BACTERIOLOGICAL ANALYSIS OF WATER IN HALLS OF RESIDENCE

BY

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UNIVERSITY OF BENIN
BENIN CITY

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

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CERTIFICATION

This is to certify that this project work was carried out by **Jerusalem Onyedikachukwu OBI** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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DR. (MRS). C.G. DIMOWO **DATE**

(Project Supervisor)

_____ _____

PROF. (MR.) E. O. IGBINOSA **DATE**

(Head of Department)

DEDICATION

The project work is dedicated to God almighty, for bringing me this far in life. I am truly grateful. I dedicate this project also to my loving supportive family and friends as well as my project supervisor who guided me throughout this process.

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ABSTRACT

Water meant for drinking and domestic use can easily become a source of infection if stored under poor sanitary conditions. This study emphasizes on the bacteriological analysis of water tanks in halls of residence in the University of Benin, Edo state Nigeria. Swab and water samples were taken aseptically from water tanks in the halls of residence and in a household with regularly treated water tanks. Serial dilution and pour plate methods were carried out for the isolation of microorganisms on different sterile media. The population of the bacteria isolates were enumerated using the colony counter. Pure cultures were obtained by the streak plate method and they were characterized by Gram staining and some biochemical tests. The isolates were further identified using Bergey's Manual of Determinative Bacteriology. The highest total heterotrophic count for swab samples was gotten from Household A and Household B which both recorded TNC bacterial count and the lowest count was $2.33\pm0.57 \times 10^1$ CFU/mL which was gotten from Hall 3A, while that of water samples recorded the highest from $2.67\pm0.57 \times 10^3$ CFU/mL which was gotten from Sample B and the lowest was $2.33\pm0.47 \times 10^3$ CFU/mL which was from Sample A. The highest total coliform count in swab samples was $7.63 \pm 0.58 \times 10^3$ cfu/mL which was gotten from Hall 4 and the lowest was $1.00 \pm 0.57 \times 10^2$ cfu/mL gotten from Hall 2A, while for that of water sample recorded the highest as $2.33\pm0.94 \times 10^3$ cfu/mL which was gotten from Sample A and recorded the lowest as $1.67\pm0.57 \times 10^3$ cfu/mL which was gotten from Sample B. The highest total count for Non-lactose fermenting Gram-negative bacteria for swab was $6.60\pm 0.58 \times 10^3$ CFU/mL which was gotten from Hall 3A and the lowest was Hall 1B and hall 4 which showed no growth, while for water samples, the highest was $2.63\pm0.48 \times 10^3$ CFU/mL which was gotten from Sample A and the lowest was gotten from $1.00\pm0.58 \times 10^3$ CFU/mL which was Sample B. The predominant isolates identified included *Escherichia coli*, *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp., *Proteus* sp., *Pseudomonas* sp., *Staphylococcus aureus* and weakly fermenting *E. coli*. This study has shown that the water stored in the halls of residences in University of Benin stores different varieties and levels of bacterial contamination indicating that the water is not entirely safe for domestic use without proper treatment.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Water is one of the most important element in all forms of life and it is indispensable in the maintenance of life on earth. Water is a very important part of life and is essential to the wellness of humans. More than two third of the human body is made up of water which proves the fact that water is a very vital part of every living organism (Khalifa and Bidaisee 2021). Clean water supports good hygiene practices like cleaning, washing, and many others. Clean water is important for human health in drinking for hydration, digestion, circulation and removal of waste products from human body.

Water could be artificially stored in places like water tanks, reservoirs, cisterns etc., to ensure continuous availability. Therefore water used by humans has to be kept clean whatever the artificial source may be, especially water tanks. Although, due to poor maintenance and inadequate storage facilities there could be microbial contamination. Poor hygienic practices of water storage has also been found to play a significant role in the spread of water borne diseases. There are a lot of different infections that are detrimental to human health that grow in unsanitary water tanks which can cause a number of water borne illnesses; such as cholera, hepatitis, typhoid and diarrhea. The agent and illness of Diarrheal disease from cholera for example, is responsible for about 1.8 million deaths worldwide. This deaths can be reduced by understaning the partucular bacterial organism causing it and how to prevent it.

Bacteriological analysis of water is a method of analyzing water to estimate the numbers of bacteria present and if needed it is also used to find out the sort of bacteria they are. Here I will be analyzing the bacteria found in the water tanks of the halls of residence in the university of Benin, Ugbowo, Benin city, Edo state, Nigeria(Uniben). The university has thousands of

students residing in halls of residence so it is very important to have water tanks in this hostels as a storage medium of water. Also due to the inconsistent water supply, water tanks are used in hostels for storage. Although this water tanks might sometimes not be treated and this could be a growth medium for pathogen causing bacteria posing as a health risk for the consumers.

Water storage is faced with so many challenges which eventually leads to compromising the quality of the water (Nnaji *et al.,* 2019). Water can be contaminated through poor maintenance of the water tanks. Water tanks have to be treated to rid them of biofilms which is a slimy layer of microorganisms on the inner surfaces that protect the bacteria and allow them to multiply. Tanks can also be a breeding ground for bacteria if they are not regularly cleaned for the removal of dirt, sand, leaves and debris. Structural damage like cracks and leaks can be a pathway for contaminants like insects, rodents and some microorganisms. This opening can also allow animal droppings leading to contamination of the water.

Stored water could be a suitable growth medium for bacteria such as *Escherichia coli, pseudomonas* spp, and *staphylococcus aureus, Vibro cholerae, shigella* spp, and *Legionella pneumophila,* making it a health risk for consumers. Diseases related to contamination of drinking water constitutes a major burden on human health. This contamination could be due to storage of water in untreated tanks like the ones in halls of residence and this could cause diseases like typhoid, cholera, dysentery, giardiasis, among other. Common symptoms mostly associated with this diseases are diarrhea, fever, and body and muscle aches. About 1.7 billion children were affected by diarrhea each year and about 525,000 of the children died each year (Kristanti *et al.,* 2022).

The contamination of water could pose a health risk to students in that it could cause skin infections and irritations for students after bathing. It could also cause typhoid when it is contaminated with *Salmonella typhi*. Cholera, schistosomiasis, diarrhea, dysentery, legionnaires'

diseases and leptospirosis are diseases that can affects students when they come in contact and use these water from contaminated tanks.

In 2023, Okafor *et al* carried out a research in selected hostels in Ifite-Awka Nigeria, and after the research, *Escherichia coli* was found to be the most dominant microorganism isolated from the borehole water samples gotten from the stored water tanks. Its was also observed that this microorganism is an opportunistic pathogen that can pose risk to individuals, especially those that have chronic health conditions and compromised immune systems.

Although many students complain about the quality of the water in the hostels and how they get skin irritations, infections and sometimes illnesses, there have been no documented analysis carried out to determine the causes of this infections. Therefore my main aim is to analyze, isolate and understand the bacteriological quality of the water tanks in school hostels.

Analyzing the water tanks in hostels is an important way to know the quantity of bacteria in water tanks and when to treat it. It also helps in reducing waterborne diseases and improving the health statuses of the students.

1.2 STATEMENT OF THE RESEARCH PROBLEM

According to WHO in 2017, It is very important to have access to clean and safe water. In highly populated environments like university halls of residence, clean and safe water stored in storage facilities especially water tanks is essential.Many halls of residence in university of Benin, Uniben, use water tanks as their source of storage facility due to inconsistent water supply. Water borne illnesses caused by bacteria in contaminated water tanks increases the risk of spreading water borne diseases which could lead to infectious outbreaks(Mohammad *et al.,* 2022).

Species of bacteria that could be found in water gotten from contaminated tanks are *Escherichia* spp, *Salmonella* spp, *Shigella* spp, *Proteus* spp, *Pseudomonas* spp, *Staphylococcus* spp,

Streptococcus spp and *Bacillus* spp (Akinkugbe and Adedeji 2023). Contamination of this water tanks could come either directly or indirectly from human or animal excreta, and this is a particularly common health risk associated with drinking water from water tanks (Manga *et al.,* 2020).

Stored water tanks are common in schools and they could lead to diseases like; typhoid fever, cholera, dysentery, gastroenteritis and legionnaires' disease. Most times this contaminated water could cause skin irritations and infections when students use them for bathing. This research is carried out mainly to analyze and identify the quantity of bacteria found in the water tanks of the halls of residence in the University of Benin and also to expose the health risk it poses to students and safety measures in the environment.

1.3 AIM AND OBJECTIVES.

The aim of this study is to analyze and identify the bacteriological quality of water tanks in halls of residence in the University of Benin.

The specific objectives were:

I. to isolate and enumerate bacterial species present in water stored in residence hall tanks.
II. to characterize microbial isolates in stored water samples.
III. to detect the presence of coliforms and *Escherichia coli* as indicators of fecal contamination
IV. to compare the bacterial load of water tanks in halls of residence with specified standard.

CHAPTER TWO

LITERATURE REVIEW

2.1 Water Quality and Public Health

Water is an essential resource for sustaining life, and its quality plays a central role in safeguarding public health, especially in institutional environments such as universities (Ali and Ghareeb, 2023). In students' halls of residence, access to clean and safe water is crucial for maintaining personal hygiene, preparing food, drinking, and general sanitation. The World Health Organization (WHO, 2017) emphasizes that safe drinking water is a basic human right and a key determinant of health. However, poor water quality in storage systems such as overhead or underground tanks can lead to serious health consequences for students living in densely populated settings where the demand for water is high (Chalchisa *et al*., 2017).

The bacteriological quality of water is of particular concern, as storage tanks in halls of residence can serve as reservoirs for various pathogenic microorganisms (Al-Bahry *et al*., 2011; Slavik *et al*., 2020). Microbial Contaminants such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Vibrio cholerae* may enter water tanks through contaminated source water, poor handling practices, or infrequent cleaning and maintenance. These bacteria are responsible for a range of waterborne diseases, including diarrhoea, dysentery, typhoid, and cholera—conditions that can spread rapidly in crowded living environments (Ashbolt, 2004; Leclerc *et al*., 2002; Rath *et al*., 2021). According to WHO (2017), waterborne diseases are a significant cause of morbidity and mortality, particularly in developing countries where water infrastructure may be inadequate.

The health risks are magnified in residential university settings, where students are often exposed to compromised hygiene conditions, shared facilities, and limited health awareness. A study by Okafor *et al*. (2023) highlights that the quality of water stored in tanks can deteriorate over time

due to biofilm formation, sediment accumulation, and exposure to external contaminants such as dust, faecal matter, or insects. Additionally, poor maintenance practices such as infrequent cleaning, open tank lids, or cracked tanks further contribute to microbial proliferation (Artiola *et al*., 2012).

In Nigeria, studies have shown that water supplied to student hostels, whether piped or fetched from boreholes, is often stored in large tanks before use. Unfortunately, these tanks are rarely disinfected, and there is often no routine bacteriological testing to ensure water safety (Ezenwaji *et al*., 2016; Olalekan *et al*., 2020). As a result, students are at high risk of consuming contaminated water, which can lead to outbreaks of gastrointestinal infections. Prüss-Ustün *et al*. (2014) estimate that globally, about 842,000 deaths occur annually due to unsafe water, sanitation, and hygiene (WASH), with the highest burden seen in low- and middle-income countries such as Nigeria.

Apart from direct health implications, unsafe water in student residences can lead to absenteeism from classes, reduced academic performance, and increased financial burdens from medical treatment. Hunter *et al*. (2010) argue that in educational institutions, reliable access to potable water is essential not only for student welfare but also for academic productivity and institutional efficiency. Moreover, the economic cost of treating waterborne illnesses, coupled with the loss of study time, creates long-term developmental consequences for students and their institutions.

Ensuring bacteriologically safe water in student accommodations requires a multi-faceted approach, including proper tank design, regular maintenance, disinfection protocols, and routine microbial monitoring. According to Bartram and Cairncross (2010), preventive public health interventions in water quality management are more cost-effective and sustainable than curative measures. As such, university administrators and public health authorities must prioritize water safety, especially in environments that house large student populations.

2.2. Waterborne Diseases

Waterborne diseases are caused by the ingestion of water contaminated with pathogenic microorganisms, including bacteria, viruses, and parasites. These diseases are a subset of water-related diseases, which also include water-based, water-washed, and water-related insect vector diseases. Each category presents distinct transmission mechanisms and health risks, particularly in settings with limited access to clean water and sanitation.

Waterborne Diseases: These diseases result from consuming water contaminated with pathogens such as bacteria (*Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, *Shigella* species), viruses (e.g., norovirus, hepatitis A), or parasites (e.g., *Giardia lamblia*, *Cryptosporidium*). Common waterborne diseases include diarrhea, cholera, typhoid fever, and dysentery, which are prevalent in areas with poor water management systems, inadequate sanitation, or improper water storage practices (Cabral, 2010). The Centers for Disease Control and Prevention (CDC) estimates that waterborne pathogens are responsible for approximately 2.5 million deaths annually, with children and vulnerable populations disproportionately affected (CDC, 2015).

Water-Based Diseases: These are transmitted through contact with water harboring parasitic organisms, such as *Schistosoma* parasites, which cause schistosomiasis. These parasites thrive in contaminated freshwater sources, particularly in tropical and subtropical regions, and infect individuals through skin contact during activities like bathing or swimming (WHO, 2020).

Water-Washed Diseases: These occur due to insufficient water for personal hygiene and sanitation, leading to conditions such as scabies, trachoma, and other skin or eye infections. Limited access to clean water for washing exacerbates these diseases, particularly in crowded settings with poor hygiene practices (Hunter *et al*., 2010).

Water-Related Insect Vector Diseases: These are spread by insects, such as mosquitoes, that breed in or near water sources. Examples include malaria, dengue fever, and Zika virus. Stagnant water in storage tanks or poorly managed water systems can serve as breeding grounds for these vectors, amplifying disease transmission (WHO, 2020).

In student populations, particularly those residing in university halls of residence, the risk of waterborne diseases is amplified due to the communal nature of living arrangements. Shared water sources, sanitation facilities, and dining areas create opportunities for rapid disease transmission. For example, a study by Onyango *et al*. (2018) in Kenya identified contaminated water in university hostels as a significant risk factor for diarrheal outbreaks among students. In Nigeria, studies have reported high incidences of waterborne illnesses in educational institutions due to poor water quality and inadequate hygiene practices (Adekunle *et al*., 2019). Water storage systems, such as tanks commonly used in student residences, are particularly susceptible to contamination if not properly maintained. Factors such as irregular cleaning, exposure to environmental contaminants, and stagnant water contribute to the proliferation of pathogens, including biofilm formation, which protects bacteria from disinfection (Flemming *et al*., 2016).

In Benin City, Nigeria, where water supply systems are often unreliable, students frequently rely on stored water from tanks or boreholes. The lack of consistent monitoring and maintenance increases the likelihood of bacteriological contamination, with studies reporting high levels of coliform bacteria in stored water, indicating fecal contamination (Al-Bahry *et al*., 2011; Chalchisa *et al*., 2017). High population density, limited maintenance budgets, and lack of awareness about proper water storage practices further exacerbate contamination risks (Olukanni *et al*., 2014).

The impact of waterborne diseases on student populations extends beyond immediate health effects. Frequent illnesses lead to absenteeism, reduced academic performance, and increased

financial burdens due to medical expenses. In resource-constrained settings like Benin City, where students often rely on limited financial resources, these consequences can be particularly severe. The psychological stress of recurrent illnesses can further compromise students' well-being, underscoring the need for reliable access to clean water in educational settings. Addressing these challenges requires targeted interventions, such as improving water storage practices, implementing regular tank cleaning, and educating residents about hygiene. This study aims to evaluate the bacteriological quality of water tanks in student halls of residence in Benin City, providing insights into contamination levels and informing strategies to mitigate associated health risks.

2.3. Bacteriological Contamination and Common Pathogens

Bacteriological contamination occurs when pathogenic bacteria infiltrate water storage systems, compromising water quality and safety. These pathogens can enter through various pathways, including fecal contamination, environmental exposure, or poor system maintenance (Edberg *et al*., 2000). Once introduced, they can survive or proliferate in stored water, especially under favorable conditions such as warm temperatures, stagnant water, or nutrient-rich environments. The health consequences of consuming or using contaminated water are severe, ranging from mild gastrointestinal distress to life-threatening systemic infections (WHO, 2011). Below is a comprehensive discussion of the most common bacterial pathogens associated with water storage systems, including their biology, transmission, health impacts, and relevance to water safety.

2.3.1. *Escherichia coli*

Escherichia coli is a gram-negative, rod-shaped, facultative anaerobic bacterium commonly found in the gastrointestinal tracts of humans and warm-blooded animals. While many *E. coli* strains are harmless and part of the normal gut microbiota, certain pathogenic strains, such as

enterohemorrhagic *E. coli* (EHEC) like O157:H7, enterotoxigenic *E. coli* (ETEC), and enteropathogenic *E. coli* (EPEC), are significant causes of waterborne diseases (Edberg *et al*., 2000). These strains produce toxins or adhere to intestinal cells, leading to severe gastrointestinal illnesses characterized by watery or bloody diarrhea, abdominal cramps, and, in severe cases, hemolytic uremic syndrome (HUS), which can cause kidney failure, particularly in children and the elderly (Edberg *et al*., 2000).

E. coli is widely used as an indicator organism for fecal contamination in water systems because its presence suggests potential contamination by other enteric pathogens, such as *Salmonella* or *Shigella* (Edberg *et al*., 2000). Contamination typically occurs through sewage leaks, agricultural runoff containing animal waste, or improper sanitation practices near water storage tanks (WHO, 2011). For example, runoff from livestock farms can introduce *E. coli* into open or poorly sealed tanks, especially during heavy rainfall. Once in the water, *E. coli* can survive for weeks, particularly in nutrient-rich environments, and its persistence is enhanced by biofilm formation on tank surfaces (Costerton *et al*., 1999). Effective water treatment, such as chlorination or UV disinfection, is critical to eliminating E. coli, but inadequate maintenance or low disinfectant residuals can allow its proliferation (WHO, 2011).

2.3.2. *Salmonella* spp.

Salmonella species, particularly *Salmonella enterica* serotypes such as Typhi, Paratyphi, and Typhimurium, are gram-negative, rod-shaped, motile bacteria that cause significant waterborne diseases, including typhoid fever and non-typhoidal salmonellosis (Hohmann, 2001). Typhoid fever, caused by *S. enterica* serotype Typhi, is a systemic infection characterized by prolonged fever, abdominal pain, headache, and, in severe cases, intestinal perforation or sepsis (Crump *et al*., 2015). Non-typhoidal salmonellosis, caused by serotypes like Typhimurium, typically results in gastroenteritis with symptoms such as diarrhea, nausea, and vomiting (Hohmann, 2001). Both

forms are transmitted primarily through the fecal-oral route, with contaminated water serving as a major vehicle (Crump *et al*., 2015).

In water storage systems, *Salmonella* is introduced through fecal contamination from infected humans or animals, often via sewage overflows, agricultural runoff, or direct contamination by wildlife accessing open tanks (LeChevallier *et al*., 2003). *Salmonella* can survive in water for extended periods, particularly in warm, nutrient-rich conditions, and its ability to form biofilms enhances its persistence (Costerton *et al*., 1999). Outbreaks of salmonellosis have been linked to contaminated water tanks in rural and urban settings, particularly in regions with poor sanitation infrastructure (Hohmann, 2001). Antibiotic resistance in some *Salmonella* strains complicates treatment, making prevention through proper tank maintenance and water treatment essential (Crump *et al*., 2015).

2.3.3. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a gram-negative, rod-shaped, opportunistic pathogen that thrives in moist environments, making water storage systems an ideal habitat (Mena and Gerba, 2009). Unlike enteric pathogens, *P. aeruginosa* is ubiquitous in the environment, found in soil, water, and on surfaces exposed to moisture (Lyczak *et al*., 2000). It is particularly notorious for forming biofilms—complex microbial communities encased in a protective matrix—on the inner surfaces of water tanks and pipes (Costerton *et al*., 1999). These biofilms shield *P. aeruginosa* from disinfectants and antibiotics, allowing it to persist and multiply even in treated water systems (Mena and Gerba, 2009).

Pseudomonas aeruginosa is a major concern for immunocompromised individuals, causing infections such as pneumonia, urinary tract infections, wound infections, and sepsis (Lyczak *et al*., 2000). In healthy individuals, it can cause skin infections (e.g., folliculitis) or eye infections,

particularly in contact lens users exposed to contaminated water (Mena and Gerba, 2009). In water storage systems, *P. aeruginosa* contamination often results from stagnant water, poor tank cleaning, or environmental exposure (LeChevallier *et al*., 2003). Its intrinsic resistance to many antibiotics and disinfectants, combined with its ability to utilize a wide range of organic compounds as nutrients, makes it a persistent contaminant (Lyczak *et al*., 2000). Regular tank maintenance, including physical cleaning and adequate chlorination, is critical to controlling *P. aeruginosa* (Mena and Gerba, 2009).

2.3.4. *Vibrio cholerae*

Vibrio cholerae is a gram-negative, comma-shaped, facultative anaerobic bacterium responsible for cholera, a severe waterborne disease characterized by profuse watery diarrhea, vomiting, and rapid dehydration (Faruque *et al*., 1998). The bacterium produces cholera toxin, which disrupts intestinal ion transport, leading to massive fluid loss that can be fatal if untreated (Sack *et al*., 2004). Cholera is primarily transmitted through contaminated water or food, with water storage systems serving as a key reservoir during outbreaks, particularly in developing countries or disaster-affected areas (Faruque *et al*., 1998).

V. cholerae thrives in warm, brackish, or stagnant water, making poorly maintained storage tanks vulnerable to contamination (Faruque *et al*., 1998). Environmental sources, such as flooding or sewage overflows, can introduce the bacterium into tanks, especially in coastal regions where *V. cholerae* naturally occurs in aquatic ecosystems (Sack *et al*., 2004). The bacterium can survive in water for weeks to months, particularly in the presence of organic matter or biofilms (Costerton *et al*., 1999). Cholera outbreaks have been historically linked to contaminated water storage systems in urban slums and refugee camps, where sanitation is inadequate (Sack *et al*., 2004). Preventing *V. cholerae* contamination requires robust water treatment, proper tank sealing, and regular cleaning to eliminate organic matter (WHO, 2011).

2.3.5. *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive, spherical bacterium that, while not primarily waterborne, can contaminate water storage systems through human contact or environmental exposure (Otto, 2008). Unlike enteric pathogens, *S. aureus* is commonly found on human skin and mucous membranes, and contamination often occurs during tank cleaning or maintenance if hygiene protocols are not followed (Tong *et al*., 2015). For example, workers handling tank surfaces without proper gloves or using contaminated tools can introduce *S. aureus* into the system. Environmental sources, such as dust or debris entering open tanks, can also contribute (LeChevallier *et al*., 2003).

Staphylococcus aureus causes a range of infections, including skin and soft tissue infections, food poisoning, and, in severe cases, systemic infections like endocarditis or sepsis (Tong *et al*., 2015). Its ability to produce enterotoxins makes it a concern for water used in food preparation (Otto, 2008). Additionally, *S. aureus* can form biofilms, enhancing its survival in water storage systems (Costerton *et al*., 1999). Methicillin-resistant *S. aureus* (MRSA) strains, which are resistant to multiple antibiotics, pose a particular challenge in healthcare settings where contaminated water may be used (Tong *et al*., 2015). Preventing *S. aureus* contamination requires strict hygiene practices during tank maintenance and regular disinfection of storage systems (Otto, 2008).

2.3.6. *Legionella pneumophila*

Legionella pneumophila is a gram-negative, rod-shaped bacterium that causes Legionnaires' disease, a severe form of pneumonia, and Pontiac fever, a milder flu-like illness (Fields *et al*., 2002). It is a significant concern in water storage systems, particularly those with warm water (25–45°C), as it thrives in these conditions (Declerck, 2010). *Legionella* is often associated with

biofilm formation in tanks, pipes, and plumbing systems, where it can survive disinfection efforts (Costerton *et al*., 1999). The bacterium is transmitted through inhalation of aerosolized water droplets containing the pathogen, such as those produced by showers or cooling towers connected to contaminated storage systems (Fields *et al*., 2002).

Contamination in water tanks typically occurs through environmental exposure, such as dust or soil containing *Legionella*, or through stagnant water that allows biofilm development (LeChevallier *et al*., 2003). Outbreaks of Legionnaires' disease have been linked to poorly maintained water storage systems in hospitals, hotels, and public buildings (Fields *et al*., 2002). Controlling *Legionella* requires maintaining water temperatures outside its optimal growth range (e.g., above 60°C or below 20°C), regular tank cleaning, and adequate disinfection (Declerck, 2010).

2.3.7. *Campylobacter jejuni*

Campylobacter jejuni is a gram-negative, spiral-shaped, microaerophilic bacterium that causes campylobacteriosis, one of the most common bacterial causes of diarrheal illness worldwide (Blaser, 1997). Symptoms include diarrhea (often bloody), fever, and abdominal cramps, with complications such as Guillain-Barré syndrome in rare cases (Humphrey *et al*., 2007). *C. jejuni* is primarily transmitted through contaminated water or food, with water storage systems becoming contaminated via animal feces, particularly from birds or livestock, or through agricultural runoff (Blaser, 1997).

In water storage systems, *C. jejuni* can survive for weeks in cool, moist conditions, particularly in tanks with organic matter or biofilms (Humphrey *et al*., 2007). Its low infectious dose means that even small levels of contamination can lead to outbreaks (Blaser, 1997). Preventing *C. jejuni* contamination requires protecting tanks from environmental exposure, such as sealing access

points to prevent animal intrusion, and implementing effective water treatment, such as chlorination or filtration (WHO, 2011).

2.3.8. *Shigella* spp.

Shigella species, including *Shigella dysenteriae*, *S. flexneri*, *S. sonnei*, and *S. boydii*, are gram-negative, non-motile bacteria that cause shigellosis, a highly infectious diarrheal disease (Niyogi, 2005). Symptoms include watery or bloody diarrhea, fever, and abdominal pain, with severe cases leading to dehydration or complications like toxic megacolon (Warren *et al*., 2006). *Shigella* is primarily transmitted through the fecal-oral route, with contaminated water serving as a major vehicle, particularly in areas with poor sanitation (Niyogi, 2005).

In water storage systems, *Shigella* contamination typically occurs through human fecal matter, such as sewage leaks or improper hygiene during tank maintenance (LeChevallier *et al*., 2003). The bacterium's low infectious dose (as few as 10–100 organisms) makes it a significant threat, as even minor contamination can lead to outbreaks (Warren *et al*., 2006). *Shigella* can survive in water for days to weeks, particularly in nutrient-rich or stagnant conditions (Niyogi, 2005). Preventing *Shigella* contamination requires stringent sanitation practices, regular tank cleaning, and effective water treatment to eliminate the pathogen (WHO, 2011).

Bacteriological contamination in water storage systems is a significant public health concern driven by a diverse array of pathogenic bacteria, including *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Staphylococcus aureus*, *Legionella pneumophila*, *Campylobacter jejuni*, *Shigella spp.*, and others like *Klebsiella pneumoniae*, *Yersinia enterocolitica*, and *Aeromonas hydrophila*. Each pathogen presents unique challenges due to its biology, transmission routes, and survival mechanisms, such as biofilm formation or environmental persistence (Costerton *et al*., 1999). Contamination often stems from fecal sources,

environmental exposure, or poor maintenance practices, highlighting the need for robust preventive measures (LeChevallier *et al*., 2003). Effective water treatment, regular tank cleaning, proper sealing, and adherence to hygiene protocols during maintenance are critical to mitigating these risks and ensuring safe water supplies for communities worldwide (WHO, 2011).

2.4. Sources of Contamination in Water Tanks

Water tanks are essential for storing potable water for domestic, industrial, and agricultural purposes. However, stored water is susceptible to contamination and bacterial proliferation, which can compromise its quality and pose public health risks.

2.4.1. Poor Maintenance

Poor maintenance is a leading cause of water tank contamination. Neglecting regular cleaning allows sediments, organic matter, and debris to accumulate, creating nutrient-rich environments for microbial growth. Sediments can harbor pathogens such as *Escherichia coli* and *Salmonella*, leading to water quality deterioration (World Health Organization [WHO], 2017). Failure to inspect and repair structural defects, such as cracks or corroded fittings, permits external contaminants to enter the tank. Inadequate sealing of lids or access hatches further increases the risk of contamination by allowing dust, insects, or small animals to infiltrate (Schafer and Mihelcic, 2012).

The WHO recommends annual cleaning and inspection of water tanks to prevent contamination, particularly in rural or resource-limited settings where maintenance may be infrequent (WHO, 2017). A study by Schafer and Mihelcic (2012) found that unmaintained household water tanks in developing countries often contained coliform bacteria at levels exceeding safe drinking water standards. Regular maintenance, including sediment removal and structural repairs, is critical to mitigating contamination risks.

2.4.2. Environmental Exposure

Water tanks, especially those located outdoors, are vulnerable to environmental contaminants. Airborne particles such as dust, pollen, or pollutants can settle on tank surfaces and enter through poorly sealed openings (LeChevallier *et al*., 1996). Rainwater runoff carrying soil, organic matter, or animal feces can infiltrate tanks, particularly in low-lying areas or during flooding events (Ahmed *et al*., 2011). For example, bird droppings on tank roofs can introduce pathogens like *E. coli* or *Salmonella* if washed into the tank during rainfall (LeChevallier *et al*., 1996).

In coastal regions, saline aerosols can corrode tank materials, creating entry points for contaminants (Falkenberg *et al*., 2014). Small animals or insects accessing tanks through vents or gaps can also introduce fecal matter or other contaminants. Proper tank design, including secure covers and filtered vents, is essential to minimize environmental exposure (WHO, 2017). Elevated tank placement and protective barriers can further reduce contamination risks from runoff or wildlife.

2.4.3. Biofilm Formation

Biofilms are complex microbial communities embedded in extracellular polymeric substances (EPS) that adhere to tank surfaces. They are a persistent source of contamination, harboring pathogens such as *Pseudomonas aeruginosa*, *Legionella pneumophila*, and *Mycobacterium* species (Falkinham *et al*., 2015). Biofilms form in areas with low water flow or stagnation, where nutrients like organic matter or sediments are present (LeChevallier and Au, 2004). The EPS matrix protects microorganisms from disinfectants like chlorine, making biofilms resistant to standard cleaning methods (Falkinham *et al*., 2015).

Biofilm formation is a significant concern in water storage systems, as it can lead to chronic contamination and corrosion of tank surfaces, particularly in metal tanks (LeChevallier and Au,

2004). Advanced cleaning techniques, such as high-pressure washing or the use of specialized biocides, are required to remove biofilms effectively. Regular monitoring and maintenance are critical to preventing biofilm establishment and ensuring water safety (WHO, 2017).

2.5. Factors Influencing Bacterial Growth in Stored Water

2.5.1. Temperature

Temperature significantly influences bacterial growth in water tanks. Most waterborne pathogens thrive between 20°C and 45°C, with *Legionella pneumophila* exhibiting optimal growth at 25°C to 42°C (Kim *et al*., 2002). In warm climates, tanks exposed to sunlight can reach temperatures conducive to bacterial proliferation, especially without insulation or shading (Schafer and Mihelcic, 2012). Conversely, temperatures below 20°C or above 60°C can inhibit bacterial growth, though some species, like *Pseudomonas*, can survive in colder conditions (Kim *et al*., 2002).

Temperature gradients within tanks can create localized areas of high bacterial activity, with warmer water at the top supporting greater microbial concentrations (LeChevallier *et al*., 1996). Insulating tanks or installing cooling systems can help maintain temperatures outside the optimal range for bacterial growth. In resource-limited settings, shading or burying tanks underground may be cost-effective solutions to reduce heat exposure (Ahmed *et al*., 2011).

2.5.2. Stagnation

Water stagnation promotes bacterial growth by allowing sediments and organic matter to settle, providing nutrients for microorganisms. Stagnation also reduces the efficacy of residual disinfectants like chlorine, which degrade over time in still water (LeChevallier and Au, 2004). Tanks with low turnover rates, such as those used for emergency storage or in households with

intermittent water use, are particularly susceptible to stagnation-related contamination (Schafer and Mihelcic, 2012).

Stagnation also enhances biofilm formation, as microorganisms have time to adhere to tank surfaces (Falkinham *et al*., 2015). In large distribution systems, dead-end pipes or oversized tanks exacerbate stagnation, creating localized contamination zones (LeChevallier *et al*., 1996). Proper tank design with mixing systems or adequate water turnover can mitigate stagnation. Matching tank size to usage patterns is also critical to maintaining water quality (WHO, 2017).

2.5.3. Tank Material

The choice of tank material influences bacterial growth and contamination risk. Common materials include concrete, plastic (e.g., polyethylene or fiberglass), and metals (e.g., stainless steel or galvanized steel), each with distinct properties affecting microbial proliferation.

- **Concrete Tanks**: Concrete is porous, allowing water to seep into micro-cracks where bacteria can grow (Falkenberg *et al*., 2014). Poorly sealed concrete surfaces may also leach minerals, altering water chemistry and promoting microbial activity. Regular sealing and cleaning are necessary to prevent contamination (WHO, 2017).
- **Plastic Tanks**: High-density polyethylene (HDPE) tanks are non-porous and resistant to corrosion, reducing bacterial adhesion compared to concrete or metal (Ahmed *et al*., 2011). However, low-quality plastics may leach chemicals that serve as microbial nutrients. Sunlight exposure can degrade plastic, creating rough surfaces that facilitate biofilm formation (Falkenberg *et al*., 2014).
- **Metal Tanks**: Stainless steel tanks are resistant to corrosion and easy to clean, making them ideal for maintaining water quality. However, galvanized steel tanks may corrode,

releasing metal ions that alter water chemistry and support bacterial growth (LeChevallier and Au, 2004). Corrosion also creates rough surfaces conducive to biofilm formation.

Proper material selection, based on durability, cost, and maintenance requirements, is essential for minimizing bacterial growth (WHO, 2017). Water tank contamination arises from poor maintenance, environmental exposure, and biofilm formation, while bacterial growth is driven by temperature, stagnation, and tank material. These factors interact to compromise water quality, necessitating integrated management strategies. Regular maintenance, environmental protection, and appropriate material selection are critical for ensuring safe drinking water. Future research should focus on cost-effective technologies for water tank management, particularly in resource-constrained settings, to enhance global access to clean water.

2.6. Health Risks Associated with Contaminated Water

Safe and clean water is essential for maintaining public health, yet contaminated water remains a significant challenge in communal living environments such as student residences. These settings, characterized by high population density, shared facilities, and often outdated infrastructure, are particularly susceptible to water quality issues. Contaminated water can introduce a range of biological, chemical, and emerging contaminants, leading to acute and chronic health effects.

2.6.1. Gastrointestinal Infections

Contaminated water serves as a primary vector for gastrointestinal infections, which represent one of the most immediate and widespread health consequences of poor water quality. Water sources tainted by fecal matter, sewage, or inadequate treatment systems harbor a variety of microbial pathogens, including bacteria such as *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, and *Vibrio cholerae*; viruses like norovirus, rotavirus, and hepatitis A; and parasites including *Cryptosporidium parvum* and *Giardia lamblia* (Leclerc *et al*., 2002; WHO, 2017).

These pathogens often enter water supplies through human or animal waste, compromised sanitation infrastructure, or natural disasters that overwhelm treatment facilities (Prüss-Ustün *et al*., 2014). The presence of these organisms in drinking water is a key indicator of fecal contamination and poses significant public health risks, especially in overcrowded settings such as university hostels. The World Health Organization estimates that contaminated drinking water and poor sanitation contribute to approximately 829,000 deaths annually from diarrheal diseases, with over 500,000 directly attributable to unsafe drinking water (WHO, 2019). While vulnerable populations such as children, the elderly, and immunocompromised individuals face heightened risks, young adults in communal settings like university dormitories are also susceptible due to their reliance on shared water sources, dining facilities, and sanitation systems.

The health impacts of gastrointestinal infections range from mild to severe, significantly affecting students' academic performance, social activities, and overall well-being. Common symptoms include frequent, watery diarrhea, which can lead to dehydration, particularly in settings with limited access to medical care (WHO, 2017). Vomiting, often accompanied by nausea, exacerbates fluid loss, while abdominal pain and cramping result from inflammation or irritation of the gastrointestinal tract (Kosek *et al*. 2003). Fever is a frequent symptom in bacterial infections such as typhoid fever or shigellosis (Crump *et al*., 2004). In severe cases, such as cholera caused by *Vibrio cholerae*, rapid dehydration can lead to electrolyte imbalances, shock, and even death if untreated (Harris *et al*., 2012). For students, these illnesses can disrupt study schedules, impair cognitive function due to dehydration and fatigue, and lead to absenteeism (Murray *et al*., 2013).

A notable case study is the 2010 Haiti cholera outbreak, which followed a devastating earthquake that compromised water infrastructure. Contaminated water sources led to over 800,000 cases and 10,000 deaths, illustrating the catastrophic potential of waterborne pathogens

(Piarroux *et al*., 2011). In student residences, similar risks arise when water treatment systems fail or shared facilities are inadequately maintained. For example, a norovirus outbreak in a university dormitory, traced to a contaminated water supply in a communal kitchen, affected over 200 students, highlighting the rapid transmission potential in such settings (Moe *et al*., 2006). Preventive measures, including regular water quality testing, chlorination, and maintenance of sanitation systems, are critical to reducing these risks in student residences.

2.6.2. Skin and Soft Tissue Infections

Using contaminated water for bathing, washing, or other hygiene practices can lead to skin and soft tissue infections, posing significant health risks in communal living environments (Ashbolt, 2004). Bacterial pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Mycobacterium marinum* proliferate in poorly maintained water systems, including those in shared showers, hot tubs, or laundry facilities (Vaerewijck *et al*., 2005; Okafor *et al*., 2011). Fungal pathogens, such as *Candida albicans* and *Aspergillus* species, thrive in warm, moist environments, increasing the risk of infection upon prolonged exposure. Additionally, inhalation of aerosolized water droplets, such as those produced during showering, can introduce pathogens like *Legionella pneumophila*, which causes Legionnaires' disease, a severe form of pneumonia (Wiik and Krøvel, 2014; Niculita-Hirzel *et al*., 2022). These risks are particularly pronounced in student residences, where shared plumbing systems may harbor biofilms complex microbial communities that resist standard water treatment methods.

The health impacts of skin and soft tissue infections vary in severity but can significantly affect students' quality of life. Folliculitis, often caused by *Pseudomonas aeruginosa*, presents as red, itchy bumps around hair follicles, commonly affecting individuals using contaminated showers or hot tubs. Cellulitis, a deeper bacterial infection caused by *Staphylococcus aureus* or *Streptococcus* species, results in redness, swelling, and pain, potentially requiring antibiotic

treatment. Impetigo, a superficial infection, causes crusted sores and is highly contagious in communal settings. Fungal infections, such as candidiasis or dermatophytosis, lead to itching, redness, and scaling, particularly in moist environments like shared bathrooms. Legionnaires' disease, caused by inhaling *Legionella*-contaminated aerosols, manifests as fever, cough, and potentially fatal pneumonia, posing a severe risk in poorly maintained water systems (Khairullah *et al*., 2025). A 2019 outbreak of *Pseudomonas aeruginosa* in a university dormitory, traced to contaminated showerheads, affected dozens of students, underscoring the risks of shared facilities (CDC, 2019).

Preventive measures are essential to mitigate these risks. Regular maintenance of water systems, including chlorination and temperature control to prevent *Legionella* growth (maintaining hot water above 50°C), is critical. Routine cleaning and disinfection of communal facilities, such as showers and laundry rooms, can reduce pathogen transmission. Educating students on proper hygiene practices, such as avoiding prolonged exposure to standing water and promptly reporting plumbing issues, can further minimize risks.

2.6.3. Chronic Health Effects

Long-term exposure to chemical contaminants in water can lead to chronic health conditions, many of which are insidious and may not manifest until years after exposure (Seth, 2013; Villanueva *et al*., 2014). Common chemical contaminants include heavy metals (lead, arsenic, cadmium, mercury), nitrates from agricultural runoff, pesticides and herbicides (e.g., atrazine, glyphosate), and industrial chemicals such as polychlorinated biphenyls (PCBs), per- and polyfluoroalkyl substances (PFAS), and volatile organic compounds (VOCs) (Jagaba *et al*., 2024). These contaminants enter water supplies through corroded pipes, industrial runoff, or natural geological deposits, posing significant risks in student residences where individuals rely on the same water source for drinking, cooking, and bathing over extended periods.

The health impacts of chronic exposure to chemical contaminants are severe and far-reaching. Lead, often introduced through aging plumbing systems, is associated with neurological damage, reduced cognitive function, and developmental delays, particularly in children. In adults, lead exposure increases the risk of hypertension, kidney damage, and cardiovascular disease (ATSDR, 2020). The Flint water crisis (2014–2016) exposed thousands of residents to elevated lead levels, leading to widespread health impacts, including cognitive impairments in young adults (Hanna-Attisha *et al*., 2016). Arsenic, a known carcinogen, is linked to skin, lung, bladder, and liver cancers, as well as skin lesions and cardiovascular disease (IARC, 2012). Chronic exposure to nitrates, often from agricultural runoff, can cause methemoglobinemia in infants and may increase the risk of gastrointestinal cancers in adults (Ward *et al*., 2018). PFAS, dubbed "forever chemicals" due to their environmental persistence, are associated with immune system dysfunction, liver damage, and increased cancer risk (EPA, 2023).

Students in low-income or poorly maintained residences are particularly vulnerable due to outdated infrastructure, such as lead-containing pipes or inadequate filtration systems. For example, older university buildings may expose residents to heavy metals over months or years, potentially affecting academic performance and long-term health. Universities must prioritize regular water testing, pipe replacement, and advanced filtration systems to mitigate these risks and protect student populations from chronic health effects.

Emerging contaminants, such as pharmaceuticals, personal care products, and microplastics, are increasingly detected in water systems due to advances in analytical chemistry. These contaminants enter water sources through wastewater discharge, improper disposal, or runoff, posing novel health risks. Pharmaceuticals, including antibiotics, antidepressants, and hormones, are excreted or improperly disposed of, entering water supplies. Personal care products, such as

triclosan (found in soaps) or parabens (in cosmetics), and microplastics from degraded plastic waste or synthetic fibers, are also prevalent (Richardson and Ternes, 2018; Zaidi *et al*., 2025).

Chronic exposure to low levels of antibiotics in water may contribute to antimicrobial resistance, a major global health threat that complicates the treatment of bacterial infections (Pruden *et al*., 2013). Hormones and hormone-mimicking chemicals, such as bisphenol A and phthalates, can cause endocrine disruption, potentially leading to reproductive issues or developmental disorders (Diamanti-Kandarakis *et al*., 2009). Microplastics, detected in 83% of tap water samples worldwide (Kosuth *et al*., 2017), can act as vectors for other contaminants, such as heavy metals or pathogens, and may cause inflammation or oxidative stress when ingested (Wright and Kelly, 2017). In student residences, where water is used extensively for drinking, cooking, bathing, and laundry, the presence of these contaminants compounds the risks posed by traditional pathogens and chemicals.

The long-term health impacts of emerging contaminants remain poorly understood, and regulatory frameworks for monitoring or removing these substances from water supplies are limited. Students can advocate for water quality testing and the installation of advanced filtration systems, such as reverse osmosis or activated carbon filters, to reduce exposure. Ongoing research is needed to better understand the risks and develop effective mitigation strategies.

2.7 Control Strategies for Ensuring Safe Water Quality

Ensuring the safety and wholesomeness of water in institutional settings such as university residences requires a multifaceted approach, integrating preventive, corrective, and educational strategies. The persistent challenge of water contamination in Nigeria, is exacerbated by infrastructure decay, poor maintenance culture, and lack of awareness among users (Adeoti *et al*.,

2023). As such, proactive control measures are essential to maintain acceptable microbiological and physicochemical water quality standards in storage systems.

One of the most fundamental strategies for controlling water contamination is routine cleaning and disinfection of water storage tanks (Bacha, 2016). Over time, sediments, biofilms, and organic matter tend to accumulate at the base and inner surfaces of storage tanks. These materials serve as substrates for microbial proliferation and biofilm formation, which can harbor pathogenic organisms and protect them from disinfectants such as chlorine (Wingender and Flemming, 2011). Periodic tank cleaning, ideally conducted every 3 to 6 months, removes these deposits and helps to restore sanitary conditions within the tank. Disinfection with appropriate concentrations of chlorine or other biocidal agents further ensures the elimination of residual microbial contaminants (WHO, 2017). In institutional settings, the absence of a structured tank cleaning protocol often contributes to sustained bacterial presence in stored water (Adetunde, *et al*., 2010; Al-Bahry *et al*., 2011; Okafor *et al*., 2023).

Another critical preventive measure is the proper design and construction of water storage tanks**.** Tanks should be constructed with durable materials that are resistant to corrosion and microbial colonization. Moreover, they should be properly sealed with tight-fitting lids to prevent the entry of dust, insects, rodents, bird droppings, and rainwater runoff, all of which are potential sources of contamination (Nwachukwu and Uzoigwe, 2005). Elevated platforms help reduce the risk of backflow from contaminated ground surfaces, especially during the rainy season when floodwaters can carry fecal matter and debris. The placement of tanks away from sewage systems and refuse disposal sites is also essential for preventing environmental contamination.

In addition to structural measures, regular microbiological and physicochemical monitoring of stored water is indispensable. Monitoring helps to detect contamination early, assess treatment efficiency, and identify sources of pollution. Key microbial parameters such as total coliforms,

Escherichia coli, and heterotrophic plate count (HPC) provide insight into the sanitary quality of water, while physicochemical parameters like pH, turbidity, residual chlorine, and total dissolved solids (TDS) determine its aesthetic and chemical safety (Edberg *et al*., 2000; WHO, 2017). Routine water quality assessment should follow standard procedures outlined by the World Health Organization and the Standards Organisation of Nigeria (SON, 2007), using accredited laboratories and validated testing protocols. Institutions that ignore water testing are often blindsided by sudden outbreaks of waterborne diseases that could have been averted through early detection and timely intervention.

For immediate household or personal protection, especially where centralized water safety cannot be guaranteed, the use of point-of-use water treatment techniques is encouraged. Methods such as boiling, filtration, chlorination, and the use of solar disinfection (SODIS) are effective in reducing microbial load in drinking water. Boiling remains the most accessible and effective method, capable of killing all classes of waterborne pathogens. However, it may not be practical for large-scale or long-term use due to fuel costs and time constraints (Sobsey *et al*., 2008). Chlorination is also highly effective and can provide residual protection, but it must be carefully dosed to avoid taste issues and health hazards. Household filtration systems, especially those incorporating activated carbon or ceramic filters, are increasingly being adopted in urban centers to address both microbial and chemical contaminants (Clasen and Bastable, 2003).

Beyond physical and chemical interventions**,** public health education and awareness campaigns are vital components of water quality control. Many cases of water contamination in university settings stem from improper handling practices, such as drawing water with unclean containers, failing to cover tanks after use, or allowing animals access to storage areas (Okonko *et al*., 2008). Health education initiatives targeting students and staff can raise awareness about the sources and consequences of water contamination, and promote behavioral changes that support safe

water use. Effective communication strategies, including posters, seminars, and student-led hygiene clubs, have been shown to improve sanitation practices and reduce waterborne disease incidence in institutional settings (Prüss-Ustün *et al*., 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Collection of Samples

The swab and water samples were taken from the water tanks that supplies water for students use in the halls of residence at the university of Benin, Edo state, Nigeria. The selective halls include: Hall 1, Hall 2, Hall 3, Hall 4. Samples were also taken from a household with regularly cleaned tanks for comparison.

3.2. Sterilization of Materials

All materials used were appropriately sterilized before and after use. The glass wared such as test tubes, pipettes, conical flasks were properly washed with detergents, rinsed properly with water and drained. It was sterilized by being wrapped in aluminium foil and oven dried at 120 ºC for 2 hours. The lab bench was swabbed with 70% alcohol for disinfection.

3.3. Preparation of Media

All media used were prepared according to manufacturer's instructions. Sterilization was at 121ºC at 15psi for 15 min. Media's used are Nutrient Agar, MacConkey Agar, and Eosine Methylene Blue Agar.

3.4. Isolation of Microorganisms

Isolation of microorganisms was carried out by the pour plate method (Dimowo and Omonigho 2023). The swab samples were placed in saline solution which was 0.9g of NaCl in 100 mL of water. Stock culture was prepared by measuring 25mL of sample and dissolving in 225mL of sterile distilled water. Serial dilutions were carried out by transferring 1 mL of stock solution in

to a test tube containing 9 mL of sterile distilled water, thereby making a 10^1 dilution. This step was repeated to further create 10^2, 10^3 dilutions. 1 mL from 10^1 and 10^3 were inoculated on different media and incubated inverted at 35-37°C for 24-48 hrs. (Dimowo and Omoregie, 2023).

3.5. Enumeration of Microorganisms

After incubation, the population of the bacterial isolates were enumerated by counting the numbers of distinct colonies on the plate using a colony counter. The number of colony forming unit per milliliter (CFU/mL) was calculated using the formula below(Dimowo and Omoregie, 2023) :

$$\textit{colony forming unit per milliliter (CFU/mL)} = \frac{(\textit{Number of colonies} \times \textit{Dilution factor})}{\textit{Volume plated (mL)}}$$

3.6. Purification of Isolates

Colonies were sub cultured using the streak plate method to obtain pure culture. The streak plate method was done using a sterile wire loop to pick up the single bacteria colony from the population and it was streaked on a sterile nutrient agar plate. The isolates were streaked in a zig zag pattern. It was incubated by inversion at 37°C for 24 hrs. (Dimowo and Omoregie, 2023).

3.7. Characterization:

3.7.1. Gram staining

This staining is the most crucial staining technique in microbiology and is also the first step in identifying a bacteria (Paray *et al.,* 2023). This test was done to separate the bacteria into gram-positive and gram-negative using the crystal violet, iodine, alcohol, and the safranin accordingly. After the test there were two results, Gram-positive bacteria appeared purple under the microscope and Gram-negative appeared pink under the microscope.

3.7.2. Biochemical tests

A series of biochemical tests were carried out for identification and characterization of the bacterial isolates. The tests are Catalase test, oxidase test, citrate test, indole test, triple sugar iron (TSI) test, urease test, and coagulase test.

3.8. Identification of isolates

The isolates were identified using Bergry's manual (9th Edition, 1994) which is the gold standard reference book for identifying and classifying bacteria. The results obtained from characterization of the isolates were compared with Bergry's manual to determine the genus and species of each isolate based on the closest affinity in cultural, biochemical, and microscopic features.

3.9. Data Analysis

The data gotten from the the analysis were written systematically and organized to avoid mix ups and for efficient interpretation. It was also taken to compare with the standards established by the World Health Organization (WHO). According to the World Health Organization (WHO, 2017), heterotrophic plate counts in potable water should not exceed 100 cfu/mL, also according to the Nigerian Industrial Standard (NIS 554:2007) and WHO (2017) guidelines, coliform bacteria should not be detectable in 100 mL of drinking water. This comparison helped the study to determine if the number of bacteria detected pose a health risk to the students using the water.

CHAPTER 4

RESULTS

The results for the bacteriological analysis of water tanks in halls of residence are presented below. The total heterotrophic bacterial count of isolates obtained from the various water tank swab samples is presented in Table 4.1. The highest bacterial count was observed in Household A and B, which both recorded TNC, while the lowest was Hall 3A which recorded 2.33± 0.57 $\times 10^1$ CFU/mL.

The total coliform counts presented in Table 4.2 show that the highest contamination was recorded from water tank swab samples in Hall 4, with 7.60 ± 0.94 $\times 10^1$ CFU/mL, followed closely by Hall 3B with 4.60 ± 0.81 $\times 10^2$ CFU/mL, while the lowest coliform count was seen in Hall 2B with 1.00 ± 0.57 × 10^2 CFU/mL.

As shown in Table 4.3, the highest counts of non-lactose fermenting Gram-negative bacteria from water tank swab samples were obtained from Hall 3A, with 6.60± 0.58 $\times 10^3$ CFU/mL at 10^3 dilution, followed by Household A (4.70 ± 0.47 $\times 10^4$ CFU/mL) and Household B (4.31 ± 0.58 × 10^3 CFU/mL), while Hall 1B and Hall 4 showed no growth (NG) at 10^3 dilution.

The total heterotrophic bacterial count of isolates obtained from the various water tank swab samples is presented in Table 4.4. The results shows that Sample B with 2.67±0.57 × 10^3 CFU/mL has the highest bacterial count.

The total coliform counts presented in Table 4.5 showed that the highest contamination was recorded from Sample A with 2.33±0.94 $\times 10^3$CFU/mL.

As shown in Table 4.6, the highest counts of non-lactose fermenting Gram-negative bacteria were obtained from Sample A with $2.63 \pm 0.48 \times 10^3$CFU/mL.

The biochemical and Morphological characteristics of the bacterial isolates, as presented in Table 4.7 and 4.8, revealed eight distinct bacterial species from both the swab and water samples. The predominant isolates identified included *Escherichia coli*, *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp., *Proteus* sp., *Pseudomonas* sp., *Staphylococcus aureus* and weakly fermenting *E. coli*.

Table 4.1: Total Heterotrophic bacteria count of isolates obtained from various water tank swab samples. Values represented in mean ± standard deviation.(CFU/mL).

Sample	Mean ±S.D	WHO Standard
Hall 1A	$4.63 \pm 0.47 \times 10^2$	**≤ 100 cfu/mL
Hall 1B	$2.42 \pm 0.82 \times 10^2$	
Hall 2A	$5.33 \pm 0.94 \times 10^1$	
Hall 2B	$5.06 \pm 0.94 \times 10^2$	
Hall 3A	$2.33 \pm 0.57 \times 10^1$	
Hall3B	$3.13 \pm 0.58 \times 10^2$	
Hall 4	$3.33 \pm 0.47 \times 10^1$	
Household A	TNC	
Household B	TNC	

The WHO standard states that total heterotrophic plate count in portable water should not exceed 100cfu/ml.

Table 4.2: Total coliform count obtained from various water tank swab samples. Values represented in mean ± standard deviation.(CFU/mL).

Sample	Mean ±S.D	WHO Standard
Hall 1A	$2.33 \pm 0.94 \times 10^1$	**≤ 0 cfu/mL
Hall 1B	$1.93 \pm 0.58 \times 10^2$	
Hall 2A	$1.33 \pm 0.47 \times 10^1$	
Hall 2B	$1.00 \pm 0.57 \times 10^2$	
Hall 3A	$1.72 \pm 0.82 \times 10^2$	
Hall 3B	$4.60 \pm 0.81 \times 10^2$	
Hall 4	$7.60 \pm 0.94 \times 10^1$	
Household A	$2.50 \pm 0.58 \times 10^2$	
Household B	$3.00 \pm 0.94 \times 10^2$	

**According to WHO guidelines, coliform bacteria should not be detectable in 100 mL of drinking water.

Table 4.3: Total counts of Non-lactose fermenting Gram-negative bacteria using Eosine Methylene Blue obtained from water tank swab samples(CFU/mL).

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Sample	10^1	10^3
Hall 1A	$3.46 \pm 0.94 \times 10^2$	$1.00 \pm 0.47 \times 10^3$
Hall 1B	$4.46 \pm 0.47 \times 10^2$	NG
Hall 2A	$3.33 \pm 0.57 \times 10^2$	$1.20 \pm 0.94 \times 10^4$
Hall 2B	$1.16 \pm 0.58 \times 10^2$	$2.00 \pm 0.82 \times 10^3$
Hall 3A	$3.10 \pm 0.94 \times 10^2$	$6.60 \pm 0.58 \times 10^3$
Hall 3B	$4.13 \pm 0.82 \times 10^2$	$2.40 \pm 0.57 \times 10^3$
Hall 4	$2.30 \pm 0.94 \times 10^1$	NG
Household A	$4.70 \pm 0.47 \times 10^2$	$4.03 \pm 0.47 \times 10^4$
Household B	$2.70 \pm 0.58 \times 10^2$	$4.31 \pm 0.58 \times 10^3$

Values represented in mean $\pm$ standard deviation

Table 4.4: Total Heterotrophic bacteria count of isolates obtained from various water tank samples. Values represented in mean ± standard deviation.(CFU/mL).

Water Sample	Mean ±S.D	Mean ±S.D	WHO Standard
Sample A	$3.33\pm0.57 \times 10^{1}$	$2.33\pm0.47 \times 10^{3}$	**≤ 100 cfu/mL
Sample B	$5.67\pm0.48 \times 10^{1}$	$2.67\pm0.57 \times 10^{3}$	

**The WHO standard states that total heterotrophic plate count in portable water should not exceed 100cfu/ml.

Table 4.5: Total coliform count obtained from various water tank samples. Values represented in mean ± standard deviation.(CFU/mL).

Water Sample	Mean ±S.D	Mean ±S.D	WHO Standard
Sample A	$1.03\pm0.82 \times 10^{2}$	$2.33\pm0.94 \times 10^{3}$	**≤ 0 cfu/mL
Sample B	$3.37\pm0.48 \times 10^{1}$	$1.67\pm0.57 \times 10^{3}$	

**According to WHO guidelines, coliform bacteria should not be detectable in 100 mL of drinking water.

Table 4.6: Total counts of Non-lactose fermenting Gram-negative bacteria using Eosine Methylene Blue obtained from water tank samples (CFU/mL).

Water Sample	10^{1}	10^{3}
Sample A	$4.67\pm0.58 \times 10^{2}$	$2.63\pm0.48 \times 10^{3}$
Sample B	$2.33\pm0.47 \times 10^{1}$	$1.00\pm0.58 \times 10^{3}$

Values represented in mean ± standard deviation

TABLE 4.7: Biochemical description of microbial isolates found in the water tank samples.

Isolates	Gram stain	Indole	Citrase	Urease	Oxidase	Catalase	Coagulase	TSI	Motility
Klebsiella	-	-	+	+	-	+	-	A/A, gas, no H_2S	-
Escherichia coli	-	+	-	-	-	+	-	A/A, gas, no H_2S	+
Citrobacter	-	-	+	-	-	+	-	K/A, gas, H_2S	+
Weakly fermenting *E. Coli*	-	+	-	-	-	+	-	A/A, gas, no H_2S	+
Pseudomonas	-	-	+	-	+	+	-	K/K, no gas, no H_2S	+
Proteus	-	-	+	+	-	+	-	K/A, gas, H_2S	+
Stapylococus aureus	+	none	none	+	-	+	+	none	-
Enterobacter	-	-	+	-	-	+	-	A/A, gas, no H_2S	+

Table 4.8: Morphological descriptions of the different isolates

Isolates	Gram Reaction & Shape	Colony Morphology (Agar)	Key Features
Klebsiella	Gram-negative short rods (capsulated)	Large, mucoid, pink colonies on MacConkey; pink to purple on EMB	Prominent capsule, lactose fermenter
Escherichia coli	Gram-negative short rods	Smooth, moist, grayish-white; pink colonies on MacConkey; metallic green sheen on EMB	Strong lactose fermenter
Citrobacter	Gram-negative rods	Colonies resemble *E. coli* but more translucent; lactose fermentation variable	Opportunistic, may produce H_2S
Weakly fermenting *E. coli*	Gram-negative short rods	Colonies pale pink or colorless on MacConkey ; faint/absent metallic sheen	Slow lactose fermenters
Pseudomonas	Gram-negative slender rods	Large, flat colonies; often greenish pigment (pyocyanin, pyoverdine); fruity odor	Non-lactose fermenter, pigment production
Proteus	Gram-negative rods	Swarming colonies on nutrient agar; colorless on MacConkey (non-lactose fermenter)	Strong motility, H_2S producer
Stapylococus aureus	Gram-positive cocci in clusters	Golden-yellow, round, convex colonies; often beta-hemolytic on blood agar	Coagulase-positive, pathogenic
Enterobacter	Gram-negative rods	Smooth, mucoid, pink colonies on MacConkey agar (less mucoid than *Klebsiella*)	Motile, lactose fermeter

CHAPTER FIVE

5.1 DISCUSSION

Water quality is one of the most critical determinants of public health, particularly in institutional and residential environments where large populations depend on centralized or stored water systems. In university settings such as the University of Benin, water tanks serve as the principal storage and distribution points for student hostels, making them vital components in maintaining hygiene and health standards. However, stored water is prone to microbial contamination due to factors such as tank age, biofilm development, poor maintenance, and intermittent water supply (Al-Bahry *et al.,* 2011; Jimoh *et al.,* 2025; Okafor *et al*., 2025;). This study investigated the bacteriological quality of water from storage tanks across halls of residence in the University of Benin.

The total heterotrophic bacterial count (THBC) indicates the general microbial population in water and is used as a basic measure of water quality (WHO, 2017). In the swab samples, Household A and household B both had too numerous to count(TNC) suggesting heavy contamination, followed closely by Hall 2A with 5.33 ±0.94 $\times 10^1$ CFU/mL. The lowest count was recorded in Hall 3A which recorded 2.33± 0.57 $\times 10^1$ CFU/mL, followed closely by Hall 1B with 2.42±0.82 $\times 10^2$ CFU/mL. In the water samples, Sample B showed the highest bacterial count with 2.67±0.57 $\times 10^3$ CFU/mL and Sample A with 2.33±0.47 $\times$ 10^3 CFU/mL being the lowest. According to the World Health Organization (WHO, 2017), heterotrophic plate counts in potable water should not exceed 100 cfu/mL. The values obtained in this study exceeded that standard significantly, implying that the stored water was bacteriologically unsafe for direct consumption. The elevated THBC values could result from the accumulation of organic materials and sediments that serve as nutrient sources for bacterial growth.

Comparable results were reported by Ogba *et al*. (2021) in the University of Calabar, South–Southern Nigeria, where total heterotrophic counts in institutional water tanks ranged from 1 cfu/mL to 161 cfu/mL and total coliforms from 1 to 92 cfu/mL. Though numerically lower, their study identified similar organisms including *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes,* and *Proteus* spp.—all of which were also observed in this present study. The overlap of these bacterial species highlights a consistent pattern of contamination in institutional storage systems across Nigerian universities. Similarly, Akani *et al*. (2021), studying reservoir tanks in a tertiary institution in Port Harcourt, reported THB values between 1.03×10^3 and 5.89×10^3 cfu/mL, alongside coliform counts up to 10.00×10^2 cfu/mL. They isolated *Citrobacter, Klebsiella, Enterobacter, Pseudomonas,* and *Salmonella* species, aligning closely with the present findings. These consistent results reinforce the notion that institutional water storage systems in Nigeria frequently harbor diverse bacterial populations, including potential pathogens, due to shared environmental and infrastructural challenges.

The total coliform count (TCC) reflects the sanitary condition and potential fecal contamination of water. In the swab samples, the highest coliform count was observed in Hall 4 with $7.60 \pm 0.94 \times 10^1$ cfu/mL, followed by Hall 3B with $4.60 \pm 0.81 \times 10^2$ cfu/mL and Household B with $3.00 \pm 0.94 \times 10^2$ cfu/mL, while the lowest counts occurred in Hall 2B and Hall 2A ($1.00 \pm 0.57 \times 10^2$ cfu/mL and$1.33 \pm 0.47 \times 10^1$ respectively). In the water samples, Sample A showed the highest coliform count with $2.33 \pm 0.94 \times 10^3$ cfu/mL and Sample B being the lowest with $1.67 \pm 0.57 \times 10^3$ cfu/mL. According to the Nigerian Industrial Standard (NIS 554:2007) and WHO (2017) guidelines, coliform bacteria should not be detectable in 100 mL of drinking water. The presence of coliforms in all tested samples suggests contamination of the tanks, possibly from poor cleaning practices, defective tank covers, or contaminated supply lines.

The findings of this study agree with Adeyemi *et al*. (2020), who examined stored borehole water in Nigerian households and reported a wide bacterial spectrum including *Aeromonas* (17%), *Escherichia* (4%), *Staphylococcus* (9%) and *Pseudomonas* (9%). Their results indicate that even domestic storage conditions are prone to contamination by opportunistic bacteria. The similarities suggest that both institutional and domestic stored waters face comparable microbial risks when not properly managed.

Similar observations have been made in other Nigerian universities. Bello *et al*. (2020), in their study on the microbial quality of utility water across four universities in Nigeria, reported that all water samples from 44 storage tanks were bacteriologically unsatisfactory, with total coliform levels above acceptable limits and *Pseudomonas aeruginosa* identified as the predominant isolate. This aligns closely with the present findings, where *Pseudomonas* species featured prominently among the bacterial isolates. Their persistence underscores the ability of these organisms to thrive in water storage systems, particularly under poor sanitary conditions.

Likewise, Ogunde *et al*. (2017), who assessed the physicochemical and bacteriological quality of water supplied to student hostels in another Nigerian university, reported the presence of *Escherichia coli* in concentrations ranging from 2 to 28 CFU/100 mL, with total coliform counts too numerous to count at certain sampling points. The detection of *E. coli* and other coliforms in their work provides further evidence that stored and distributed water in Nigerian universities frequently fails to meet basic microbial safety standards. This is consistent with the current study where *E. coli* and other enteric bacteria were isolated from multiple storage tanks, suggesting possible fecal contamination of the water either through faulty covers, contaminated supply lines, or inadequate maintenance.

Non-lactose fermenting Gram-negative bacteria (NLF-GNB) are environmental organisms often linked to biofilm formation and opportunistic infections. In the swab samples, Hall 3A recorded the highest NLF-GNB count (6.60± 0.58 $\times 10^3$ CFU/mL), followed by Household A (4.70 ± 0.47 $\times 10^4$

CFU/mL) and Household B ($4.31 \pm 0.58 \times 10^3$ CFU/mL). Hall 1B and Hall 4 showed no growth at 10^3 dilution. In the water samples, Sample A shows the highest NLF-GNB count with $2.63 \pm 0.48 \times 10^3$ CFU/mL, and Sample B the lowest with $1.00 \pm 0.58 \times 10^3$ CFU/mL. These organisms—particularly *Pseudomonas aeruginosa* and *Proteus* species—are known to persist in moist environments and resist many disinfectants (Igbinosa *et al*., 2011). The detection of such bacteria corroborates the observations of Akani *et al*. (2021), who also found *Pseudomonas, Citrobacter, Enterobacter* and *Proteus* species dominating water tank samples from Port Harcourt. Their prevalence underscores the ability of Gram-negative bacteria to colonize and survive in biofilms inside institutional water systems.

The biochemical and morphological identification (Tables 4.7 and 4.8) revealed the presence of eight major bacterial species: *Escherichia coli, Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp., *Proteus* sp., *Pseudomonas* sp., *Staphylococcus aureus*, and weakly fermenting *E. coli.* The recurrence of these bacteria across Nigerian studies strengthens their significance as common waterborne and opportunistic pathogens. *E. coli* and *Klebsiella pneumoniae* are classical fecal coliforms and their presence indicates possible fecal intrusion or contamination through poor tank hygiene (Cheesbrough, 2010). *Proteus* and *Pseudomonas* species, which were also found by Ogba *et al*. (2021) and Akani *et al*. (2021), are particularly worrisome due to their environmental persistence and involvement in urinary tract and wound infections (Igbinosa and Okoh, 2009).

The detection of *Staphylococcus aureus*, a skin and mucosal commensal, may result from direct handling of tank outlets or lids during water collection (Prescott *et al*., 2008). These findings, coupled with earlier reports, show that the microbial composition of stored water in institutional and domestic settings across southern Nigeria follows a consistent trend involving a mix of coliforms, opportunistic pathogens, and environmental saprophytes.

The results of this study indicate that the water in the analyzed storage tanks is bacteriologically unsafe for human consumption. The isolation of potential pathogens such as *E. coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* highlights the health risks associated with consuming or using such water for domestic purposes. Prolonged exposure could lead to outbreaks of gastrointestinal infections, urinary tract infections, and skin diseases, particularly among immunocompromised individuals (Ashbolt, 2015).

Given the similarities observed with previous studies across Nigeria (Ogba *et al*., 2021; Akani *et al*., 2021; Adeyemi *et al*., 2020), the problem of water contamination in institutional settings appears systemic. It emphasizes the urgent need for regular tank sanitation, periodic water quality monitoring, and hygiene awareness campaigns to prevent waterborne diseases and safeguard public health.

5.2 Recommendations

Based on the findings of this study, the following recommendations are proposed:

Regular Cleaning and Disinfection of Water Storage Tanks: The storage tanks should be cleaned and disinfected at least once every three months using approved cleaning agents (e.g., chlorine-based disinfectants). This will help to reduce biofilm formation and the buildup of microbial populations on tank surfaces.

Periodic Microbiological Surveillance: Routine microbiological testing of stored water should be conducted by the University's Environmental Health and Microbiology Units to ensure compliance with WHO and Nigerian Standard for Drinking Water Quality (NSDWQ) guidelines.

Installation of Filtration and Disinfection Systems: Incorporating ultraviolet (UV) disinfection or chlorination units at water outlets can help minimize microbial contamination before water reaches users.

Proper Tank Design and Maintenance: Tanks should be made of non-corrosive materials with tightly fitted lids to prevent entry of dust, insects, rodents, and other contaminants. Leakage points should be sealed, and tanks should be positioned to avoid direct exposure to sunlight and environmental pollutants.

Improved Water Handling Practices: Water handlers and maintenance staff should be trained on sanitary handling procedures, while residents should be discouraged from tampering with tank openings or drawing water from unauthorized points.

Public Health Education: Awareness programs should be organized periodically within the university community to educate students on the importance of water hygiene, potential health risks of contaminated water, and safe domestic water storage practices.

Policy Implementation: The University management, in collaboration with relevant environmental health authorities, should develop a sustainable water safety plan that integrates regular inspection, treatment, and microbial risk assessment for all storage facilities on campus.

5.3 Conclusion

This study has demonstrated that water stored in the halls of residence within the University of Benin harbors varying levels of bacterial contamination, indicating that the water is not entirely safe for domestic use without proper treatment. The detection of bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus* species and *Staphylococcus aureus* reflects inadequate sanitation, possible fecal contamination, and poor maintenance of water storage facilities. This findings suggest that contamination may arise from environmental exposure, unclean tanks, improper handling, and deterioration of water quality during storage. These factors pose potential public health risks to students and residents who depend on these tanks for daily use. This study underscores the need for improved hygiene practices in the maintenance of water storage systems,

including regular cleaning, disinfection, and microbial quality monitoring. Implementing effective water safety management practices will help ensure that stored water in the university environment remains safe and fit for human consumption.

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APPENDICE I

3.7. Morphological and biochemical tests of the isolates.

3.7.1. Gram staining

Smears of the bacterial isolates were prepared and heat fixed on a clean grease free slides. The smears were stained for sixty seconds with crystal violet. This was gently washed out with distilled water. The slides were flooded with iodine solution for another sixty seconds. This was washed off distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with running water. The smears were then counter stained with safranin solution for one minute. Finally, the slides were washed off with distilled water, air dried and observed under 100x objective.

3.7.2. Biochemical tests

Catalase Test: This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive

Oxidase Test: A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-pphenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test

Citrate Utilization Test: This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated at 37°C for 24 - 48 hr. The development of deep blue colour after incubation indicates a positive result

Indole Test: Indole test is performed to determine the ability of the organism to split tryptophan molecule into indole. This test is performed to help differentiate species of the family enterobacteriaceae. Kovac's reagent which contains hydrochloric acid, dimethyl-aminobenzaldehyde and amyl alcohol is used. The broth was inoculated with the test organism and incubated for 18 hours at 37°C. 5ml of Kovac's reagent was then added down the inner wall of the tube. Development of bright red colour at the interface of the reagent and the broth within seconds after adding the reagent was indicative of the presence of indole and a positive result.

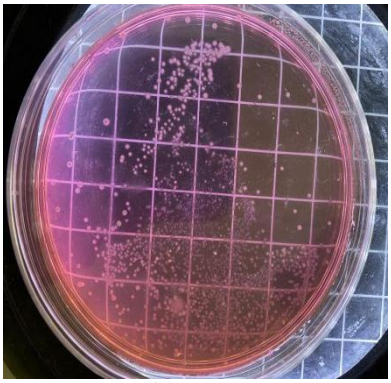
Triple sugar iron (TSI) agar test: An agar slant prepared of a TSI agar was used in carrying out this test in a sterile test tube at a slanted angle. The slanted medium was inoculated with TSA pure culture using a straight inoculation needle by stabbing first through the center to the bottom of the tube and streaking the agar slant's surface. After inoculations, the test tubes were covered with foil paper and left at an ambient temperature of 36°C to incubate for 24 hours. Reactions on test tubes were examined, and sugar fermentations were indicated by the production of H_2S, gas and a change in colours from red (alkaline) to yellow (acid). When an alkaline/acid (red top/yellow bottom) slant reaction appeared, it only indicated dextrose (glucose) fermentation. When an acid/acid (yellow top/yellow bottom) slant reaction appeared, it showed the fermentation of dextrose, lactose and/or sucrose. The appearance of an alkaline/alkaline (red top/red bottom) slant reaction represented the absence of sugar fermentation. The blackening of the medium in the slant indicated H_2S production. Bubbles, cracks, or bottom-raised space in the slanted agar indicated gas production (formation of CO_2 and H_2)

Urease Test: Urease test is a biochemical test done to observe the ability of microorganisms to produce the urease enzyme. This urease enzyme breaks down urea into ammonia and carbon dioxide (NH_4 and CO_2 respectively). The ammonia produced increases the pH of the medium there by making it alkaline. The pH indicator in the medium changes color depending on the medium's pH.

The procedure involves inoculating the test organism into the urea agar slant and incubate at 35-37°C for 24 hrs. Observe for results. It turns yellow if acidic (pH<6.8), Red if medium is neutral (7.0 pH level), and Pink or Fuchsia if alkaline (pH >8.1). two types of medium used are Christensen's Urea Agar which contains urea, phenol red, and peptone, and Stuart's Urea Broth which is a more sensitive for detecting weak urease producers. Urease test is done to identify organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide.

APPENDICE II

Pictures of bacterial isolates using Eosine Methylene Blue agar.



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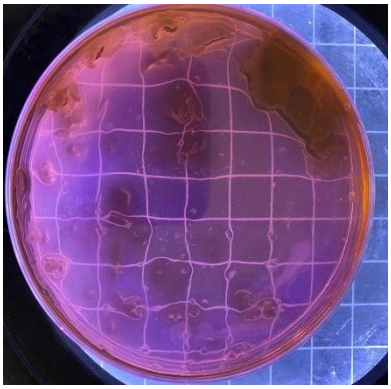

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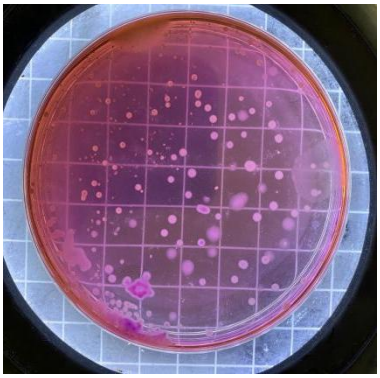

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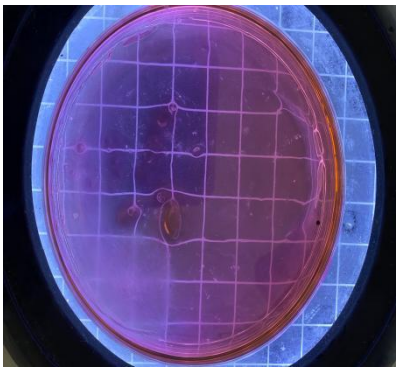

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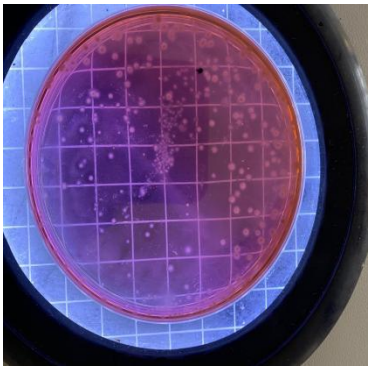

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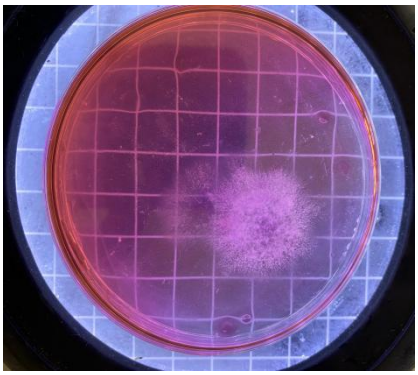

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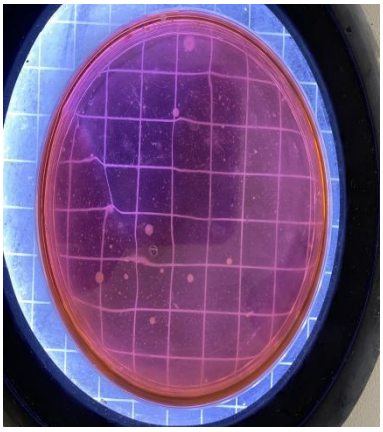

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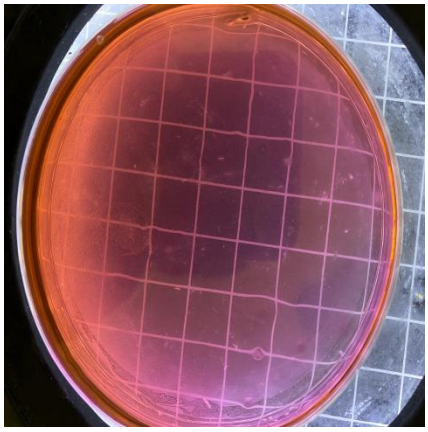

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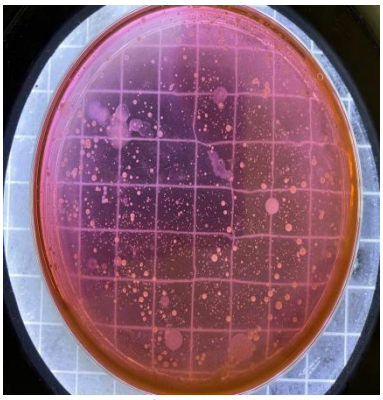

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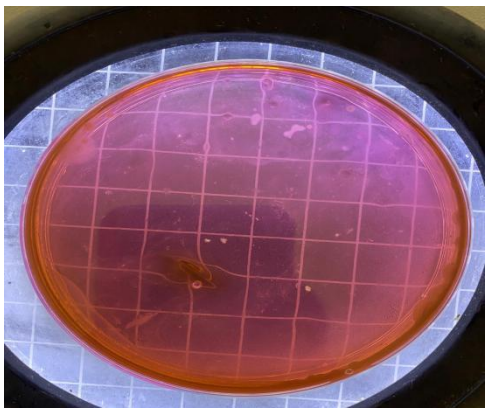

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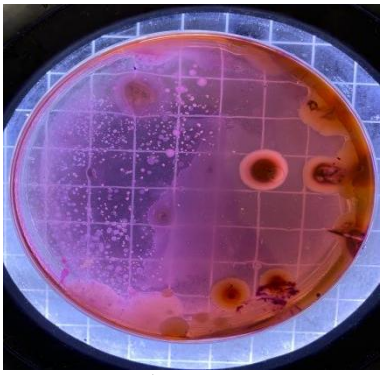

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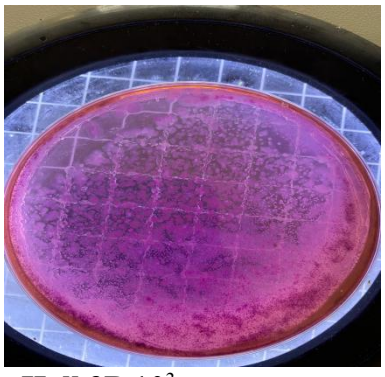

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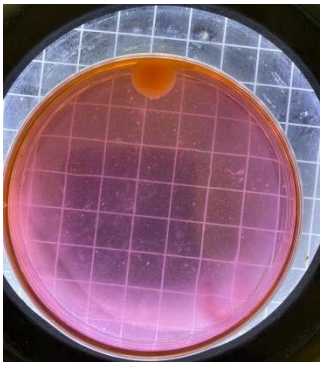

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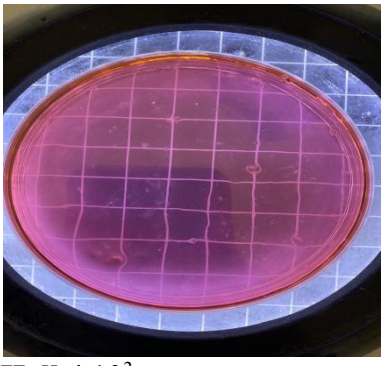

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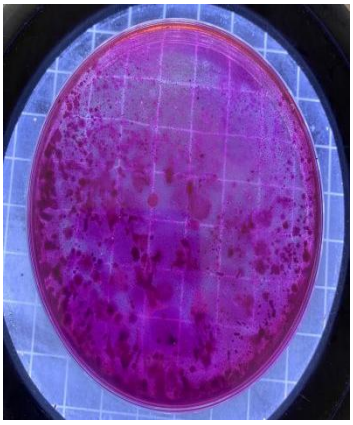

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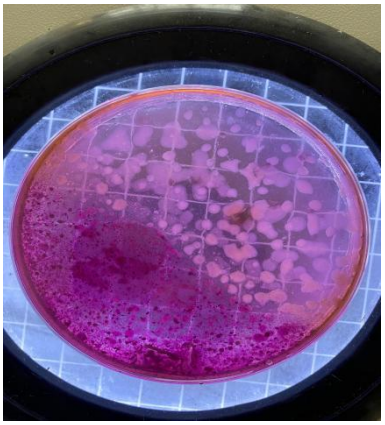

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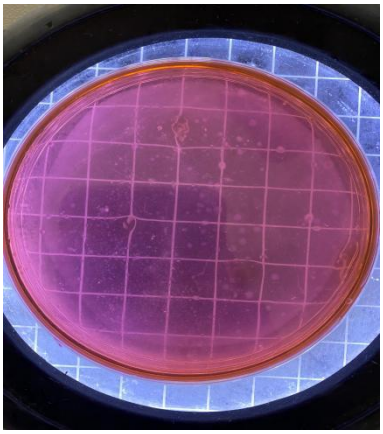

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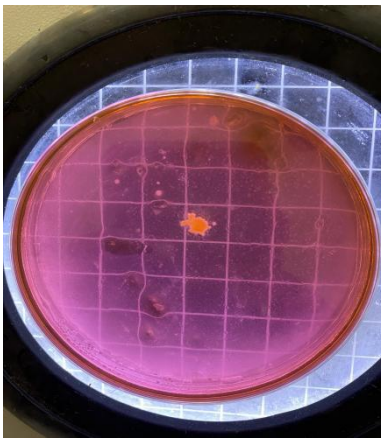

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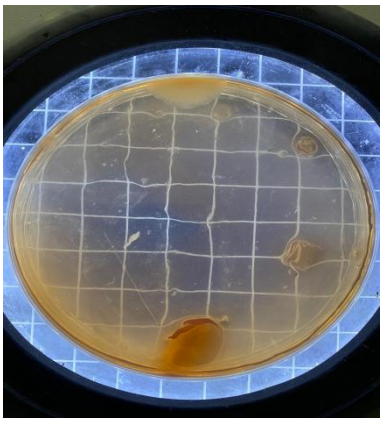
Pictures of bacterial isolates of MacConkey agar.



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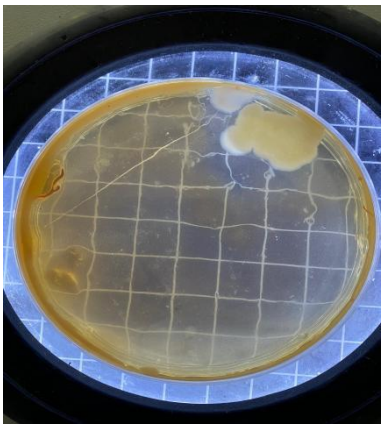

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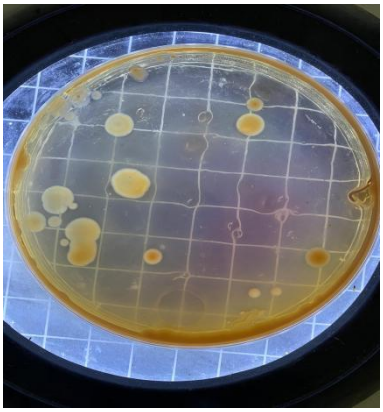

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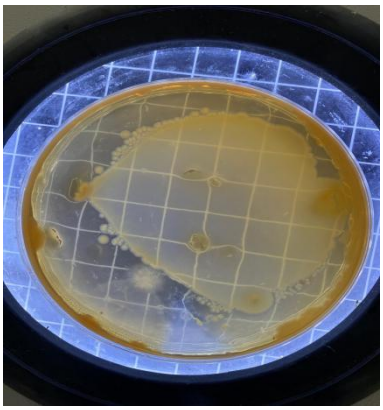

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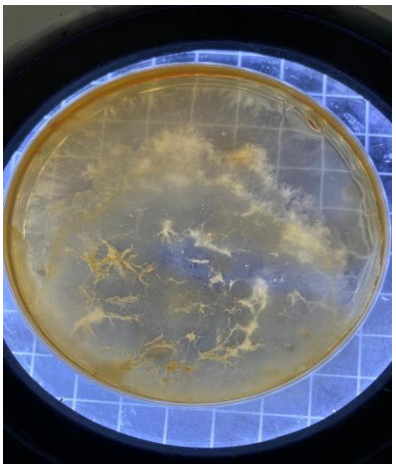

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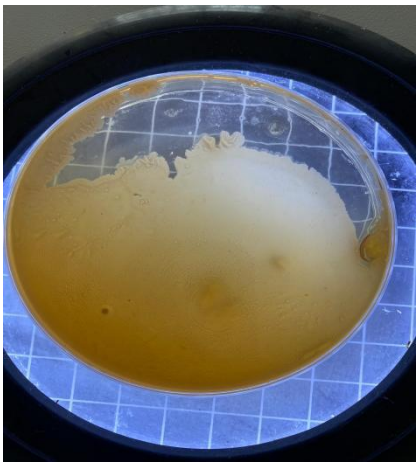

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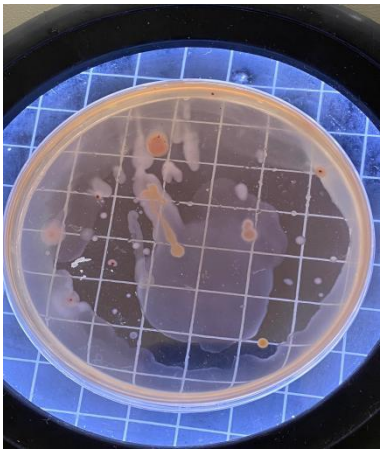

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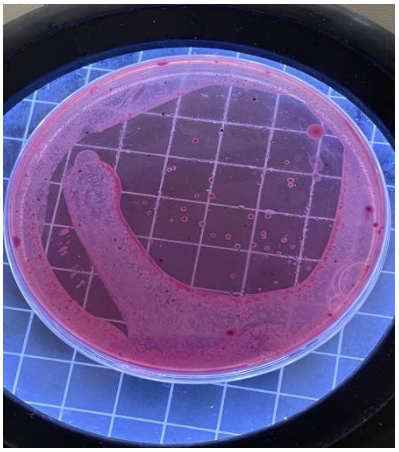

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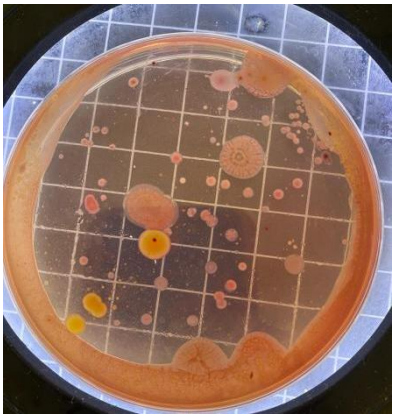

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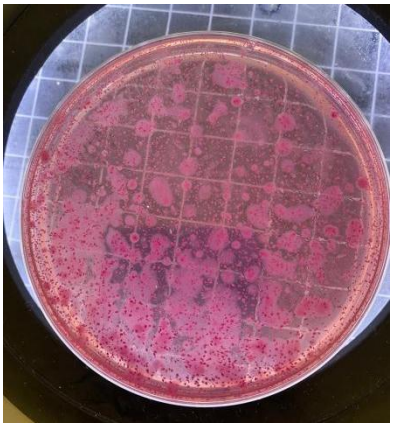

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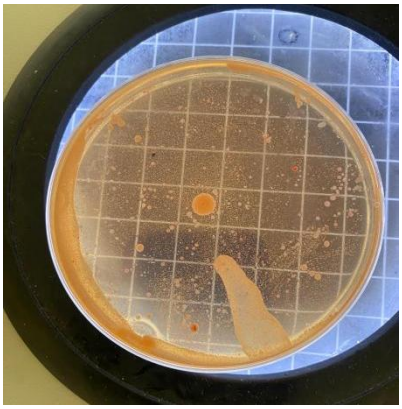

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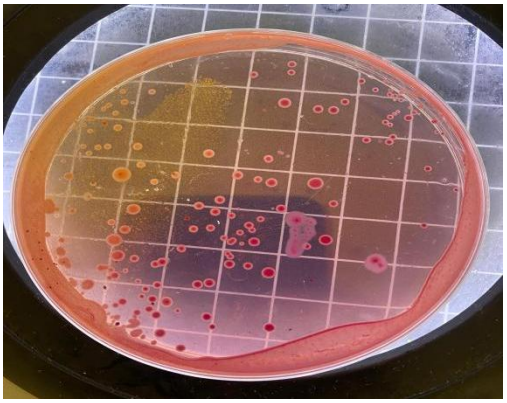

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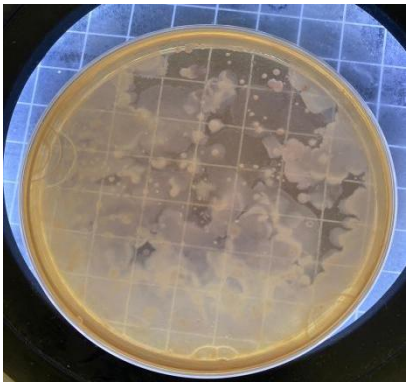

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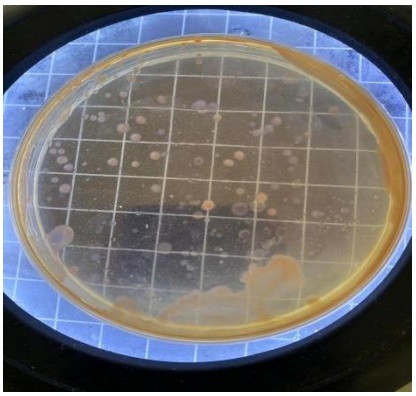

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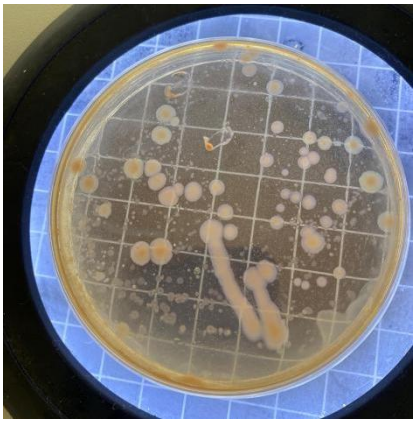

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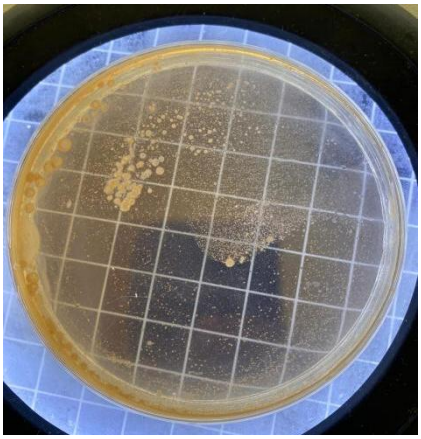

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