne Illness Related with Destitute Refrigeration

Food decay and disintegration is no mischance. It is a actually happening prepare. To get it how to keep up the quality of nourishment and anticipate decay, we require to know what can cause it. Components that influence nourishment deterioration incorporate: Microorganisms, Chemicals, Discuss, Light, Creepy crawlies, Rodents, Parasites and Other Animals, Physical Harm, Temperature, Time.

2.3.1 Microorganisms

Numerous sorts of microorganisms can cause nourishment issues. The microorganisms that can cause food-borne sickness are called pathogenic microorganisms. These microorganisms develop best at room temperatures (60-90°F), but most do not develop well at fridge or cooler temperatures. Pathogenic microorganisms may develop in nourishment without any recognizable alter in odor, appearance or taste. Deterioration microorganisms, counting a few sorts of microbes, yeasts and molds, can develop well at temperatures as moo as 40°F. When deterioration microorganisms are display, the nourishment ordinarily looks and/or smells horrendous. In keeping with destitute execution by condensers, the temperature of your fridge is of the highest significance. If it appears that your fridge is hotter than it utilized to be, at that point you may have an issue. Concurring to the Joined together States Division of Farming (USDA) the insides temperature of your fridge ought to never rise over the basic temperature of 40 ◦F. If temperatures ended up as well warm in your fridge nourishment put away in it gets to be a veritable Petri dish. Exceptionally genuine foodborne pathogenic microbes can thrive in a warm fridge. *E. coli* is a foodborne pathogen that creates from insulant refrigerated nourishment and can cause strongly loose bowels in grown-ups for approximately a week. In children and the elderly *E. coli* can be more extreme and lead to an expanded chance of life undermining kidney disappointment known as hemolytic uremic syndrome. Another pathogenic microscopic organism that may create in an excessively warm fridge is salmonella. The Centers for Illness Control (CDC) gauge that 450 passings in the U.S.

can be ascribed to *Salmonella* each year. Such obnoxious indications as stomach cramping, hazardous the runs, and fever that go with salmonella final between 4 and 7 days. Once more, children and the elderly are more at hazard to create life-threatening complications from *Salmonella.*

2.4 Foodborne Illnesses Caused by Bacteria

Foodborne maladies are caused by defilement of nourishment and happen at any organize of the nourishment generation, conveyance and utilization chain. They can result from a few shapes of natural defilement counting contamination in water, soil or discuss, as well as risky nourishment capacity and handling. Foodborne sickness seems to be a foodborne contamination or intoxication.

2.4.1 A foodborne Illness is an aggravation of the stomach and bowels. The disease can happen when you eat or drink something that is sullied by a microbes, infection or parasite. Frequently the irritation leads to loose bowels, queasiness, heaving, stomach torment, stomach spasms and in some cases fever. A foodborne contamination can final between one and three days. Numerous foodborne contaminations happen at people's homes, basically due to destitute cleanliness. It's as simple as this: planning nourishment without hand washing. Cross-contamination is moreover a chance, for occasion if crude meat and lettuce are both chopped on the same cutting board. Indeed, utilizing the same cut to chop both seem cause defilement by foodborne pathogens. Eating meat or angle that is not cooked all the way through, or eating crude shellfish, increments the hazard of food-borne infections.

2.4.1.1 *Escherichia coli* 0157:H7

It is found in the Intestinal tracts of a few warm-blooded animals, crude drain, unchlorinated water; one of a few strains of *E. coli* that can cause human illness. Transmission- Sullied water, crude drain, crude or uncommon ground meat, unpasteurized apple juice or cider, raw natural products and vegetables, person-to-person.

Symptoms- The runs or ridiculous stomach issues, sickness, and disquietude; can start two to five days after nourishment is eaten, enduring around eight days. A few, particularly the exceptionally youthful, have created Hemolytic Uremic Disorder (HUS) that causes intense kidney disappointment. A comparable sickness, thrombotic thrombocytopenic purpura (TTP), may happen in more seasoned adults.

2.4.1.2 ***Campylobacter jejuni*** – Found in Intestinal tracts of creatures, winged creatures, crude drain, untreated water, and sewage sludge.

Transmission- Sullied water, crude drain, and crude or undercooked meat, poultry, or shellfish. Symptoms- Fever, migraine, and muscle torment taken after by loose bowels (now and then wicked), stomach torment, and sickness that show up two to five days after eating; may final seven to 10 days.

2.4.2 Foodborne intoxication, more commonly known as nourishment harming, is caused by eating nourishment that contains poisons that are discharged by pathogens; the pathogens themselves do not cause illness.

2.4.2.1 ***Clostridium botulinum*** – It is found broadly dispersed in nature; soil and water on plants and intestinal tracts of creatures and angle. Develops as it were in small or no oxygen.

Transmission- Microscopic organisms deliver a poison that causes ailment. Despicably canned nourishments, garlic in oil, vacuum-packed and firmly wrapped food.

Symptoms- Poisons influence the apprehensive framework. Side effects as a rule show up in 18 to 36 hours, but can now and then show up as few as four hours or as numerous as eight days after eating. Twofold vision, saggy eyelids, inconvenience talking and gulping, and trouble breathing may happen. Can be deadly in three to 10 days if not treated.

2.4.2.2 ***Bacillus Cereus*** – It is found broadly disseminated in nature; can be disconnected from meats, drain, vegetables, and fish.

Transmission- Microscopic organisms create a poison that causes ailment. Vomiting-type episodes have more often than not been related with rice items and other bland nourishments such as potatoes, pasta, and cheese items. Sauces, puddings, soups, casseroles, baked goods, and servings of mixed greens have moreover been involved in outbreaks. Symptoms- Foodborne Illnesses is characterized by queasiness and heaving 0.5 to six hours after the ingestion of a sullied nourishment item. In more serious cases, stomach issues and the runs might happen with side effects enduring up to 24 hours2.5 Microbes Found in Fridges

2.5.1 (***Listeria monocytogenes***)

The microbes *Listeria* has been connected to ice cream, solidified vegetables and natural product. Not at all like most microscopic organisms, *Listeria* can develop and increase in your cooler and fridge. This is shocking to a few. *Listeria* can moreover be found in soil, water, and a few creatures, counting poultry and cattle. It can moreover be display in crude drain and nourishments made from crude drain. *Listeria* has been known to survive in handling plants and sully an assortment of handled meats, counting ready-to-eat store meats, hot mutts and meat spreads. These microbes can be slaughtered by legitimate cooking and pasteurization. One bunch of individuals most at hazard for *listeriosis* (the sickness caused by *Listeria monocytogenes*) in pregnant ladies. In pregnant ladies, *listeriosis* can cause premature delivery, stillbirth and genuine sickness or passing of infant babies. Other bunches at chance for listeriosis incorporate more seasoned grown-ups, individuals with compromised resistant frameworks and those with certain incessant restorative conditions, like HIV/AIDS, cancer, diabetes, kidney illness and transplant patients. Side effects of the contamination incorporate fever, solid neck, disarray, shortcoming, and heaving now and then continued by loose bowels. The brooding period can be 3 to 70 days, and the ailment may final days or weeks depending on the wellbeing of the individual earlier to the ailment setting in. Chilling nourishment legitimately is another imperative step to decrease the chance of *Listeria* disease. In spite of the fact that *Listeria* can develop at refrigeration temperatures, it develops more gradually at temperatures of 40 degrees Fahrenheit or less. Making beyond any doubt certain nourishments do not spill juices onto other nourishments to maintain a strategic distance from cross-contamination is another way to ensure nourishment, cover all nourishment or wrap in plastic wrap or thwart some time recently putting it in the fridge. Utilize a fridge thermometer in the center of the cooler and check intermittently. Alter the temperature control if vital to keep nourishments at or underneath 40 degrees F. Utilize precooked and prepared to eat nourishments as before long as you can. The longer they are put away in the ice chest, the more chance Listeria has to develop. Perishable and ready-to-eat nourishments ought to be expended as before long as conceivable, if you are putting away remains, they must be devoured or arranged of after three days.

2.5.2 *Pseudomonas fluorescens*. The major living beings prevailing on meat at the bundling plant and retail store are pseudomonads which develop at refrigeration temperature and may create discoloration, undesirable odors and sludge. (Stringer *et al.,* 1969)., found that *Pseudomonas* species accounted for 91% of the decay microbial populace of hamburger carcasses put away at chill temperatures with the leftover portion comprising of *Acinetobacter*, Moraxella strains. Comparative perceptions have been detailed by Davidson *et al.,*1973 in meat, pork and sheep carcasses. Ayres, 1960 illustrated that off odor and ooze were discernible on vigorously bundled meat when bacterial numbers come to tallies almost 107 and 108 CFU/cm^2, separately. In expansion, numerous of these microorganisms expound proteinases and lipases contributing to tissue degeneration and distinguishable organoleptic changes in the tangible properties of the meat.

2.5.3 *Yersinia Enterolitica*

In the European Union, *Yersinia enterocolitica* ranks third among reported zoonotic pathogens transmitted through food. It triggers severe ailments like gastroenteritis, mesenteric lymphadenitis, reactive arthritis, erythema nodosum, and pseudoappendicitis (Ostroff *et al*., 1994; Horisaka *et al*., 2004; European Food Safety Authority and European Centre for Disease Prevention and Control, 2016). It is widely present in the environment and prevalent in animal populations (Benembarek, 1994; Robins-Browne, 2013). Additionally, it is often isolated from various foods like milk and dairy products, pork, poultry, eggs, and fresh produce (Bari *et al.,* 2011).

Yersinia enterocolitica can thrive in temperatures close to and below 0°C (Tudor *et al.,* 2008; Divya and Varadaraj, 2013). Thus, even refrigeration temperatures (0– 4°C) enable substantial bacterial growth over time. Multiple studies have recorded *Y. enterocolitica* growth in food items stored under refrigeration: e.g., on raw beef, where cell counts escalated up to 2 logCFU/ml within 4 days (Tudor *et al.*, 2008), and in pasteurized milk, reaching 5–7 log CFU/ml after 7 days (with an initial inoculum of 1–3 log CFU/ml) (Amin and Draughon, 1987).

2.5.4 *Staphylococcus* spp

Staphylococcal foodborne illness results from consuming food items carrying staphylococcal enterotoxins. These enterotoxins withstand heat, gastrointestinal proteases, and a broad pH range. *Staphylococcal* foodborne illness often sets in swiftly within 2 to 8 h after consumption, causing intense nausea and notably forceful vomiting. Its duration can range from several hours to a maximum of a day. The affliction's severity hinges on the individual's sensitivity to the toxin, the toxin concentration in the ingested food, the type of enterotoxin, the quantity of contaminated food consumed, and the individual's overall health. Severe cases may lead to acid-base imbalance, exhaustion, and shock, necessitating hospitalization, though fatalities are infrequent. Foods implicated in this illness often have high protein content, entail substantial handling during preparation, and are left at room temperature without proper subsequent cooking or adequate heating.

2.6 Factors Influencing Microbial Presence

2.6.1 Water Availability(aw)

Microorganisms require water sustenance for proliferation. Optimal growth occurs with aw values between 0.9-0.99. However, certain species can survive at considerably lower levels. Microbial growth is generally unhindered by water activity above 0.7 for most microorganisms. Adapting to water-scarce environments incurs higher energy expenditure. Water availability and temperature are interconnected, with elevated temperatures decreasing the aw requirement for microbial growth.

Beyond impacting growth, water availability also affects compound production by microorganisms, potentially leading to reduced fungal particle size in high humidity settings.

2.6.2 Temperature

Microorganisms possess specific temperature ranges for growth, encompassing minimum, maximum, and optimal values. Understanding the interplay between time, temperature, and other variables is essential for food storage optimization. Temperature significantly influences both microbial generation and lag times. The temperature-growth rate relationship varies across microbial groups. Classification based on temperature preferences includes thermophiles, mesophiles, psychrophiles, and psychrotrophs. Mesophiles, including most human pathogens, exhibit optimal growth between 30°C and 45°C, with minimum growth temperatures ranging from 5 to 10°C. Psychrophilic organisms thrive optimally between 12°C and 15°C, with a maximum growth range of 15°C to 20°C. Psychrophilic species relevant to food safety are limited. Psychrotrophs, including *Listeria monocytogenes* and *Clostridium botulinum* type E, grow at low temperatures (-0.4°C and 3.3°C, respectively) but have higher growth optima (37°C and 3°C, respectively) than true psychrophiles. Psychrotrophic organisms are prevalent in food spoilage and include bacteria, yeast, molds, and certain foodborne pathogens. Growth temperature affects virulence gene expression in some foodborne pathogens. For instance, *Yersinia enterocolitica* virulence plasmid-regulated protein expression is high at 37°C, low at 22 °C, and absent at 4°C. Thermal sensitivity is also influenced by growth temperature. *Listeria monocytogenes* exhibits a 2.4-fold increase in D value at 64°C when held at 48°C in inoculated sausages. Lag period and growth rate are influenced by temperature and other intrinsic and extrinsic factors. Extended exposure in warm environments promotes microbial proliferation, particularly in areas close to human body temperature. Refrigeration slows growth, preserving food safety for longer periods. Boiler rooms and areas near heat sources are susceptible to bacterial and mold accumulation. Areas around moisture-generating machinery raise concerns in building health assessments. Environmental consultants can identify and rectify potential health hazards by optimizing airflow and implementing heat dissipation strategies.

2.6.3 pH

The pH level influences microbial growth. Microorganisms generally prefer neutral environments and are often inhibited when more acidic or basic elements are present. Similar to water and temperature, each microorganism species has specific pH ranges for optimal growth. Acidophilic organisms thrive between pH 0-5, neutrophilic organisms between pH 5.5-8.0, and alkaliphiles between pH 8.0-11.5. The majority of microorganisms prefer pH levels below 12.

2.6.4 Oxygen

Concentration Areas with high oxygen levels and access to crucial nutrients contribute to increased microbial proliferation compared to environments with reduced oxygen. While managing oxygen levels can pose challenges, maintaining clean spaces devoid of food and nutrient sources can help starve bacteria and deter pests.

2.6.5 Nutrient Content

Microbes require essential nutrients for proper growth and metabolic functions. The specific type and quantity of nutrients needed vary depending on the microorganism. These nutrients encompass water, energy sources, nitrogen, vitamins, and minerals (Mossel *et al.,*1995). Foods contain varying amounts of these nutrients. Meats are rich in protein, lipids, minerals, and vitamins, with low carbohydrate levels. Plant-based foods provide ample carbohydrates, with varying amounts of proteins, minerals, and vitamins. Milk-based products and eggs serve as nutrient-abundant options. Microorganisms can obtain energy from carbohydrates, alcohols, and amino acids. Most microbes can utilize simple sugars like glucose. Others have the ability to metabolize more complex carbohydrates, such as starch or cellulose found in plants, and glycogen in meats. Some microorganisms rely on fats for energy. Amino acids provide nitrogen and energy for most microorganisms. Certain microorganisms can metabolize peptides and complex proteins. Other nitrogen sources include urea, ammonia, creatinine, and methylamines. Essential minerals for microbial growth include phosphorus, iron, magnesium, sulfur, manganese, calcium, and potassium. As small amounts are generally required, a wide range of foods can meet these mineral needs. Gram-positive bacteria tend to have more specific nutritional preferences. For instance, *Staphylococcus aureus* requires amino acids, thiamine, and nicotinic acid for growth (Jay 2000,). Fruits and vegetables lacking in B vitamins do not support the growth of these microorganisms. Gram-negative bacteria generally obtain necessary nutrients from the carbohydrates, proteins, lipids, minerals, and vitamins present in a wide array of foods (Jay, 2000). *Salmonella enteritidis* illustrates a pathogen with specific nutrient requirements. Iron availability can limit its growth. For example, the egg white (albumen), unlike the yolk, contains antimicrobial agents and limited free iron, which prevents *Salmonella enteritidis* from reaching high levels. A study by Clay and Board (1991) demonstrated that adding iron to *Salmonella enteritidis* inoculum in egg albumen resulted in higher pathogen growth compared to a control inoculum without added iron. Foodborne microorganisms that are most successful in utilizing available nutrients tend to thrive. Typically, simple carbohydrates and amino acids are consumed first, followed by more complex forms. The diversity of food components often allows multiple microorganisms to coexist in a food item. The availability of vital nutrients determines the growth rate. Most foods contain adequate nutrients to support a range of foodborne pathogens, making it challenging to predict pathogen growth or toxin production based solely on the nutrient composition of the food.

2.6.6 Naturally Occurring and Added Antimicrobials

Some foods possess inherent antimicrobial compounds that offer a degree of microbiological stability. Various plant-based antimicrobial compounds include essential oils, tannins, glycosides, and resins found in certain foods. Examples include eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allyl isothiocyanate in mustard, eugenol and thymol in sage, and carvacrol (isothymol) and thymol in oregano (Jay, 2000). Phy toalexins and lectins are additional antimicrobial compounds derived from plants. Lectins are proteins that bind to polysaccharides, including cell surface glycoproteins (Mossel *et al*., 1995). Through this binding, lectins can exert a mild antimicrobial effect. The concentration of these compounds in processed foods is typically low, resulting in a minimal antimicrobial effect. However, they may contribute to stability when combined with other factors in the formulation. Animal-based foods can also contain antimicrobial components. Examples include lactoferrin, conglutinin, and the lactoperoxidase system in cow's milk, lysozyme in eggs and milk, and other factors in fresh meat, poultry, and seafood (Mossel *et al.,* 1995). Lysozyme is a small protein that can break down the cell wall of bacteria. The lactoperoxidase system in cow's milk involves three components: lactoperoxidase, thiocyanate, and hydrogen peroxide. Gram-negative psychrotrophs like *Pseudomonas* are highly susceptible to the lactoperoxidase system. Thus, enhancing this system has been proposed to improve the shelf life of raw milk in regions with limited refrigeration (Mossel *et al.,*1995). Analogous to plant-derived compounds, animal-derived compounds have a limited impact on the ambient shelf life of foods.

2.6.7 Storage/holding conditions

This discussion of storage conditions will focus on temperature, cooling time, and relative humidity factors affecting food and packaging. Other important storage considerations, such as packaging efficacy in preserving specific characteristics, may also be relevant.

When assessing microbial growth, temperature and time are crucial and must be evaluated jointly. Higher storage or display temperatures reduce refrigerated food shelf life because they favor microbial growth.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This research was carried out in selected residences in the Benin metropolis ,Benin City, Edo state, Nigeria. Surface samples and air samples of the refrigerator were collected and evaluated for microbial presence and burden.

3.2 Study Design and Sample Collection

Prior to sample collection, informed consent was obtained from home and store owners and well structured questionnaire was used to obtain information concerning the handling and maintenance practice of the refrigerators. A total of 15 samples (15 refrigerators) was collected and analyzed from participants who had filled the questionnaire in the study. Questionnaires was administered before samples collection and all relevant data or information concerning their cleaning regime, electricity load shedding, and age of refrigerators as well as types of of refrigerators was documented. Samples was collected via the random sampling technique.

Surface swabbing of refrigerators was done by rubbing a defined dimension with a sterile cotton swab moistened with sterile physiological saline solution and kept in 10 ml neat saline suspension. Collected samples was kept in ice chest ice packs and transported to Laboratory for analysis.

3.3 Sample Preparation, Inoculation and Incubation

The method of choice for the examination of surfaces was swabbing a known area using a sterile swab that has been moistened in sterile saline. This semi-quantitative approach enables the enumeration of the microorganisms per cm2 and can facilitate the interpretation of the results according to the method delineated by Public Health England. Sterile swab sticks, aseptically soaked with 10 ml of physiological saline were used to swab a known area of the towel which come in direct contact with the body. The swab sticks were then immediately transferred into 10 ml of nutrient broth. This according to Public Health England (2014) is equivalent to 100 and gives a lower limit of detection of 10 colony forming unit (cfu) per swab if 1 mL is plated.

Where:

C = sum of colonies on the plates counted, V = volume of inoculum

n1 = number of plates counted at first dilution, n2 = number of plates counted at second dilution, n3 = original volume of neat suspension

d = dilution from which the first count was obtained

For the indoor air of refrigerators, plates will be exposed for 30 - 45 minutes and the formula used is below

Where: a = number of colonies on Petri plates, b = surface area of the Petri plates in cm2t = time of exposure

3.4 Evaluation of Log Reduction and Percentage Bacteria Reduction in Refrigerator Samples

The microbial load in refrigerators can be represented by log reduction. Mathematically, it can be calculated using the formula: or A represents the number of microorganisms before cleaning (considering factors that increase the microbial load), while B is the quantity after treatment (considering factors that reduce the microbial load). Percentage bacteria reduction was calculated using the following formula (Ogofure and Ologbosere, 2023).

3.5 Phenotypic Identification of Bacteria from Samples

3.5.1 Biochemical Tests

These tests determine the bacteria's capacity to produce enzymes like catalase, oxidase, and urease. Additional biochemical tests were used to assess the bacteria's ability to utilize sugars or substrates.

3.5.1.2 Catalase (Hydrogen Peroxide; H2O2) Test

This biochemical test assesses and identifies the presence of the enzyme catalase. Catalase catalyzes the release of oxygen from hydrogen peroxide, producing effervescence. Catalase neutralizes hazardous H_2O_2 (hydrogen peroxide) into harmless water and oxygen. All aerobic organisms produce or express this enzyme, making it a useful test to distinguish aerobic and anaerobic organisms (Willey *et al.,* 2008). Procedure: A drop of H_2O_2 (3%) is applied to a sterile slide, followed by a loopful of the bacterial isolate. Effervescence indicates positive catalase activity; no effervescence denotes the enzyme's absence.

3.5.1.3 Oxidase Test: This biochemical test identifies the electron acceptor (cytochrome-c-oxidase), which reduces oxygen. It detects the enzyme's presence in bacteria, specifically oxidases involved in electron transfer between tetramethyl-p-phenylene-diamine (the redox dye) and electron donors in the bacteria (Willey *et al.,* 2008).

Procedure: A whatman filter paper is soaked with a 1% tetramethlyphenylene diamine hydrochloride solution. A 24-hour culture of the test isolate is smeared onto the impregnated filter paper. A positive result is indicated by a purple color**.**

3.5.1.4 Test for Urea Hydrolysis (Urease Test)

This test determines the ability of bacteria to create an alkaline product (ammonia) by cleaving urea under the influence of the urease enzyme. Procedure: Urea is added to urease agar base before inoculation with the test organism in a slant. Incubation is performed at the optimal temperature (370C) for 24–48 hours. A positive result is indicated by the formation of an intense pink/red color, while a negative result shows no color (Bridson, 2006; Willey *et al.,* 2008).

3.5.1.5 Indole Formation Test

This biochemical test determines the capacity of bacteria to produce indole through tryptophan hydrolysis. The spot indole test was used to detect rapidly indole-producing organisms. This test detects the presence of tryptophanase, an enzyme that breaks down tryptophan to release indole, which reacts with cinnamaldehyde to generate a blue-green compound. No color formation occurs when the enzyme is absent (indole negative) (Bridson, 2006; Willey *et al.,* 2008).

Procedure: Filter paper is saturated with a 1% paradimethylaminocinnamaldehyde reagent. A colony is removed from the agar surface and rubbed onto the surface of the filter paper impregnated with the reagent. A positive outcome is indicated by the emergence of a blue color within 30 seconds. The majority of indole-producing organisms will turn blue within 30 seconds to one minute. A faint pink or no coloration denotes a negative result.

3.5.1.6 Citrate Utilization Test (Simon Citrate Agar (SCA) Slant)

SCA slants were utilized for this biochemical test procedure. It aims to assess the bacterium's capacity to use citrate as its primary carbon source. Sodium citrate (sole carbon source), bromothymol blue (indicator), and ammonium dihydrogen phosphate (nitrogen

Procedure: As previously reported (Bridson, 2006; Willey *et al.,* 2008), slant preparations of culture media are used to incubate bacterial isolates for 24 hours. The development of a blue coloration signifies a positive citrate reaction, while the absence of color change or the retention of the medium's green hue signifies a negative result.

3.6 Triple Sugar Iron (TSI) Test

The Triple Sugar Iron (TSI) test is designed to assess an organism's ability to ferment sugars and produce hydrogen sulfide (H2S) or gas (O2), or both. This test is primarily utilized to distinguish between members of the *Enterobacteriaceae* family and other Gram-negative rods based on their sugar fermentation patterns. The test is performed using a TSI agar slant in a sterile test tube and incubated at 36°C for 24 hours. Following inoculation with a TSA pure culture, the test tubes are examined for réactions. Sugar fermentations are indicated by the production of H_2S, gas, and a color change from red (alkaline) to yellow (acid). An alkaline/acid (red top/yellow bottom) slant reaction indicates the fermentation of dextrose (glucose) only. An acid/acid (yellow top/yellow bottom) slant reaction indicates the fermentation of dextrose, lactose, and/or sucrose. An alkaline/alkaline (red top/red bottom) slant reaction signifies the absence of sugar fermentation. The blackening of the medium indicates H2S production. The presence of bubbles, cracks, or a bottom-raised space in the slanted agar indicates gas production (formation of CO_2 and H_2).

3.7 Growth on Differential Media

3.7.1 *Bacillus Cereus* Agar Base

Bacillus cereus agar was used to identify and isolate *Bacillus* species and pathogenic *Staphylococci* species. *Bacillus cereus* agar helps to restrict the growth of Gram-negative bacteria, and this differentiating media allows the differentiation of Gram-positive *Bacillus* species. Autoclaved dissolved *Bacillus cereus* agar at 121°C for 15 minutes; then was allowed to cool and poured into Petri dishes. Isolated pure cultures were inoculated by streaking on the medium and incubated the plates at 37°C for 24 hours. Plates were examined and observed for typical growths by colony forms, colours and spore morphology.

3.7.2 Mannitol Salt Agar (MSA)

MSA is used in differentiating and selecting mostly *Staphylococcu*s species, which was prepared and autoclaved at 121°C for 15 minutes, then was allowed to cool and poured into Petri dishes. The isolated pure cultures were inoculated by streaking on the medium, and the plates were incubated at 37°C for 24 hours. Plates were examined and observed for typical growth.

3.7.3 *Pseudomonas Agar*

Pseudomonas species produce a variety of pigments, and fluorescein is commonly produced. *Pseudomonas* agar was used to determine pigment production by *Pseudomonas* species. *Pseudomonas* agar is a selective and differential medium that inhibits Gram-positive and Gram-negative bacteria other than isolating *Pseudomonas* species. *Pseudomonas* agar Petri dishes were prepared for inoculation after the medium was autoclaved at 121°C for 15 minutes, cooled, and poured into plates. Plates were inoculated with the isolated pure inoculums and incubated at 37°C for 24 hours. It was then examined and observed for growths, where the positive result was cream to greenish-yellow colouration in the agar, which can fluoresce under UV lighting.

3.7.4 Eosin Methylene Blue (EMB) agar

Eosin Methylene Blue (EMB) agar is a differential medium that inhibits the growth of Gram-positive bacteria and is used to indicate Gram-negative pathogenic enteric bacteria by distinguishing between organisms that ferment lactose and those that cannot cope with a colour indication. A sterile petri plate was prepared with EMB, which was autoclaved at 121°C for 15 minutes, allowed to cool and inoculated with pure inoculums by streaking. Inoculated plates were incubated at 37°C for 24 hours and examined plates for colonial morphological changes. Lactose fermenting bacteria produced dark colonies with green metallic sheen or pink mucoid colonies (positive result), and lactose non-fermenters were colourless (negative result).

3.8 Antibiogram/Antibiotic Susceptibility Testing

The identified colonies of bacteria were used to determine the susceptibility and resistance of bacterial isolates, which were subjected to standard antibacterial susceptibility testing (AST) to decipher their resistance or susceptibility to common antibiotics used for treatment within the locality. The standard discs were produced by Oxoid, UK, which were used to execute the disc diffusion method employed in this study. For this assay, a fully grown bacterial culture (from 18-24 hours) were cultured on Mueller-Hinton agar. The inoculum corresponding to 1.5 x 108 cells/ml McFarland standard was streaked using a sterile loop onto the Mueller Hinton Agar plates before the antibiotic discs were added with extreme care to the plates with the aid of a sterile forceps. The susceptibility results were recorded after incubation for a 24hour period at 37ºC. Following the standard or rules of AST established in 2020 by CLSI (Clinical Laboratory Standards Institute). The inhibition zone around each disc (measured using a meter rule in diameter) was assessed and interpreted based on 2020 CLSI standard as Resistant (R), Intermediately resistant (I) and Sensitive (S). The antibiotic discs used in the study with their corresponding codes and concentrations include amoxicillin/clavulanic acid (30µg AUG), erythromycin (5µg ERY), ciprofloxacin (5µg CIP), GEN= gentamycin (10µg GEN), tetracycline (30µg TET), imipenem (10µg), metronidazole (MTZ), Clindamycin (CL) and Vancomycin (30 µg).

3.9 Multiple Antibiotic Resistance (MAR) Index

This index is obviously a good tool, which identifies the region where the isolates were obtained. Whether they are from places of high or low risks or from area where antibiotics are abused. This tool becomes necessary for health risk assessment. According to Davis and Brown (2016), an index of ≥ 0.2 and above is indicative of a 'high-risk' contamination source.

The MAR index was determined by the formula by Chitanand *et al.,* (2010).

CHAPTER FOUR

RESULTS

The figure below shows the heterotrophic bacteria count of indoor air and surfaces of refrigerators based on regularity of cleaning. It is shown that the lowest bacteria count of the sample were observed in refrigerators that are cleaned weekly (3.01 ± 0.41 CFU/ml) and the highest was observed on refrigerators cleaned once in a month (3.29 ±0.33 CFU/ml)

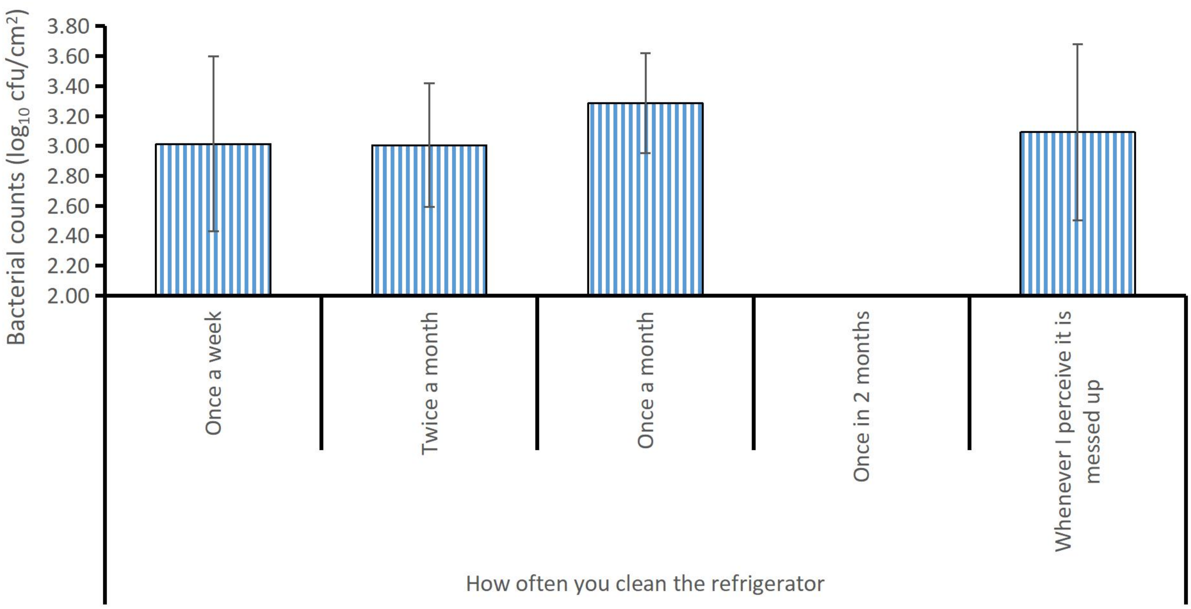

Figure 4.1. heterotrophic bacterial counts of refrigerators on the basis of regularity or frequency of cleaning

Figure 4.2 shows the heterotrophic bacteria count of indoor air and surfaces of refrigerators based on average hours of electricity being supplied . It was observed that the lowest bacteria count was observed in areas with 5-9 hours of electricity (2.74 ± 0.20 CFU/ml) and the highest bacteria count was observed in electricity supply of 15-19 hours (3.56 ± 0.00 CFU/ml)

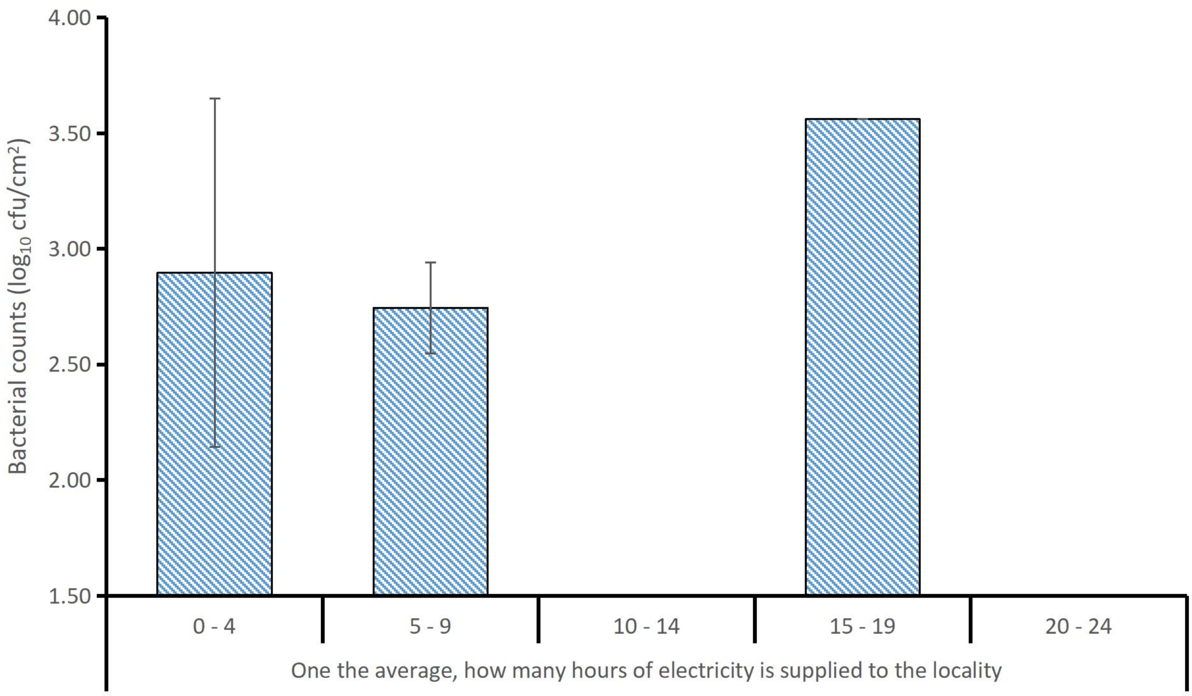

Figure 4.2. Heterotrophic bacterial counts of refrigerators on the basis of average hours of electricity supplied to areas

Figure 4.3 below shows the heterotrophic bacteria count of indoor air and surfaces of refrigerators on the basis of generator usage during power failure. The lowest bacteria count was observed in refrigerators without external power supply (2.61 ± 0.79 CFU/ml) and the highest was observed in refrigerators with external power supply (3.25 ± 0.31 CFU/ml)

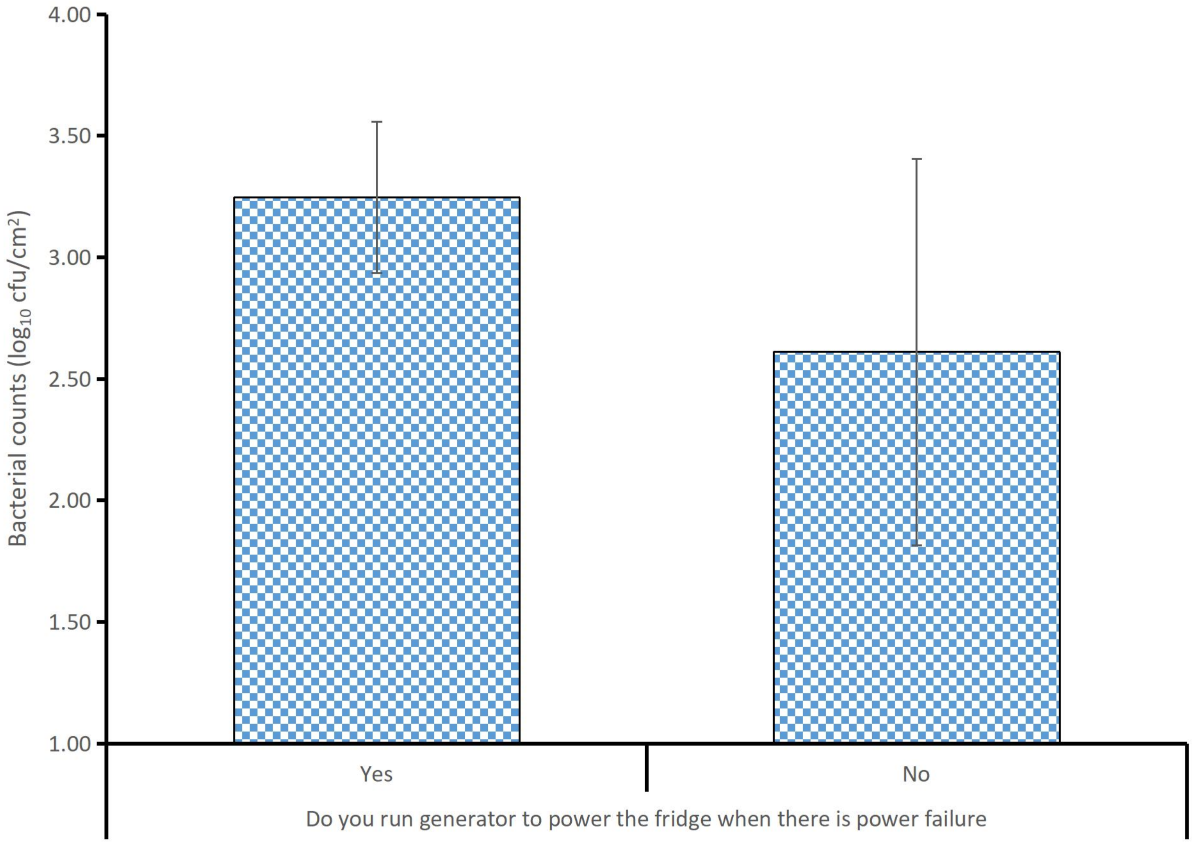

Figure 4.3. Heterotrophic bacterial counts of refrigerators on the basis of using generators during power failure

The result presented in table 4.1 shows the cultural, morphological and biochemical characteristics of isolates

Table 4.1 Cultural, morphological and biochemical characteristics of bacterial isolates from refrigerators

Morphological				
Elevation	raised	Flat	Flat	Raised
Margin	Entire	Undulate	Undulate	smooth
Color	Cream	Cream	Cream	Cream
Shape	Circular	Irregular	Irregular	Irregular
Size	Medium	Large	Large	Small
Gr. diff. agar	SSA	EMB	EMB	MSA
Colour	black	pink	green	Yellow
Staining				
Gram stain	-	-	-	+
cell type	rod	Rod	Rod	Cocci
Arrangement	pair/chains	disperse	disperse	clusters
Color	pink	pink	pink	purple
Spore staining	-	-	-	-
Biochemical				
KOH String Test	+	+	+	-
Catalase	+	+	+	+
Indole	-	-	+	-
Citrate	-	+	-	+
Oxidase	-	-	-	-
Motility	+	+	+	-
Urease	-	-	-	+
Glucose	+	+	+	+
Sucrose	-	+	-	+
Lactose	-	+	+	+
Mannitol	-	-	-	+
Gas formation	+	-	+	-
H_2S formation	+	-	-	-

TSI (Slant/Butt) reaction	k/AG H_2S	A/A(K*)G*	A/AG	A/A*
Esculin Hydrolysis	-	+	-	-
Identity	*Salmonella enterica*	*Enterobacter aerogenes*	*E. coli*	*Staphylococcus aureus*

Key:

Gr. diff. agar- Gr differential Agar

SSA- Salmonella- Shigella Agar

EMB- Eosin Methylene Blue (Agar)

MSA- Mannitol Salt Agar

This table presented shows that;

*Enterobacter aerogenes i*s susceptible to these antibiotics; GEN, CIP, CN, and resistant to E, M, TE, CB, CS.

Escherichia coli is susceptible to the antibiotics GEN , TE, CIP, CN and resistant to E, M, CB, CS.

Staphylococcus aureus is susceptible to GEN, CIP, CN and resistant to E, M , TE, CB, CS.

Salmonella enterica is susceptible to GEN, CN and resistant to E, M ,TE, CIP ,CB and CS.

Table 4.2 antibacterial sensitivity of isolates from refrigerators

Isolates	GEN	E	M	TE	CIP	CN	CB	CS
Enterobacter aerogenes	S	R	R	R	S	S	R	R
E. coli	S	R	R	S	S	S	R	R
Staphylococcus aureus	S	R	R	R	S	S	R	R
Salmonella enterica	S	R	R	R	R	S	R	R

Keys:

S- sensitive

R- Resistant

GEN- gentamicin

CIP - ciprofloxacin

CN- gentamicin

E- erythromycin

M- metronidazole

TE- tetracycline

CB- carbenicillin

CS- cefoperazone

The figure below shows the Multiple Antibacterial Resistance (MAR) Index of the isolated from the refrigerators sampled. *Salmonella enterica* had the highest MAR Index of all isolates.

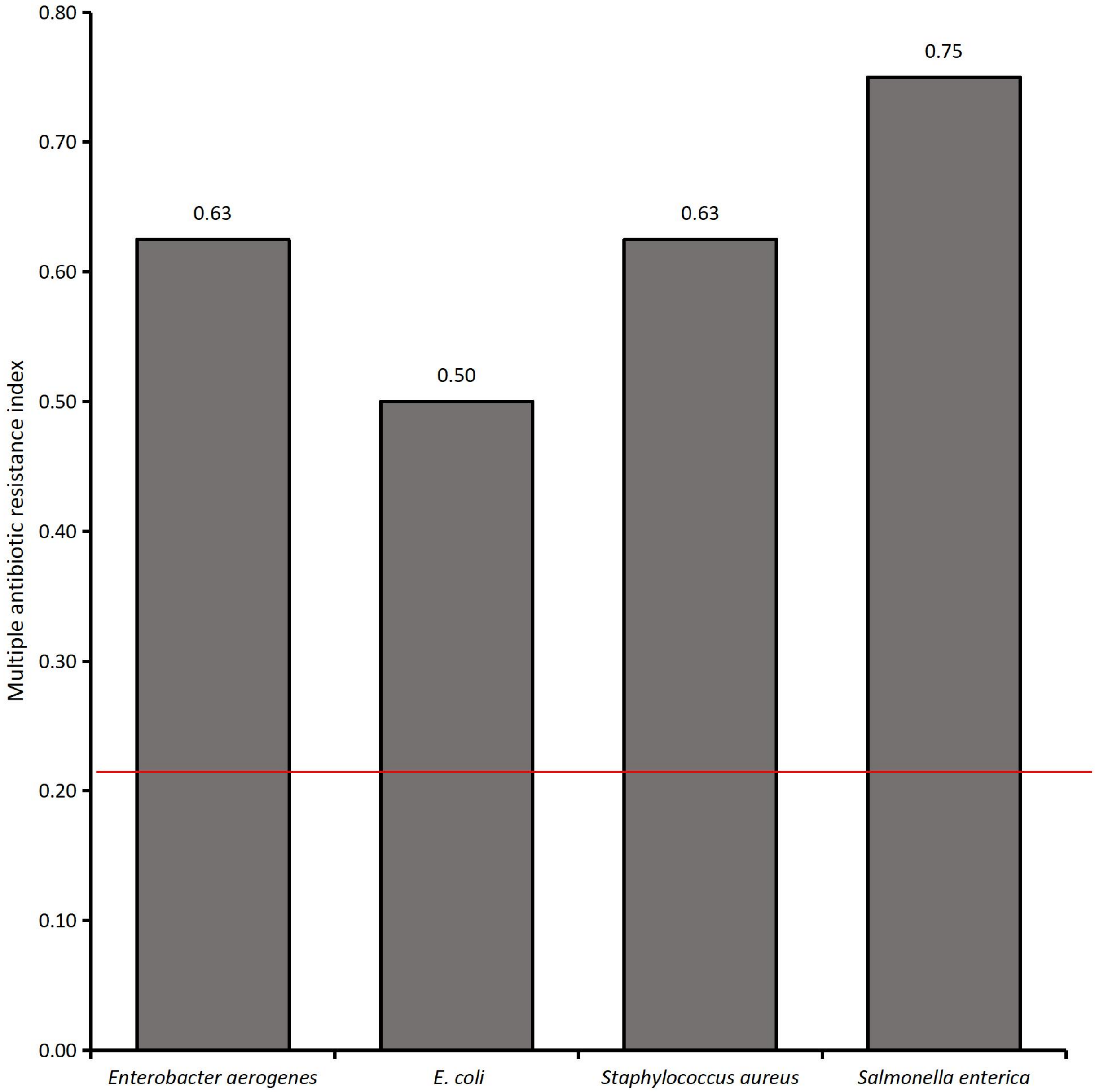

Figure 4.4 Multiple antibiotic resistant index of the bacterial isolates from refrigerators

CHAPTER FIVE

5.0 DISCUSSION

These findings indicate the presence of diverse bacterial species in refrigerators. Domestic refrigerators could be regarded as substantial potential sources of foodborne illnesses. The refrigerators sampled exhibited contamination by bacteria, with Staphylococcus aureus as the predominant type. This study involved sampling 15 refrigerators from five known locations in Ugbowo metropolis.

The absence of hygiene in over half of the sampled domestic refrigerators poses a risk of foodborne disease among Ugbowo householders. Furthermore, the frequent addition of contaminated items (produce, meats, poultry, and eggs) to refrigerators without prior cleaning, commonly observed among study participants, facilitates continuous contamination. This can result in direct or cross-contamination of other stored foods, rendering them unsafe for consumption due to elevated bacterial counts. Ingestion of contaminated food may lead to foodborne illnesses (Otu-Bassey *et al.,* 2017). Identified bacteria include *Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes*, and *Salmonella enterica*. The presence of microorganisms in refrigerators impairs the microbial quality of stored food. Stringent and regular cleaning (maintaining good hygiene practices) resulted in the lowest microbial loads, while irregular cleaning led to the highest microbial loads.

The average duration of electricity supply affects the microbial load of refrigerators. It was observed that the heterotrophic bacteria count was highest at 15-19 hours of electricity supply and lowest at 5-9 hours, indicating that the average duration of electricity supply is not the determining factor. Many bacteria can withstand cooling temperatures.

The use of generators as an alternative power source during power outages is likewise not a determinant of microbial load in refrigerators. It was observed that refrigerators with external power sources had higher heterotrophic bacteria counts than refrigerators without external power sources. The morphological characteristics used to identify the isolates included elevation (raised, flat), margin (entire, undulate, smooth), size (large, medium, small), shape (circular, irregular), and color. The differential agars used to identify the isolates were SSA (*Salmonella enterica*), EMB (*Enterobacter aerogenes* and *Escherichia coli*), and MSA (*Staphylococcus aureus*). Biochemical tests were also employed to identify the isolates based on specific characteristics.

Antibacterial susceptibility testing was performed to determine which antibiotics the identified isolates were susceptible or resistant to. All identified isolates exhibited susceptibility to gentamicin but resistance to erythromycin, metronidazole, carbenicillin, and cefoperazole. All identified bacteria isolates, except S. enterica, were susceptible to ciprofloxacin. All identified bacteria isolates, except E. coli, were resistant to tetracycline.

The MAR Index indicates that *E. coli* is the most susceptible to the employed antibiotics, with the lowest MAR value (0.5), while *S. enterica* is the most resistant and least susceptible, exhibiting the highest MAR value (0.75). According to Davis and Brown (2016), both indices ($\geqslant$0.2) indicate a "high-risk" contamination source.

CONCLUSION

As a result, refrigeration, once hailed solely as a preservative technique, has shown vulnerability to contamination. Thus, it becomes imperative to educate households, laboratories, and the public at large about optimal refrigeration practices. These encompass emphasizing the significance of temperature regulation, adhering to meticulous hygiene, implementing proper food storage and refrigerator maintenance protocols, coupled with preventive upkeep measures. Thorough hand hygiene is paramount as individuals handling items intended for refrigeration may inadvertently introduce bacteria if their hands are neglected (potential vectors of transmission). Consequently, regular and thorough cleaning of refrigerators (at least weekly) is essential to minimize the proliferation of microorganisms and reduce microbial load. Furthermore, this mitigates the contamination of food stored within, as bacterial contamination can lead to food spoilage and, if ingested, potentially cause foodborne illnesses ranging from diarrhea to chronic conditions.

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APENDIX ONE

Group	Temperature °C (°F)		
	Minimum	Optimum	Maximum
Thermophiles	40 - 45 (104 - 113)	55 - 75 (131 - 167)	60 - 90 (140 - 194)
Mesophiles	5 - 15 (41 - 59)	30 - 45 (86 - 113)	35 - 47 (95 - 117)
Psychrophiles	-5 - +5 (23 - 41)	12 - 15 (54 - 59)	15 - 20 (59 - 68)
Psychrotrophs	-5 - +5 (23 - 41)	25 - 30 (77 - 86)	30 - 35 (86 - 95)

Source: Table 1.1 in ICMSF 1980, p 4.

Table 1: temperature rate for prokaryotic organisms

Organism	Minimum	Optimum	Maximum
Bacillus cereus	5 (41)	28 - 40 (82 - 104)	55 (131)
Campylobacter spp.	32 (90)	42 - 45 (108 - 113)	45 (113)
Clostridium botulinum types A & B*	10 - 12 (50 - 54)	30 - 40 (86 - 104)	50 (122)
Clostridium botulinum type E**	3 - 3.3 (37 - 38)	25 - 37 (77 - 99)	45 (113)
Clostridium perfringens	12 (54)	43 - 47 (109 - 117)	50 (122)
Enterotoxigenic *Escherichia coli*	7 (45)	35 - 40 (95 - 104)	46 (115)
Listeria monocytogenes	0 (32)	30 - 37 (86 - 99)	45 (113)
Salmonella spp.	5 (41)	35 - 37 (95 - 99)	45 - 47 (113 - 117)
Staphylococcus aureus growth	7 (45)	35 - 40 (95 - 104)	48 (118)
Staphylococcus aureus toxin	10 (50)	40 - 45 (104 - 113)	46 (115)
Shigella spp.	7 (45)	37 (99)	45 - 47 (113 - 117)
Vibrio cholerae	10 (50)	37 (99)	43 (109)
Vibrio parahaemolyticus	5 (41)	37 (99)	43 (109)
Vibrio vulnificus	8 (46)	37 (99)	43 (109)
Yersinia enterocolitica	-1 (30)	28 - 30 (82 - 86)	42 (108)

ICMSF 1996; Lund and others 2000; Doyle and others 2001
* proteolytic; ** non-proteolytic

Table 2: approximate minimum, maximum and optimum temperature values in °C (°F) permitting growth of selected pathogens relevant to food

APPENDIX TWO

SURVEY ON HOUSEHOLD USE OF REFRIGERATORS IN VIEW OF THEIR BACTERIOLOGICAL QUALITY.

I am Orishedere Oghenetega Benedicta a 400 level student of the department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, with the Matriculation number LSC19068995.

The purpose of this survey, is to assess primarily, bacteriological quality of household refrigerators in line with my undergraduate project, a partial fulfilment for the award of a bachelor of science degree in microbiology.

1. What is your sex or gender

   [male] [female]

2. How man refrigerator(s) do you have

   [1] [2] [3] [more than 3]

3. How old is the refrigerator?

   [less than a year] [1-2years] [2-3years] [3-4years] [more than 4 years]

4. How often do you clean the refrigerator? [Once a week] [twice a month] [once a month] [once in 2 months] [whenever I perceive it is messed up]

5. When did you last clean your refrigerator? [This week] [sometime this month] [last month] [I can’t remember] [any other answer]

6. Why do you feel it is appropriate to clean your fridge? [to remove dirt] [it is a routine for me] [to prevent foul smell or odor from the fridge] [any other reason]

7. Are you aware that they fridge can be a reservoir for bacteria and other disease causing germs

[yes]   [no]

8. With the above knowledge (if yes), have you given special attention to your fridge in terms of care?   [yes]   [no]

9. When was the last time you took your fridge for repairs [this month] [3 months ago] [last year] [never for once]   [can't remember]

10. Do you clean your fridge using any antiseptic like Dettol or antibacterial or antifungal soaps? [yes] [no]   [not necessary to do so]

11. If the above question is [yes] how often do you clean with antiseptic?   [once a month]   [once in 2 month] [once in 3 months]   [anytime]

12. On the average , how many h hours of electricity is supplied to this locality in a day [0-4]   [5-9]   [10-14]   [15-19]   [20-24]

13. Do you run generators to power the fridge whenever there is power failure? [yes] [no]

14. If yes, how often? [every time]   [sometimes]   [once in a while]