ISOLATION OF GRAM POSITIVE BACTERIA FROM LAUNDRY WASTEWATER IN EKOSODIN

BY

Oghenekevwe Blessing ASAMA(MISS)

LSC2003094

DEPARTMENT OF MICROBIOLOGY
FACULTY OF LIFE SCIENCES
UNIVERSITY OF BENIN
BENIN CITY

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A PROJECT 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 THE DEGREE OF BACHELOR OF SCIENCE, B. Sc (Hons.) IN MICROBIOLOGY, UNIVERSITY OF BENIN.

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CERTIFICATION

This is to certify that the project work was carried out by Oghenekevwe Blessing ASAMA (Miss) with matriculation number **LSC2003094** in the Department of Microbiology, Life Sciences, University of Benin, Benin City under the supervision of;

______________________________ ______________________________

PROF E.O. IGBINOSA **DATE**

(PROJECT SUPERVISOR)

______________________________ ______________________________

PROF (MRS) F. I AKINNIBOSUN **DATE**

(HEAD OF DEPARTMENT)

APPROVAL

This project was carried out by Oghenekevwe Blessing ASAMA (Miss) under the supervision of PROF E.O. IGBINOSA in partial fulfillment of the award of a Bachelor of Science B.Sc degree in Microbiology.

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

(Head of Department)

DEDICATION

I dedicate this work to God Almighty for His unfailing grace, mercy, strength and for being my source of wisdom and knowledge.

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I wish to acknowledge God Almighty for His direction and help throughout this work, without Him, success would have been impossible.

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ABSTRACT

The contamination of water sources by untreated wastewater poses a serious public health risk due to the spread of pathogenic bacteria, particularly Gram-positive species. This study examined the microbiological properties of wastewater collected from commercial laundry shops in Benin City, Edo State, Nigeria. The research focused on enumerating total heterotrophic bacteria, isolating Gram-positive bacteria, and identifying their species based on morphological and biochemical characteristics. A total of eight (8) wastewater samples were collected in duplicates from two commercial laundry shops, with the samples divided into two categories: wastewater from washing and wastewater from rinsing. Microbial enumeration was performed using the spread plate technique on nutrient agar, while Mannitol salt agar was used for selective screening of Gram-positive bacteria. The bacterial isolates were characterized based on their morphological and biochemical properties using standard microbiological methods. Results revealed that the total heterotrophic bacterial counts were highest in the laundry washing wastewater samples, with values ranging from 102±2.3 × 10^6 to 81±0.9 × 10^6 CFU/mL. In contrast, significantly lower bacterial counts were observed in the rinsing wastewater samples, with counts ranging from 48±1.8 × 10^6 to 25±2.4 × 10^6 CFU/mL. Additionally, the bacterial counts varied by location, with Laundry Shop 1 recording 68±1.4 × 10^6 CFU/mL and Laundry Shop 2 showing slightly lower counts at 57±1.6 × 10^6 CFU/mL. The microbiological assessment of laundry wastewater revealed the presence of diverse bacterial species, with a total of 17 isolates identified and characterized. Staphylococcus aureus was the most prevalent isolate, accounting for

52.9% (9/17) of the total, followed by Staphylococcus spp. (41.2%) and Micrococcus spp. (5.9%). These findings highlight the potential health and environmental risks associated with untreated laundry wastewater, particularly due to the dominance of pathogenic and opportunistic bacteria. The study emphasizes the need for effective wastewater management practices in commercial laundry facilities to mitigate the spread of microbial contaminants. Future research could focus on advanced treatment technologies and their ability to reduce bacterial load in laundry effluent.

CHAPTER ONE

INTRODUCTION

1.1. BACKGROUND STUDY

Laundry wastewater, a byproduct of domestic and commercial cleaning processes, contains a mixture of detergents, organic compounds, and various microbial communities. Among the microorganisms present in wastewater, Gram-positive bacteria are of particular interest due to their potential to resist harsh environmental conditions and their involvement in various human diseases. Wastewater from laundry operations is often released into the environment untreated, leading to contamination of water bodies and soil. Laundries wastewater contains a high amount of impurities originated from washing chemicals and dirt originated from washed textiles. In addition to a great amount of fats, oils, suspended solids, surfactants, heavy metals and organic solvents might also exist in laundries wastewater (Mozia *et al*., 2016). These substances potentially pollute the aquatic environment when laundry wastewater is being discharged without proper treatments. Therefore Laundry wastewater must be adequately managed to minimize its ecological impact.

Laundry wastewater is characterized by high levels of organic and inorganic pollutants, which include detergents and surfactants (Tambekar *et al*., 2016), bleach and fabric softeners (Olanipekun *et al*., 2019), nutrients and metals (Kumar *et al*., 2020). These pollutants can harm aquatic life, contaminate water sources, and affect human health (WHO, 2019). Laundry wastewater has been shown to harbor various microorganisms, including Gram-positive bacteria such as *Staphylococcus* spp., *Bacillus* spp., and *Enterococcus* spp. These bacteria are widely recognized for their resilience in hostile environments and their association with human and environmental health risks (Mutuku *et al*., 2017). Gram-positive bacteria are characterized by their thick peptidoglycan cell wall, which allows them to survive in a variety of conditions, including those created by chemical detergents (Smith *et al*., 2015).

In Ekosodin, a densely populated area near the University of Benin, laundry wastewater is commonly discharged into open drainage systems, raising concerns about the potential spread of pathogenic microorganisms. The practice of discharging untreated laundry wastewater into open drainage systems in Ekosodin, a bustling student community, heightens the risk of environmental pollution, spreading harmful bacteria and pathogens, and compromising soil and water quality (Obire and Nwankwo, 2018). Several studies have highlighted the potential for laundry wastewater to act as a reservoir for bacteria that are resistant to commonly used antibiotics (Ali *et al*., 2018). Gram-positive bacteria, such as Staphylococcus aureus, have been identified in high concentrations in untreated wastewater, posing risks to public health, particularly in communities where open drainage systems are prevalent (Kumar *et al*., 2019). These bacteria can enter the environment and potentially affect local populations through direct contact, consumption of contaminated water, or through the food chain. The persistence of these microorganisms in the environment is of increasing concern, particularly in areas where wastewater treatment facilities are lacking or inadequate. This makes the study of such bacteria in laundry wastewater especially relevant in urban areas like Ekosodin, where untreated wastewater is a common byproduct of daily activities. Understanding the types and behavior of these bacteria is crucial for mitigating potential environmental and health risks.

1.2. Aim and Objectives

The aim of this study is to isolate and characterize Gram-positive bacteria from laundry wastewater in Ekosodin, Benin City, Edo State.

The specific objectives of this study were to:

i. evaluate the total heterotrophic bacterial count of laundry wastewater from different locations in Ekosodin;
ii. determine the heterotrophic bacterial count of laundry wastewater based on location;
iii. isolate Gram-positive bacteria using selective culture media;
iv. identify and characterize isolated bacteria based on morphological and biochemical characteristics;
v. determine the percentage occurrence of the Gram-positive bacteria isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 DEFINITION OF WASTEWATER

Wastewater originates from everyday activities like bathing, flushing toilets, doing laundry, and washing dishes in residential settings. On the other hand, commercial wastewater comes from non-residential sources, such as restaurants, car washes, laundry shops, offices, or supermarkets. This type of wastewater may contain different chemicals and pollutants and requires special treatment or disposal. The U.S. Environmental Protection Agency (EPA) defines wastewater as water that has been used in domestic, industrial, commercial, or agricultural settings, as well as water from surface runoff or stormwater. This water may contain contaminants like household chemicals, pharmaceuticals, and industrial pollutants, which must be treated before being discharged into the environment.

Tchobanoglous *et al*. (2020) define wastewater as water compromised by human activities, encompassing domestic, commercial, and industrial sources, which require advanced treatment to eliminate pollutants and contaminants like PFAS. The World Health Organization (WHO) emphasizes that wastewater includes "liquid waste discharged from homes, commercial properties, industries, and storm drains" and can pose significant public health risks due to the presence of harmful contaminants (WHO, 2016).

2.2 CATEGORIES OF WASTEWATER

Wastewater is often categorized based on its source, characteristics, and degree of contamination. The main categories of wastewater include domestic wastewater, industrial wastewater, stormwater, and agricultural runoff.

Domestic Wastewater

Domestic wastewater comes from households, residential areas, and institutions, and is typically rich in organic matter, pathogens, and nutrients. Examples include wastewater from toilets, showers, and kitchens. It generally contains physical, chemical, and biological contaminants such as suspended solids, biological oxygen demand (BOD), chemical oxygen demand (COD), nitrogen, phosphorus, and pathogens. As reported by Tchobanoglous *et al*. (2020) about 70% of the wastewater produced in urban areas is domestic. (Metcalf and Eddy, 2014) emphasize that proper treatment of domestic wastewater is essential to avoid risks to public health and the environment. Domestic wastewater is often divided into two sub-categories:

Blackwater: Water from toilets, which contains pathogens and requires extensive treatment due to its high levels of contaminants (Corcoran *et al*., 2010).

Greywater: Water from baths, sinks, washing machines, and kitchens. It contains lower levels of contaminants compared to blackwater.

Industrial Wastewater

Industrial wastewater comes from various industrial processes and can vary significantly in composition depending on the industry. It often contains a mix of heavy metals, toxic chemicals, oils, and suspended solids. Industries such as pharmaceuticals, textiles, and food processing generate large amounts of industrial wastewater, which require different treatment methods depending on the pollutants present (Metcalf and Eddy, 2003). The United States Environmental Protection Agency (EPA, 2020) emphasizes the importance of treating industrial wastewater to prevent environmental contamination and harm to aquatic life. (Tchobanoglous *et al*., 2020) highlight the need for specialized treatment technologies to address the complex composition of industrial wastewater.

Stormwater

Stormwater generated from rainfall and snowmelt runoff, flows over impervious surfaces such as roads and buildings, collecting contaminants like oil, grease, pesticides, and heavy metals along the way. Although initially clean, stormwater can become polluted as it navigates urban drainage systems. The primary concern with stormwater management lies in handling massive volumes, which can result in flooding and contamination of surface waters (Fletcher *et al*., 2015).

Agricultural Runoff

Agricultural runoff is a significant source of wastewater in rural areas and is largely composed of excess fertilizers, pesticides, animal waste, and sediments from soil erosion. This category of wastewater is highly nutrient-rich, often leading to eutrophication in water bodies if not managed properly. Runoff from concentrated animal feeding operations (CAFOs) also contributes to high levels of pathogens and pharmaceuticals in receiving water bodies (Carpenter *et al*., 1998).

2.3. SOURCES OF WASTEWATER

Domestic Sources: Households (toilets, showers, kitchens, laundry), Residential complexes and apartments, Institutions (schools, hospitals, offices).

Industrial Sources: Manufacturing industries (textiles, chemicals, food processing), Mining and metallurgical processes, Pharmaceuticals and petrochemicals.

Agricultural Sources: Irrigation runoff containing fertilizers and pesticides, Animal waste from livestock farms, Runoff from concentrated animal feeding operations (CAFOs).

Stormwater Runoff: Rainfall or snowmelt running off roads, buildings, and parking lot, Urban drainage systems carrying oil, grease, and debris.

Infiltration and Inflow: Groundwater or rainwater entering sewer systems through leaks or faulty connections.

2.4 COMPOSITION OF LAUNDRY WASTEWATER

Laundry wastewater, a significant component of domestic greywater, is generated from washing clothes and textiles. It typically contains a mix of organic and inorganic pollutants, including surfactants, detergents, dirt, grease, suspended solids, and trace amounts of heavy metals. As household water consumption patterns continue to grow, laundry wastewater has become an important focus of wastewater treatment and reuse research. Laundry wastewater is characterized by a complex mixture of contaminants. According to Christou *et al*. (2017), it typically contains:

Surfactants and Detergents: These are the primary components used in laundry cleaning products and can have significant environmental impacts if not treated properly (Metcalf and Eddy, 2014).

Organic Matter: Derived from human skin cells, food stains, and fibers from clothing, which contribute to an increase in Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD).

Microfibers: Synthetic fibers, such as polyester and nylon, shed during the washing process and are increasingly recognized as a source of microplastic pollution in water bodies (Napper *et al*., 2016).

Phosphates: Commonly found in detergents, phosphates contribute to eutrophication when released untreated into water systems.

Pathogens: Though lower in concentration than in blackwater, laundry wastewater may still contain harmful bacteria and viruses from clothing and household textiles (Al-Gheethi *et al*., 2015).

2.5 MICROBIAL COMPOSITION OF LAUNDRY WASTEWATER

Laundry wastewater is not only a significant source of chemical pollutants but also harbors a diverse range of microorganisms. The microbial composition of this wastewater can be influenced by several factors, including the type of laundry being washed (e.g., household or industrial), the nature of detergents and cleaning agents used, water temperature, and the frequency of laundry cycles. These microorganisms can include bacteria, fungi, viruses, and even protozoa, which originate from human skin, clothing, and environmental contamination.

2.5.1 Bacterial communities

Bacteria are the dominant microorganisms found in laundry wastewater, with a wide variety of both gram-positive and gram-negative species present. Some of the most common bacterial genera identified include:

***Staphylococcus* species:** Frequently found on human skin, this genus includes species such as *Staphylococcus aureus*, a known pathogen, and *Staphylococcus epidermidis*, a common skin commensal. These bacteria can be shed from clothing and skin during the washing process (Gaia *et al*., 2011).

***Pseudomonas* species:** This genus is common in water environments and is known for its resistance to harsh conditions, including the presence of detergents and surfactants. *Pseudomonas aeruginosa*, a well-known opportunistic pathogen, has been detected in laundry wastewater, raising concerns about its potential to cause infections, especially in immunocompromised individuals (Chavez *et al*., 2022).

***Escherichia* species:** Species such as *Escherichia coli* are commonly found in laundry wastewater, particularly when clothes that have been in contact with fecal matter are washed. This bacterium can serve as an indicator of fecal contamination (Knappett *et al*., 2011).

***Bacillus* species:** *Bacillus* species are spore-forming bacteria commonly present in the environment and laundry wastewater. Their spores can survive harsh conditions, including high temperatures and chemical detergents, making them resistant to conventional washing cycles (Gião *et al*., 2010).

2.5.2 Fungal communities

Fungi, including yeasts and molds, can also be found in laundry wastewater. These organisms are generally derived from human skin, clothing, and environmental sources such as air and dust. Common fungal species include:

Yeasts: Yeasts such as *Candida albicans* and *Candida parapsilosis*, present in laundry wastewater, are normally harmless but can become opportunistic pathogens in individuals with compromised immune systems (Bloomfield *et al*., 2006).

***Aspergillus* species:** Species of *Aspergillus* have been detected in laundry wastewater and are known for their ability to thrive in diverse environments. These molds can produce spores that are resistant to detergents and may cause respiratory issues when inhaled, particularly for individuals with allergies or weakened immune systems (Rashid *et al*., 2020).

***Penicillium* species:** Another common mold, Penicillium species are often found in damp environments and can contribute to musty odors in laundry. These fungi may also be involved in the degradation of organic matter in wastewater (Nyanhongo *et al*., 2009).

2.5.3 Viral Contaminants

Viruses can be present in laundry wastewater, especially if the water is contaminated with human bodily fluids, such as sweat, saliva, or feces. Viruses of concern include:

Enteric viruses: These include viruses like Norovirus and Rotavirus, which are typically shed in the feces and can persist in wastewater. They pose a risk of gastrointestinal infection if laundry wastewater is improperly treated or comes into contact with clean water sources (Sinclair *et al*., 2008).

Adenoviruses: Adenoviruses, which cause respiratory and gastrointestinal infections, can also be present in laundry wastewater, particularly when clothes from individuals with active infections are washed (Heim *et al*., 2003).

2.5.4 Antibiotic-Resistant Bacteria (ARB)

One growing concern in laundry wastewater is the presence of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs). These microorganisms can survive the washing process, especially when low-temperature cycles are used or insufficient amounts of detergents are applied.

Methicillin-resistant Staphylococcus aureus (MRSA): Recent studies confirm that Methicillin-resistant *Staphylococcus aureus* (MRSA) can indeed be found in laundry wastewater, particularly in healthcare settings where contaminated textiles such as healthcare workers' attire are laundered. This is a concern because MRSA can survive on fabrics, and improper washing or insufficient disinfection may allow the bacteria to spread.

Healthcare workers' clothing, including scrubs and white coats, has been frequently found to harbor MRSA, with contamination rates as high as 79% for long-sleeved coats. MRSA contamination can occur through contact with infected patients, surfaces, and medical devices.

2.5.5 Extended-spectrum beta-lactamase (ESBL)-producing bacteria

ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* have been detected in laundry wastewater. These bacteria are resistant to beta-lactam antibiotics, making infections difficult to treat (Larramendy and Soloneski, 2016).

2.5.6 Protozoan

While not as common as bacteria and fungi, protozoa can also be found in laundry wastewater. These microorganisms typically come from environmental contamination or from human hosts.

Giardia: This protozoan, which causes gastrointestinal illness, can be present in laundry wastewater contaminated by fecal matter. Its cysts are resistant to disinfection, posing a potential risk for waterborne transmission (Jarroll *et al*., 2010).

Cryptosporidium: Recent research highlights that *Cryptosporidium* remains a resilient protozoan of concern due to its ability to survive in various environments, including laundry and wastewater. *Cryptosporidium oocysts*, which cause severe diarrheal illness, are highly resistant to conventional disinfection processes in wastewater treatment. They are commonly found in both treated and untreated wastewater, with concentrations ranging from 10 to 200 oocysts per liter. Their resistance to chlorine and other disinfectants makes it challenging to completely eliminate them from water supplies.

Laundry wastewater contains a diverse and complex microbial community, including bacteria, fungi, viruses, and protozoa. Many of these microorganisms originate from human skin, clothing, or environmental contamination. The presence of pathogens, antibiotic-resistant bacteria, and potentially harmful fungi and viruses highlights the need for proper treatment of laundry wastewater, especially in healthcare and industrial settings.

2.6. TREATMENT OF LAUNDRY WASTEWATER

A comprehensive treatment strategy for laundry wastewater involves integrating multiple stages of physical, chemical, and biological processes to address the varied pollutants present, including surfactants, dyes, microplastics, and heavy metals.

2.6.1. Screening

The primary goal of screening is to remove large debris and solids, such as lint, hair, and fibers that can clog or damage downstream treatment systems. Wastewater passes through a screen or sieve, which filters out these larger materials. The screening process helps in reducing the load on subsequent treatment steps by eliminating non-dissolved solid materials early in the process.

This step is crucial because large solid particles can interfere with chemical and biological treatment processes if left untreated. By removing solids in the early stages, it prevents system blockages and reduces wear on equipment.

2.6.2. Sedimentation

Sedimentation removes suspended particles and solids that are denser than water. This helps reduce the concentration of suspended solids before the wastewater moves to chemical or biological treatments. After screening, wastewater is allowed to sit in a sedimentation tank, where heavier particles settle to the bottom due to gravity. The settled sludge can then be removed for further processing or disposal.

Sedimentation is a low-energy, cost-effective method for reducing suspended solids. It helps decrease the burden on more energy-intensive processes, such as filtration and advanced oxidation processes (AOPs). While effective at removing larger particulates, sedimentation alone is insufficient for handling dissolved or fine particulate pollutants. Thus, it is typically followed by more advanced treatment processes.

2.6.3. Coagulation

In this step, chemical coagulants such as alum ($Al_2(SO_4)_3$), ferric chloride ($FeCl_3$), or calcium chloride ($CaCl_2$) are added to the wastewater. These coagulants neutralize the negative charges on colloidal particles, which destabilize them and allow them to come together.

Aluminum sulfate (Alum): Commonly used in laundry wastewater treatment for its effectiveness in removing suspended solids and organic matter.

Ferric chloride: Effective for removing phosphates and other fine particulates.

Natural or bio-coagulants: Some recent research suggests using eco-friendly alternatives like biopolymers or bacterial extracellular polymeric substances (EPS) as effective coagulants for treating laundry wastewater.

2.6.4. Flocculation

After coagulation, flocculation involves gently mixing the water to promote the aggregation of particles into larger, visible clumps called flocs. Flocculants like polyacrylamide can be used to aid in this process. These flocs are large enough to be removed via sedimentation or filtration.

Flocculants are chemicals or natural substances that enhance the clumping process. They further bind the destabilized particles into larger flocs, which make them easier to separate from the water. This step is particularly effective at lowering chemical oxygen demand (COD) and turbidity by removing suspended solids and organic material, thus reducing the load on subsequent treatment processes like advanced oxidation. Coagulation-flocculation improves the efficiency of membrane-based treatments (e.g., ultrafiltration) by reducing membrane fouling.

2.6.5. Primary Filtration

Sand Filtration: In sand filtration, water is passed through a bed of fine granular sand. This physical barrier removes suspended solids and particulate matter. Sand filters operate by trapping particles in the spaces between sand grains as water percolates through the filter.

Sand filtration is effective for removing particles larger than 20 microns and can also reduce turbidity. It is often used after coagulation-flocculation to further purify the water. Regular backwashing of the sand filter is necessary to prevent clogging and maintain efficiency.

Granular Activated Carbon (GAC) Filtration: The GAC filtration process involves passing water through a layer of activated carbon granules, leveraging their extensive surface area to capture and remove organic contaminants, surfactants, dyes, and certain trace metals. GAC is particularly effective at adsorbing non-polar organic compounds, which are common in Laundary wastewater due to detergents and fabric softeners. GAC can also remove odor-causing substances and improve water clarity.

GAC filtration is often used after primary treatments like coagulation-flocculation and sand filtration, as it can capture finer contaminants and dissolved organic substances. Over time, the GAC becomes saturated with contaminants and must be regenerated through thermal or chemical processes to restore its adsorptive capacity.

2.6.6. Advanced Oxidation Process (AOP)

Advanced Oxidation Processes (AOPs) are used to degrade complex organic pollutants, surfactants, and dyes present in laundry wastewater (LWW) that are resistant to conventional treatments (Santos *et al.*, 2022). These processes rely on the generation of highly reactive hydroxyl radicals (•OH), which can oxidize and break down organic molecules into simpler, less harmful compounds. AOPs are known for their high efficiency in reducing Chemical Oxygen Demand (COD) by transforming complex molecules into simpler, biodegradable forms. They excel at breaking down persistent contaminants, making them suitable for use alongside other treatments like filtration. Their non-selective nature means they react with various organic pollutants, improving water quality significantly.

2.6.7. Ozonation

The ozonation process utilizes oxidation to dismantle persistent organic pollutants, resulting in improved water transparency. Through the application of ozone gas (O_3), ozonation achieves oxidative degradation of contaminants, decreasing COD and improving water quality, frequently paired with filtration or adsorption systems for enhanced effectiveness.

2.6.8. Membrane Filtration (Ultrafiltration or Reverse Osmosis)

Membrane filtration, particularly Ultrafiltration (UF) and Reverse Osmosis (RO), is an intriguing yet somewhat perplexing method for treating laundry wastewater.

In Ultrafiltration the wastewater is pushed through a membrane with tiny pores that allow water and small molecules to pass through, while larger particles and macromolecules like suspended solids and some bacteria are retained. It seems effective, but not quite for everything it's not great at removing dissolved salts or very fine pollutants. It works well for some parts of the treatment, but then you'd probably need something stronger for other contaminants.

Reverse Osmosis (RO), which gets more complicated. Reverse osmosis uses a high-pressure pump to force the water through a semi-permeable membrane that blocks pretty much everything except water molecules. RO can remove dissolved salts, organic compounds, and even small molecules like surfactants and dyes, making it incredibly efficient.

2.6.9. Bioremediation

Bioremediation for treating organic pollutants can involve biological agents like microorganisms or biopolymers. A notable approach is the use of extracellular polymeric substances (EPS), which are produced by bacterial fermentation.

Recent studies emphasize the role of EPS as a bio-flocculant, which improves the aggregation of suspended solids and helps in the removal of organic pollutants from wastewater. This method enhances the biodegradation process, making it an efficient and eco-friendly alternative for treating contaminants.

2.7. FINAL POLISHING (ADSORPTION OR ION EXCHANGE)

In the final treatment phase, methods like activated carbon adsorption or ion exchange resins are employed to target and capture trace organic pollutants and heavy metals. These techniques ensure the removal of any remaining contaminants, including micropollutants, making the treated water safe for reuse or discharge into the environment. Activated carbon works by adsorbing pollutants onto its high surface area, while ion exchange resins swap unwanted ions, like heavy metals, with harmless ones.

2.7.1 Disinfection

To ensure the treated water is free of pathogens, disinfection methods like chlorination, ultraviolet (UV) radiation, or ozone treatment are employed. These processes effectively eliminate harmful microorganisms, making the water safe for reuse or environmental discharge.

2.8. IMPACT OF LAUNDRY WASTEWATER

Laundry wastewater is a significant source of pollutants such as surfactants, phosphates, microplastics, and heavy metals, which threaten environmental health, water quality, and human safety. If left untreated, these pollutants can lead to oxygen depletion in aquatic systems, causing eutrophication that negatively impacts marine ecosystems.

Additionally, microplastics shed from synthetic fabrics can accumulate in oceans, harming marine life and potentially entering the human food chain. Moreover, substances found in laundry detergents, including endocrine disruptors, may contaminate drinking water supplies, creating health risks for humans.

2.8.1. Impact on environment

Laundry wastewater is a significant source of pollution due to the presence of synthetic detergents, surfactants, microplastics, dyes, and chemicals like phosphates (WHO, 2019). These pollutants contribute to eutrophication when released into water bodies, leading to excessive algae growth that depletes oxygen levels, causing harm to aquatic ecosystems (Xu *et al.*, 2020).

The surfactants and phosphate-based detergents are particularly harmful, as they can disrupt aquatic organisms' growth and reproduction (Kumar *et al*., 2019).

Recent studies show that anionic surfactants found in detergents persist in the environment, contributing to long-term degradation of water quality (Liu *et al*., 2019). Microplastics, which are commonly released from synthetic fabrics during washing, accumulate in water bodies, harming marine organisms when ingested (Naddafi *et al*., 2019). This can affect the entire aquatic food chain, as microplastics are consumed by smaller organisms and move up to larger predators (UNEP, 2020).

2.8.2. Impact on Man

Laundry wastewater can indirectly affect human health, especially when the contaminants infiltrate drinking water sources. Heavy metals like cadmium, lead, and mercury, often present in detergents and fabric softeners, can be toxic when ingested, even at trace levels (IARC, 2019). Microplastics found in laundry wastewater also pose health risks. Recent studies have identified microplastics in drinking water supplies, raising concerns about their impact on human health, as their long-term effects are still being researched (WHO, 2020).

Additionally, chemicals like endocrine-disrupting compounds (EDCs) found in some laundry detergents can interfere with human hormonal systems when consumed via contaminated water sources (Kumar *et al*., 2022). EDCs are linked to reproductive health issues, developmental disorders, and cancer risks, making the uncontrolled release of laundry wastewater a pressing concern for human populations.

2.8.3. Impact on the recipient water bodies

Recipient water bodies, including rivers, lakes, and oceans, are particularly vulnerable to the effects of untreated or inadequately treated laundry wastewater. Organic pollutants from Laundry wastewater, including surfactants, dyes, and suspended solids, lead to increased chemical oxygen demand (COD) and biological oxygen demand (BOD), resulting in oxygen depletion that stresses aquatic life (Riverkeeper, 2020). The nutrient loading caused by phosphates in detergents also promotes harmful algal blooms (HABs), which release toxins harmful to fish, birds, and even humans who rely on these waters for drinking or recreational purposes (USGS, 2020).

2.9. GRAM-POSITIVE BACTERIA

2.9.1. Definition of Gram-positive bacteria

Gram-positive bacteria are a major group of bacteria that retain the crystal violet stain used in the Gram staining method, resulting in a purple or blue coloration under a microscope. This characteristic is due to their unique cell wall structure, which is primarily composed of a thick layer of peptidoglycan (murein), distinguishing them from Gram-negative bacteria. Gram staining, developed by Hans Christian Gram in 1884, remains one of the fundamental methods for bacterial classification based on cell wall properties (Prescott *et al*., 2005).

Gram-positive bacteria have a simpler structure compared to Gram-negative bacteria, as they lack the outer membrane found in the latter group. The peptidoglycan layer, which is much thicker, provides rigidity and resistance to mechanical damage. Additionally, they have teichoic acids and lipoteichoic acids, which contribute to their overall charge and function, including roles in maintaining the cell wall structure, regulating ion passage, and contributing to the bacteria's pathogenicity (Todar, 2020).

2.9.2. Classification of Gram-positive bacteria

Gram-positive bacteria are broadly classified into two main phyla: Firmicutes and Actinobacteria. Each of these phyla contains various medically and industrially important genera.

Firmicutes are characterized by their low guanine and cytosine (G+C) content in their DNA. They are typically spore-forming and include genera such as Bacillus, Clostridium, Lactobacillus, Staphylococcus, and Streptococcus. Members of this phylum play essential roles in the human microbiome, the environment, and industrial applications, while some species are known pathogens. Bacillus species are mostly aerobic or facultative anaerobic and can form endospores. They are widely studied for their ability to produce enzymes and antibiotics (Madigan and Martinko, 2005). Staphylococcus and Streptococcus species are clinically important as they cause a wide range of diseases in humans, from mild skin infections to more severe diseases such as pneumonia, toxic shock syndrome, and sepsis (Ryan and Ray, 2004).

Actinobacteria, on the other hand, are distinguished by their high G+C content in their DNA. This phylum includes important genera such as *Mycobacterium*, *Corynebacterium*, *Nocardia*, and *Streptomyces*. Members of this phylum are notable for their ability to produce secondary metabolites, including antibiotics, and some species are significant pathogens. Mycobacterium species are the causative agents of diseases such as tuberculosis and leprosy. These bacteria have a unique cell wall structure rich in mycolic acids, which gives them resistance to several antibiotics and allows them to survive inside macrophages (Gengenbacher and Kaufmann, 2012). Streptomyces species are well known for their production of antibiotics, such as streptomycin and tetracycline, and are widely used in industrial applications (Barka *et al*., 2016).

2.9.3. Gram Staining and its Role in Classification

The Gram staining process is the cornerstone of bacterial classification. The differential staining technique distinguishes bacteria into Gram-positive and Gram-negative based on the properties of their cell walls. Gram-positive bacteria, with their thick peptidoglycan layer, retain the crystal violet dye after alcohol decolorization, while Gram-negative bacteria lose the stain and take up the counterstain (usually safranin), appearing red or pink (Beveridge, 2001).

The Gram-positive classification is further supported by other biochemical and molecular techniques, including genetic sequencing and phylogenetic analyses. These tools help to differentiate bacteria at the genus and species levels more accurately, especially among closely related organisms.

2.9.4. Key characteristics of Gram-positive bacteria

One of the primary defining features of Gram-positive bacteria is the structural composition of the cell wall which is made up of thick peptidoglycan layer, which retains the crystal violet stain used in Gram staining (Silhavy *et al*., 2010). This layer is significantly thicker (20–80 nm) than that of Gram-negative bacteria and provides rigidity and protection (Vollmer *et al*., 2008). Embedded in this layer are teichoic acids and lipoteichoic acids, which play roles in cell wall maintenance, ion transport, and the initiation of immune responses in hosts (Brown *et al*., 2013).

Furthermore, Gram-positive bacteria lack an outer membrane unlike the Gram-negative bacteria (Klein *et al*., 2013). This characteristic makes them more susceptible to antibiotics that target the peptidoglycan layer, such as penicillin. However, the absence of an outer membrane also reduces the potential for the bacteria to develop complex resistance mechanisms found in Gram-negative bacteria, like efflux pumps and porins (Chambers *et al*., 2009).

Several Gram-positive bacteria, particularly from the genera *Bacillus* and *Clostridium*, have the ability to form endospores (Setlow, 2014). Endospores are highly resistant to environmental stresses such as heat, desiccation, and radiation, enabling these bacteria to survive in harsh conditions for extended periods. This ability contributes to the persistence and resilience of pathogenic species.

In terms of antibiotic resistance and susceptibility, certain species have developed resistance to many antibiotics through the thick peptidoglycan wall of Gram-positive bacteria (Chambers *et al*., 2009). For instance, *Staphylococcus aureus* can develop resistance to methicillin, forming MRSA (Methicillin-resistant *Staphylococcus aureus*) (DeLeo *et al*., 2010). The thick cell wall itself can slow the entry of antibiotics, providing a natural barrier to some extent.

The presence of teichoic and lipoteichoic acids are unique features of Gram-positive bacteria, as they contribute to their rigidity and are crucial for cell division (Brown *et al*., 2013). These acids also influence the bacteria's charge and regulate autolytic enzymes, which degrade the peptidoglycan for growth and cell wall remodeling (Schade *et al*., 2021). Lipoteichoic acids, being anchored to the cell membrane, extend into the peptidoglycan layer and play roles in the host's immune recognition, often eliciting a pro-inflammatory response (Weidenmaier *et al*., 2008).

Furthermore, many Gram-positive bacteria produce exotoxins that contribute to their pathogenicity (Green *et al*., 2008). For example, *Clostridium botulinum* produces botulinum toxin, while *Streptococcus pyogenes* produces streptolysins. These toxins can have diverse effects on host cells, ranging from immune evasion to cell lysis.

2.9.5. Factors influencing Gram-positive bacteria in Laundry wastewater

Gram-positive bacteria, especially *Staphylococcus* and *Bacillus* species, are capable of forming biofilms in pipes and surfaces in laundry systems (White *et al*., 2021). Biofilms are complex microbial communities that offer protection to bacteria from environmental stressors, including detergents and temperature fluctuations (García-González *et al*., 2017). Once biofilms are established, they can continuously release bacteria into the wastewater, increasing the bacterial load and resistance to cleaning agents (Singh *et al*., 2019).

Washing Machine Design and Maintenance: The design and maintenance of washing machines also play a critical role in the bacterial load in laundry wastewater (Arnold *et al*., 2020). Front-loading washing machines, for example, are more prone to bacterial contamination due to their airtight seal, which can trap moisture and encourage bacterial growth (Jones *et al*., 2019). Poor maintenance, such as infrequent cleaning of filters and rubber seals, can lead to the buildup of biofilms, providing a reservoir for gram-positive bacteria (White *et al*., 2021).

Temperature: Temperature is a significant factor in determining the survival and proliferation of gram-positive bacteria. Laundry processes typically involve a range of temperatures from cold (20°C) to hot (above 60°C). High temperatures, particularly above 60°C, are known to reduce bacterial load, including gram-positive species such as *Staphylococcus aureus* and *Enterococcus faecium* (Clement *et al*., 2017). However, sub-lethal temperatures (below 60°C) may allow these bacteria to survive and even proliferate, particularly if other conditions are favorable (e.g., presence of organic matter and surfactants) (Nomura *et al*., 2016).

Surfactants and Detergents: Detergents and surfactants are the primary chemical components in laundry products, used to remove dirt, oils, and microorganisms (Tropschug *et al*., 2010). While many surfactants, such as quaternary ammonium compounds, are effective antimicrobial agents (Merabet *et al*., 2016), some Gram-positive bacteria are resistant to these chemicals. The hydrophobic nature of the cell wall in Gram-positive bacteria can allow them to evade the effects of detergents, contributing to their persistence in wastewater (Singh *et al*., 2019). Biofilm-forming Gram-positive bacteria, such as *Staphylococcus epidermidis*, are particularly resilient (García-González *et al*., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Ekosodin Community, Benin City, Nigeria. Benin City lies between latitude 6°20'00 North and longitude 5°37'20 East of the Greenwich Meridian.

3.2 Sample Collection

In total, eight (8) wastewater samples were gotten from two (2) laundry shops (Laundry Shop 1 and Laundry Shop 2). These wastewater samples were labeled laundry wastewater-washing 1A (LW-W1A), laundry wastewater-washing 1B (LW-W1B), laundry wastewater-washing 2A (LW-W2A), laundry wastewater-washing 2B (LW-W2B), laundry wastewater-rinsing 1A (LW-R1A), laundry wastewater-rinsing 1B (LW-R1B), laundry wastewater-rinsing 2A (LW-R2A) and laundry wastewater-rinsing 2B (LW-R2B). The samples were collected aseptically using sterile sampling bottles, labeled properly and transported immediately for laboratory analysis.

3.3 Determination of Total Heterotrophic Bacteria

The total heterotrophic bacteria were enumerated out using nutrient agar (NA) media. NA (Lab M, Lancashire, United Kingdom) was prepared with 28 g of the agar powder dissolved in 1000 mL of distilled water and sterilized by autoclaving at 121°C for 15 minutes.

The samples were serially diluted using the ten-fold dilution techniques to get eight diluents by transferring one mL of the wastewater samples in test tubes to 9 mL sterile distilled water. After which 200 µL of diluent 10^6 the bacterial suspension was inoculated via spread plate method into sterile nutrient agar plates in duplicates, then incubated at 37°C for 18-24 hours. The bacterial isolates were counted and the mean counts were expressed as colony-forming units per millilitre (CFU/mL).

3.4 Isolation and Cultural Characterization of Gram-positive bacteria

The wastewater samples were screened for the detection of Gram-positive bacteria on Mannitol Salt Agar (MSA). An aliquot of 200 µL from diluent 10^3 of the wastewater samples was inoculated using spread plate method and the plates were incubated at 37ºC for 24 hours. After incubation, distinct yellow, pink or red colonies were considered to be presumptive Gram-positive bacteria. Presumptive bacterial isolates were enumerated and the mean counts were expressed in colony-forming units per millilitre (CFU/mL). Presumptive isolates were purified by sub-culturing on nutrient agar plate and stored on nutrient agar slants until ready for further characterization.

3.5 Biochemical Characterization of Gram-positive bacteria

Presumptive bacterial isolates were further characterized via Gram reaction, coagulase test, indole test, motility test, oxidase test, catalase test, hydrogen sulphide test, starch hydrolysis, methyl-red voges-proskauer (MRVP) test and sugar fermentation test.

3.5.1 Coagulase test

Coagulase test was carried out by placing drops of saline on a clean glass slide and a discrete colony of presumptive isolate was emulsified in the saline drops using a sterile inoculating loop to make bacteria suspension. Using a Pasteur pipette, a drop of plasma was added to the bacteria suspension and saline drop then mixed gently. Formation of clumps in the bacteria suspension within 10-15 seconds indicates coagulase positive result.

3.5.2 Indole test

Spot indole test was carried out using a fresh culture of the test organism. Several drops of 1% p-dimethylaminocinnamaldehyde reagent were placed on a piece of filter paper. A loopful culture of the test organism was rubbed on the reagent saturated area of the filter paper. Positive result is shown by the presence of a blue to blue-green colour change within 2-3 minutes while negative results remain colourless or appears light pink.

3.5.3 Motility test

The motility test was carried out using sulphide indole motility (SIM) medium. The SIM was needle-stabbed deeply with a colony growth of the test culture and incubated at 37^0C 24-48 hours. Bacteria motility is evident by a diffuse zone of growth extending out from the line of inoculation. Confinement of growth to the stab line and leaving the medium clearly transparent indicate negative result.

3.5.4 Oxidase test

This was carried out by wet filter paper method using Kovacs oxidase reagent (1% tetra-methyl-p-phenylenediamine dihydrochloride. The filter paper was soaked with freshly prepared reagent and a loopful culture of the isolate was rubbed on the filter paper. A positive reaction was indicated by purple to blue coloration appearing with 5-30 seconds while absence of purple coloration was considered as a negative result.

3.5.5 Catalase test

Catalase test was carried out by making a suspension of fresh culture of the test organisms using sterile distilled water on a clean glass microscope slide and few drops of hydrogen peroxide (H_2O_2) were added using a dropping pipette. Formation of bubbles indicates positive result. Lack of bubbles indicates negative result.

3.5.6 Hydrogen sulphide test

This test was carried out using triple sugar iron (TSI). The test organisms were inoculated by stab inoculation and the test tubes were incubated at 37^oC for 18-24 hours. The formation of black coloration in the medium was considered to be hydrogen sulphide positive while the absence of black coloration was recorded as hydrogen sulphide negative.

3.5.7 Starch hydrolysis

The test was performed using starch agar medium. A single streak inoculation of the test organism was made on the centre of the starch agar plate and incubated for 48 hours at 37^0C. The surface of the incubated agar plate was flooded with iodine solution for 30 seconds and excess iodine was then decanted. Clear zone around the line of growth after addition of the iodine solution indicated hydrolysis of starch while blue-black coloration around the streak line indicated negative hydrolysis of starch.

3.5.8 Methyl-red Voges-Proskauer (MRVP) test

Methyl red test was carried out using MRVP broth. The test culture was inoculated into the broth medium and incubated at 37^0C for 24 h. After incubation, an aliquot of 2mL of the broth was transferred into two clean test tubes labeled MR and VP then re-incubated for another 24 h. After incubation, methyl red was subsequently added to the MR-labeled broth medium and observed for colour change. Colour change from yellow to red indicates positive test result while yellow colour indicates negative test result, that is, glucose is converted into neutral end product. In VP (Voges-Proskauer) test, it was carried out by adding 6 drops of 5% alpha-napthrol and 2 drops of 40% potassium hydroxide to the VP tube and mixed properly. The culture was allowed to stay for about 30 min and colour change was observed. Colour change to pink-red at surface indicates positive result. If culture appears yellow to copper in colour, it indicates a negative result.

3.5.9 Sugar fermentation test

Purple broth was used determine the fermentation reactions of the test organisms. The purple broth medium consists of peptone with the pH indicator bromcresol purple. The respective carbohydrate based media (glucose, mannitol, lactose, sucrose and fructose) were to the purple broth medium in separate test tubes which was subsequently inoculated with the test organism to determine its ability to ferment the carbohydrate, produce acid and/or produce gas. Durham tube was inserted into the tubed broth media to trap gas produced during fermentation. Positive result was indicated by colour changes from purple to yellow while the color will remain unchanged or darker purple when there was no carbohydrate fermentation.

3.6 Data analysis

Statistical analysis was carried out on the data using Microsoft Excel 2013. Mean values were expressed using descriptive statistics.

CHAPTER FOUR

4.0 RESULTS

4.1 Total Heterotrophic Bacterial Counts of Laundry Wastewater Samples

The mean total heterotrophic bacterial counts of laundry wastewater were shown in Table 4.1. The distribution as observed was 87±2.5 CFU/mL × 10^6 [laundry wastewater-washing 1A (LW-W1A)], 102±2.3 CFU/mL × 10^6 [laundry wastewater-washing 1B (LW-W1B)], 94±1.2 CFU/mL × 10^6 [laundry wastewater-washing 2A (LW-W2A)] and 81±0.9 CFU/mL × 10^6 [laundry wastewater-washing 2B (LW-W2B)].

The total heterotrophic bacterial counts as observed in water used in rinsing was 48±1.8 CFU/mL × 10^6 [laundry wastewater-rinsing 1A (LW-R1A)], 33±0.7 CFU/mL × 10^6 [laundry wastewater-rinsing 1B (LW-R1B)], 27±1.1 CFU/mL × 10^6 [laundry wastewater-rinsing 2A (LW-R2A)] and 25±2.4 CFU/mL × 10^6 [laundry wastewater-rinsing 2B (LW-R2B)]. The total heterotrophic bacterial counts based on location were shown in Figure 4.1. It was observed to be 68±1.4 CFU/mL × 10^6 [Laundry Shop 1] and 57±1.6 CFU/mL × 10^6 [Laundry Shop 2].

Table 4.1: Total Heterotrophic Bacterial Counts of Laundry Wastewater Samples

Sample Code	Mean Counts of Heterotrophic Bacteria CFU/mL ×10^6
LW-W1A	87±2.5
LW-W1B	102±2.3
LW-W2A	94±1.2
LW-W2B	81±0.9
LW-R1A	48±1.8
LW-R1B	33±0.7
LW-R2A	27±1.1
LW-R2B	25±2.4

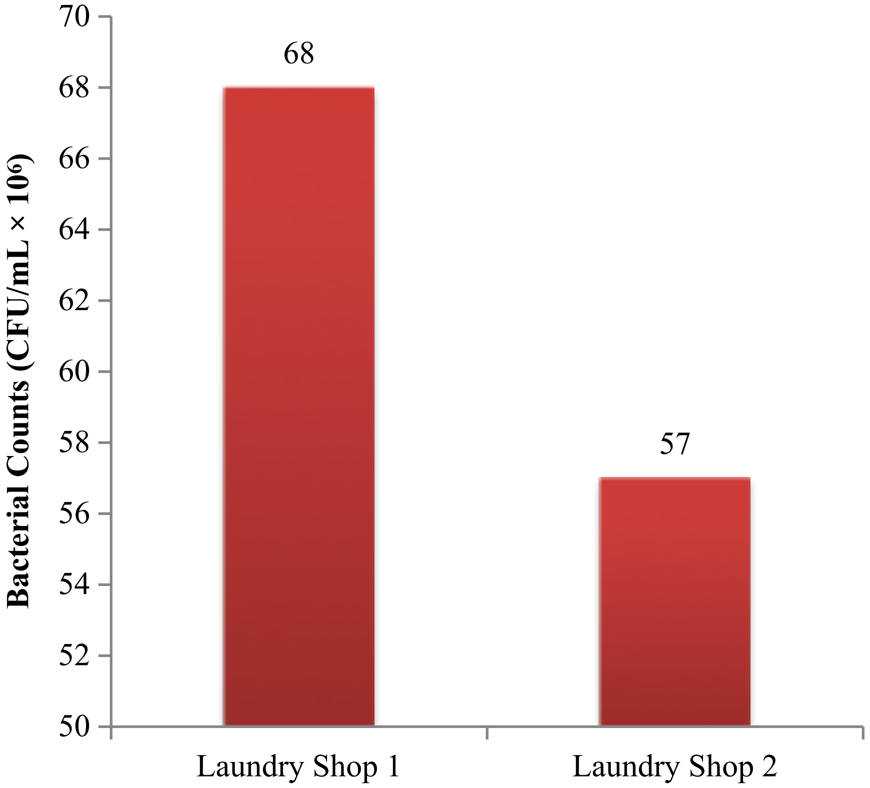

Figure 4.1: Distribution of Total Heterotrophic Bacterial Counts Based on Laundry Shop

4.2 Occurrence of Gram-positive Bacteria in Laundry Wastewater Samples

The occurrence of Gram-positive bacteria laundry in wastewater was shown in Table 4.2. The distribution as observed was 41±1.7 CFU/mL × 10^3 [LW-W1A)], 35±0.9 CFU/mL × 10^3 [LW-W1B], 38±0.5 CFU/mL × 10^3 [LW-W2A], 51±1.1 CFU/mL × 10^3 [LW-W2B)], 14±1.7 CFU/mL × 10^3 [LW-R1A)], 13±0.9 CFU/mL × 10^3 [LW-R1B], 11±0.5 CFU/mL × 10^3 [LW-R2A] and 16±1.1 CFU/mL × 10^3 [LW-R2B)].

The bacterial isolates that were characterized based on their morphological and biochemical properties were shown in Table 4.3. In total, seventeen (17) bacterial isolates of diverse cultural characteristics were selected and characterized. The probable bacterial identified include *Staphylococcus aureus*, *Staphylococcus* spp. and *Micrococcus* spp. The frequency of occurrence revealed that the highest occurring bacteria were *Staphylococcus aureus* [9/17 (52.9%)]. The others include *Staphylococcus* spp. [7/17 (41.2%)] and *Micrococcus* spp. [1/17 (5.9%)] as shown in Figure 4.2.

Table 4.2: Occurrence of Gram-positive Bacteria in Laundry Wastewater Samples

Sample Code	Mean Counts of Gram-positive Bacteria CFU/mL ×10^3
LW-W1A	41±1.7
LW-W1B	35±0.9
LW-W2A	38±0.5
LW-W2B	51±1.1
LW-R1A	14±1.7
LW-R1B	13±0.9
LW-R2A	11±0.5
LW-R2B	16±1.1

Table 4.3: Biochemical characterization of the bacterial isolates

													Sugar fermentation					
Isolate groups	**Growth on Mannitol Salt agar**	**Gram staining**	**Shape**	**Coagulase test**	**Indole**	**Motility**	**Oxidase**	**Catalase**	**Hydrogen sulphide**	**Starch hydrolysis**	**Methyl red**	**Voges proskauer**	**Glucose**	**Mannitol**	**Lactose**	**Sucrose**	**Maltose**	**Presumptive Bacterial**
1	Yellow	+ve	Cocci	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	A-	A-	A-	A-	A-	*Staphylococcus aureus*
2	Pink	+ve	Cocci	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	AG	-G	AG	AG	AG	*Staphylococcus* spp.
3	Red	+ve	Cocci	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	- -	- -	-G	- -	- -	*Micrococcus* spp.

Keys: Positive (+ve); Negative (-ve); Acid and Gas Production (AG); Acid Production Only (A-); Gas Production Only (-G); Absence of both Acid and Gas Production (- -).

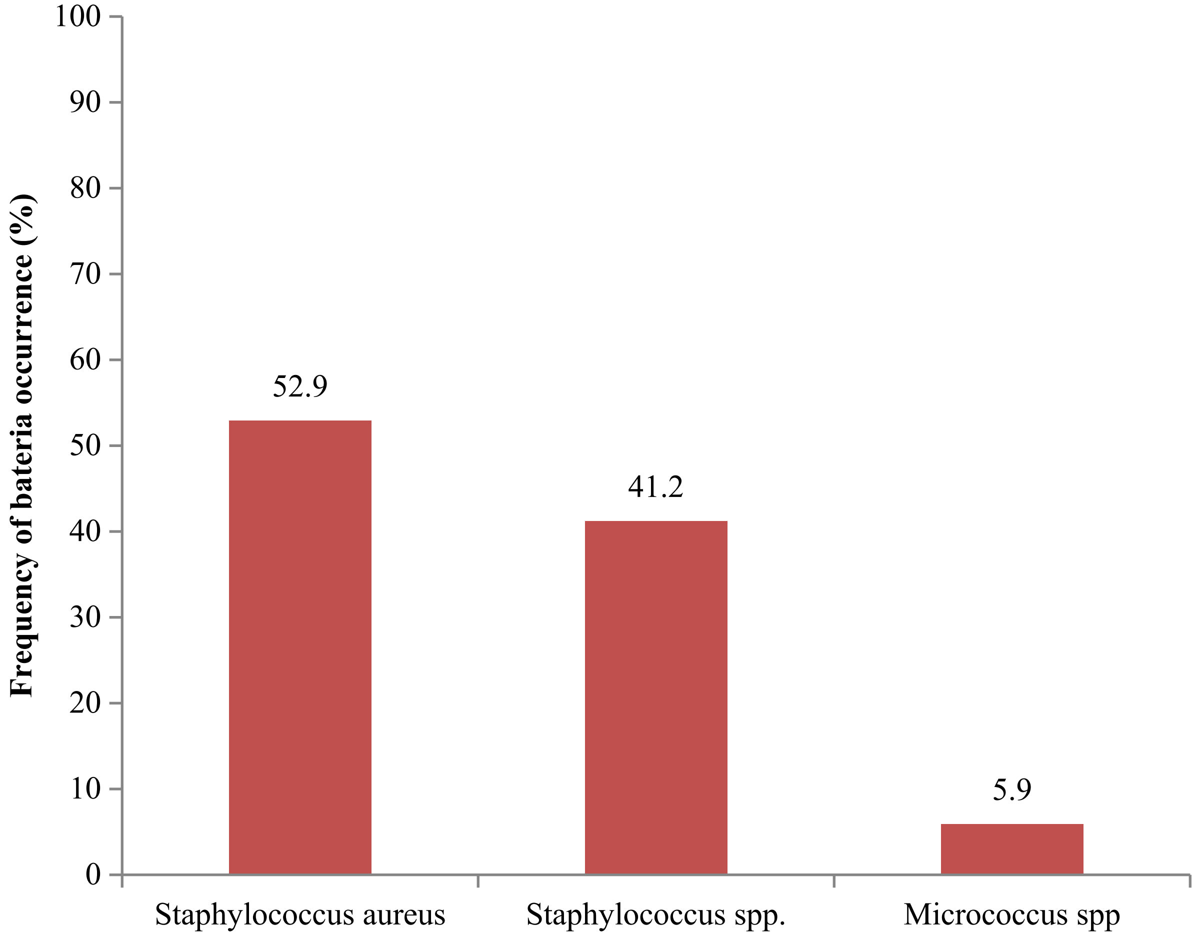

Figure 4.2: Frequency of Gram-positive Bacteria in Laundry Wastewater Samples

CHAPTER FIVE

DISCUSSION

Laundry wastewater, often overlooked as a source of environmental pollution, contains a mix of detergents, organic matter, dirt, and microbial residues that can support the growth of various bacteria, including opportunistic pathogens. Produced during washing and rinsing, this wastewater can contaminate surface and groundwater if not properly managed, threatening ecosystems and public health. Additionally, antimicrobial agents in detergents may unintentionally contribute to the emergence and spread of antimicrobial resistance (AMR) among microbial populations (Larsson and Flach, 2022).

This study investigates the microbial composition and bacterial load in laundry wastewater from two laundry shops, focusing on the prevalence of Gram-positive bacteria and their implications for environmental and public health.

The total heterotrophic bacterial counts observed in this study ranged from 25±2.4 CFU/mL × 10^6 to 102±2.3 CFU/mL × 10^6, reflecting significant microbial proliferation in the laundry wastewater samples. Wastewater from washing cycles, such as LW-WA1 and LW-WA2, showed significantly higher bacterial levels compared to rinsing cycles like LW-RA1 and LW-RA2. This difference underscores the influence of washing processes, where detergents, organic matter from dirty fabrics, and accumulated dirt provide a nutrient-rich environment that promotes bacterial growth. These findings are consistent with Adamu et al. (2015), who observed elevated bacterial loads in wastewater containing organic matter and surfactants, highlighting the washing stage's critical role in microbial growth.

The two laundry shops studied showed notable differences in bacterial counts. Laundry Shop 1 recorded a higher mean count compared (68±1.4 CFU/mL × 10^6) to Laundry Shop 2 (57±1.6 CFU/mL × 10^6). The variation could result from several factors, such as differences in detergent compositions, the amount of water used in washing and rinsing, and disparities in environmental cleanliness and wastewater management practices. Comparable trends were reported by Naidoo and Olaniran (2014), who associated high bacterial levels in wastewater with inadequate hygiene and environmental conditions that favour microbial proliferation.

Gram-positive bacteria were predominantly isolated from washing wastewater samples, with LW-WB2 recorded the highest count (51±1.1 CFU/mL × 10^3) while LW-W1B exhibited the lowest count (35±0.9 CFU/mL × 10^3). In the rinising wastewater samples, LW-R2B has the highest count (16±1.1 CFU/mL × 10^3), while LW-R2A has the lowest count (11±0.5 CFU/mL × 10^3).

The predominant Gram-positive bacterial identified include *Staphylococcus aureus*, *Staphylococcus* spp. and *Micrococcus* spp. Among these, *Staphylococcus aureus*, was the most frequently occurring organism, accounting for 52.9% of the total isolates. Its presence is likely due to skin shedding, soiled fabrics, poor hygiene practices, and its adaptability to nutrient-rich environments. The prevalence of Staphylococcus spp. (41.2%) and Micrococcus spp. (5.9%) further supports the notion that laundry wastewater serves as a reservoir for diverse microbial populations, including potential pathogens. These results align with the findings of Adefisoy and Okoh, (2016) and Gunjal and Rajapakshe (2023), who reported similar bacterial profiles in wastewater samples, emphasizing the potential environmental and health risks. The high bacterial loads and significant presence of Gram-positive bacteria in laundry wastewater highlight its potential as a vector for environmental pollution. Effluents containing detergents and surfactants can worsen environmental issues by contributing to eutrophication and disrupting aquatic ecosystems (Mousavi and Khodadoost, 2019; Azizullah et al., 2021; Arora et al., 2023). Additionally, the metabolic adaptability of organisms like Staphylococcus spp. and Micrococcus spp., along with their capacity to carry multidrug-resistant genes, raises concerns about the role of laundry wastewater in spreading antimicrobial resistance (AMR).

Improper disposal of laundry wastewater can result in the contamination of surface and groundwater, posing a risk to humans and animals by exposing them to pathogenic microorganisms through direct contact or the ingestion of polluted water (Odigie, 2014; Abdalla and Khalil, 2018).

5.1. Recommendations for Wastewater Management

To reduce the environmental and public health risks linked to laundry wastewater, it is crucial to implement effective management strategies. These include:

Pretreatment Systems: Laundry facilities should adopt wastewater pretreatment systems to eliminate organic matter and surfactants before discharging the water.

Public Awareness: Raising awareness among laundry operators and the public about the dangers of untreated wastewater can promote better disposal practices.

Policy and Regulation: Governments and regulatory bodies should implement stricter regulations on wastewater disposal to reduce environmental contamination.

Future Research: Further studies should investigate the antimicrobial resistance profiles of bacterial isolates in laundry wastewater to assess their potential impact on public health and inform intervention measures.

5.2. Conclusion

The results of this study emphasize the microbiological risks linked to laundry wastewater, especially the notable presence of Gram-positive bacteria. This highlights the importance of enhancing wastewater management systems to safeguard public health and preserve ecological balance. These findings serve as a basis for policymakers and researchers to tackle the issues associated with wastewater in urban environments.

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