BACTERIOLOGICAL ANALYSIS OF SACHET WATER SOLD IN THE UNIVERSITY OF BENIN

BY

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UNIVERSITY OF BENIN
BENIN CITY

NOVEMBER, 2025.

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

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this project work was carried out by Oghenemine Kelly AKPOTOR (Miss) of the Department of Microbiology, Faculty of Life Science, University of Benin, Benin City.

…….. …….

Dr C. U. Ajuzie Date

(Project Supervisor)

APPROVAL

This is to certify that this project work was carried out by Oghenemine Kelly AKPOTOR (Miss) of the Department of Microbiology, Faculty of Life Science, University of Benin, Benin City.

……... ……..

Prof. E. O. Igbinosa Date

(Head of Department)

DEDICATION

This project work is dedicated to the Almighty God for his grace and mercies throughout my period of study.

ACKNOWLEDGEMENTS

My sincere appreciation goes to the Almighty God for his grace and mercies throughout my period of study.

I wish to acknowledge whole heartedly my project supervisor Dr C. U. Ajuzie for his patience and understanding towards me and the success of this project. May God Almighty richly bless you ma for your efforts.

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ABSTRACT

This study evaluated the bacteriological quality of sachet water sold on the University of Benin, Ugbowo campus. Five brands: Uniben Table Water, Faith Mark, Olivia, Uzama, and Notre Dame were sampled in the afternoon (2:00 pm) on campus. The samples were transported to the laboratory for bacterial isolation and identification using standard bacteriological techniques. Phenotypic virulence properties of the isolates were assessed, and antimicrobial sensitivity was determined using the biodisc diffusion method. Uniben Table Water had a bacterial count of 3.2±0.72 x10^4 cfu/mL, Faith Mark 2.0±0.91 x10^4 cfu/mL, Olivia 1.9±0.67 x10^4 cfu/mL, Uzama 3.3±1.51 x10^4 cfu/mL, and Notre Dame 1.1±0.96 x10^4 cfu/mL. The identified bacterial isolates included *Bacillus subtilis, Staphylococcus aureus, Micrococcus letus,* and *Escherichia coli.* Pathogenicity tests indicated that all isolates were pathogenic, testing positive for gelatin liquefaction, DNase, spirit blue, and haemolysin assays. Antibiotic susceptibility testing showed that *Bacillus subtilis, Staphylococcus aureus, Micrococcus letus,* and *Escherichia coli* were sensitive to gentamycin and ciprofloxacin but resistant to pefloxacin, ampiciox, zinnacef, amoxicillin, rocephin, and septrin. The bacterial counts exceeded the limits recommended by the Environmental Protection Agency (EPA) and World Health Organization (WHO) (1.0 × 10^2 cfu/mL). These findings indicate that periodic bacteriological assessment of sachet water quality is necessary, and the National Agency for Food and Drug Administration and Control (NAFDAC) should ensure quality assurance and adherence to internationally defined drinking water standards.

CHAPTER ONE

INTRODUCTION

1.0 Background of Study

The right to safe consuming water is crucial to fitness. It is a primary human right and a issue of effective coverage for fitness safety. The high-quality of ingesting water is a powerful environmental determinant of health. Water is important to sustaining life and it desires to be dealt with that manner. A exceptional supply should to all (Ajayi *et al.,* 2018). Sicknesses associated with contamination of ingesting water represent a major burden on human fitness, interventions to improve the pleasant of drinking water provides sizeable health benefits. Recently, the United nations popular meeting declared the duration from 2005 to 2015 because the worldwide decade for movement, 'Water for life'. The same old industrialized world version for shipping of secure drinking water and sanitation generation is, but, not to be had in maximum of the developing global (Bharath *et al.,* 2023). Safe and transportable water elements in urban centres in Nigeria are still inadequate notwithstanding four a long time of independence and numerous efforts from numerous governments.

Inspite of its potentials for exact groundwater garage, there's incessant water scarcity inside the Nigeria, because the distribution and deliver gadget of handled water are very bad (Dada, 2019). This has made over ninety% of the population reliant on water provision from assets apart from the Zaria water scheme. Many human beings depend upon water carriers for provision of water for domestic and every day needs and this has brought about the arrival of locally sourced low price alternatives sachet water called 'pure water' turning into a chief supply of drinking water. The manufacturing, marketing and intake of sachet water has multiplied exceptionally with

numerous manufacturers being marketed in Nigeria and other developing nations (Dibua *et al.,* 2017). The Nigerian countrywide business enterprise for meals and Drug management and manage (NAFDAC) is remitted to put in force compliance with internationally defined consuming water suggestions, but law of the packaged water enterprise aimed toward proper great warranty has remained a undertaking to the organization.

Water in sachets is easily available and low-priced however there are concerns approximately their purity. The integrity of the hygienic surroundings and the conditions where the majority of the water in sachets are produced has also been wondered (Franco and Cantusio, 2020). Although nationally documented evidence is uncommon, there are claims of beyond outbreaks of water borne ailments that ensued from consumption of polluted water in sachets. An information in their microbiological satisfactory and safety are consequently imperative and have to be a reason of subject to customers, water providers, regulators and public fitness government. Ailment inflicting microorganisms transmitted via drinking water are predominantly of faecal starting place and are called enteric pathogens. The world fitness business enterprise (WHO) estimates that about 1.1 billion people globally drink dangerous water and the massive majority of diarrhoeal disorder inside the world is as a result of risky water, sanitation and hygiene. Bad water, sanitation and hygiene debts for 1.7 million deaths a year international (3.1% of annual deaths) (Obiri –Danso *et al.,* 2023).

The WHO requirements state that drinking water have to no longer comprise any micro-organisms recognized to be pathogenic or any bacteria indicative of faecal pollution. The concept of faecal indicator bacteria in figuring out the sanitary first-class of water became first proposed within the Eighteen Eighties while workers commenced to apply bacteriologic media to evaluate microbial presence in water and meals commodities. Bacterial infection cannot be detected by

using sight, odor or flavor. A primary laboratory take a look at is the fine way to tell if Coliform organisms are present as they can be there and not using a look or flavor distinction (Olayemi, 2019). When water is tested for Faecal or overall Coliform, the results are usually given as the quantity of colony-forming gadgets per one hundred millilitres (CFU/100 ml) of water sampled. No sample should incorporate Faecal Coliform or *E. coli*, and preferably there have to be no overall Coliform.

Coliform bacteria have been used as indicators of unsanitary conditions in water and foods for over a century. The idea of coliform microorganism emerged in 1892 whilst Shardinger proposed using *E. coli* as an indicator of faecal contamination. This turned into primarily based on the premise that *E. coli* is considerable in human and animal faeces and isn't always normally found in different niches, and can be easily detected by means of its ability to ferment lactose (Onifade and Ilori, 2018). It changed into additionally less difficult to isolate than different regarded gastrointestinal pathogens, for this reason the presence of *E. coli* in food or water became ordinary as an 'indicator' of faecal contamination and the feasible presence of other frank pathogens observed in stool like parasites, viruses, bacteria like *Salmonella typhi*, Shigella, etc.

Coliform bacteria commonly belong to four genera of the *Enterobacteriaceae* circle of relatives, *Citrobacter, Enterobacter, Esherichia* and *Klebsiella*. *Escherichia coli* are the most well-known coliform. Coliforms are clean to come across, however their affiliation with faecal infection can be questionable due to the fact a few coliforms are determined obviously in environmental samples (Radhakrishnarr *et al.,* 2023). This led to the introduction of faecal coliforms as a hallmark of infection. Faecal coliform, first defined primarily based on the works of Eijkman are a subset of total coliforms that develop and ferment lactose at accelerated incubation temperature, 44.five-45.5°C and convey acid and gas from lactose within 48 hours. Almost all of the methods

used to hit upon *E. coli*, general coliforms or faecal coliforms are enumeration methods which can be based on lactose fermentation (Chandra *et al.,* 2022). Studies carried out at the microbiological excellent of pure water in Nigeria has shown various tiers of infection. Enforcing compliance via the requlator frame NAFDAC to make sure right great guarantee and the upkeep of the world over defined ingesting water requirements has also remained a mission as many aren't registered or even those which are registered do now not always meet the standards required.

1.2 Aim and Objectives

The aim of the study is to evaluate the bacteriological qualities of sachet water sold within the University of Benin, Ugbowo campus.

The objectives of the study are to:

1. isolate, enumerate and identify the bacteria associated with sachet water sold within the University of Benin, Ugbowo campus.
2. determine the prevalence of bacterial strains present in sachet water sold within the University of Benin, Ugbowo campus.
3. determine the antibiotic susceptibility pattern of the bacterial isolated from the sachet water.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sachet Water

Sachet water may be referred to as equipped to drink packed and device -sealed water. This water is called “pure water” by a few of the locals in Nigeria and other African neighboring nations like Ghana, Togo and so on. Sachet water is likewise sold in hand filled, hand-tied plastic luggage. That is domestically referred to as “ice -water” (Erah *et al.,* 2022). Machine-sealed sachet water this is produced in industries is referred to as manufacturing facility- produced, even as that produced via manually filling plastic luggage with water and knotting the water filled baggage is known as sachet water. The primary supply of the sachet water is water from borehole. Sachet water produced in small scale industries is especially dealt with by aeration, double or unmarried filtration the usage of porcelain molecular candle filter out or membrane filters and in uncommon times, disinfection is implemented (Obi and Okocha, 2017). The level of treatment commonly depends on the source of water. Water to be used for human consumption ought to meet positive necessities. It should be free of all disease inflicting microorganisms, low in concentration of compounds which might be acutely toxic or which have critical long term impact on health.

Ideally, water for consuming should be clear, free of odor and compound that can reason coloration or taste. drinking water from the ground is received by way of drilling boreholes and shallow wells through the present water desk to form a well factor. In sure regions of Africa, because the water percolates thru the soil, harmful physical, organic and chemical elements (e.g.

great suspended count, faecal coliform and fluoride) emerge as contained inside the water making it fallacious for human consumption (Okonkwo *et al.,* 2019). The best of ingesting water has attracted remarkable interest international because of implied public fitness affects. Sachet packaged ingesting water could be very commonplace in Nigeria. It's far often observed as a major source of water at food canteens and sold with the aid of many meals companies within the United States. The majority of the populace consume it, hence the want to ascertain the traits of sachet and borehole water a good way to protect the health of customers. Many common and substantial health dangers had been discovered to be related to drinking water in developing countries, a massive percentage of which can be of organic starting place (Olowe *et al.,* 2025). Unsafe water, bad sanitation and poor hygiene were stated to rank third some of the 20 main threat factors for health burden in growing international locations along with Nigeria. The pointers for great ingesting water are meant for use by using countries as a foundation for the improvement of national requirements which if well applied will ensure the safety of drinking water.

Sachet water has received reputation and ha s been embraced through the population due to the fact it's far reasonably-priced, geared up to drink and continually available (Rajini *et al.,* 2020). Water is indispensible to existence as it is required for all physiological strategies which demand that all residing organisms have equipped get entry to to water. This need is no less crucial to human beings who have to drink plenty of water every day. Apart from consuming, man uses water for lots domestic, business and leisure purposes which consist of washing, bathing, cooking, meals processing, brewing and beverage bottling in addition to carrying activities. Because of this there may be a need for the steady deliver of potable water to all human communities and in areas where in such components are lacking, a top notch deal of time and

effort are dedicated to locating a suitable supply of supply (Trivede *et al.,* 2020). Regrettably, such water resources even when they're available, are seldom safe or dependable and waters received there from want to be treated as it should be as a way to lead them to potable.

In all city regions inside the evolved countries, reliance is positioned on the supply of competently handled water by municipal government. In developing international locations however there's very little get right of entry to such dealt with water and so, potable water is normally difficult or maybe impossible to get (Vaishali and Punita, 2023). This is because very little cash has been made to be had for the proper municipal infrastructure and this being so, a huge percentage of humans in these nations need to rely upon their own person efforts to get the water which they want. one of the approach of satisfying the want for potable water especially in urban groups is to devour packaged water which in Nigeria is offered in plastic sachets known as pure water or in plastic bottles. Bottled water is consuming water which has been packaged in plastic bottles ranging in length from small unmarried serving polyethylene terephthalate bottles of 500 ml −1.5 L capability to huge carboys (20 L) for water coolers (Seth *et al.,* 2024). In Nigeria, bottled water is regarded as being more secure than water dispensed and sold in sachets, however it is also about ten instances extra high priced that is why it's miles patronized specially through people with a rather massive disposable profits.

Apart from microbiological issues, the upsurge within the call for bottled water has brought about the hobby of many producers within the manufacturing of bottled water. Near decades in the past, bottled water turned into a made of some multinational and huge scale meals processing and beverage generating companies in Nigeria (Thivya *et al.,* 2024). currently but, there may be the involvement of very many water bottling companies ranging from huge scale multinational groups to medium scale enterprise establishments, institutional and government enterprise

investment agencies as well as small scale marketers. These water bottling agencies use diverse water purification techniques which can be one of or aggregate of two of filtration, ozonisation, extremely violet irradiation and chlorination.

Bottled water has been mentioned to be related to outbreaks of infections within the last few years. In 2006, *Salmonella enterica* serovar Kottbus from bottled water become extensively associated with 41 instances in a virulent disease in infants in Gran Canaria (Moyo, 2023). Nineteen of the instances had underlying disorder or were immuno-compromised. The organism turned into remoted from bottled water randomly decided on from the markets and within the local factory in which the water was bottled. Govindarajan and Senthilnathan (2024) have defined a pandemic of health center-received *P. aeruginosa* infection caused by contaminated bottled water in extensive care devices in a sanatorium in Germany. In the health center, the bottled water becomes used for the guidance of orally administered medicinal drugs and oral fluid alternative. some unopened bottles of water were found to include the outbreak stress of *P. aeruginosa*. instances which include these underscore the want for persistent surveillance or monitoring of great of bottled water. studies at the pleasant of bottled water in many parts of the arena together with Canada, South Africa, Iran, Egypt and Nigeria have shown that bottled water samples aren't continually of the required microbiological great (Bello *et al.,* 2023).

2.2 Drinking Water and Microbes

Coliform microorganism are not often pathogenic, however there are a few exceptions. Sure strains of *E. coli* were related to gastrointestinal infections in adults, called vacationer's diarrhea or Montezuma's revenge, urinary tract infections, and new child meningitis. Sure strains of *Klebsiella pneumoniae* had been associated with gastrointestinal infections, pneumonia, medical

institution-acquired urinary tract infections, burn wound infections, or as secondary invaders in different breathing infections (Jain *et al.,* 2020). Enterobacter has been related to sanatorium received urinary tract infections. Citrobacter has been associated with health center-obtained urinary tract infections, superficial wound infections, osteomyelitis, neonatal meningitis, and gastroenteritis (Wose-Kinge *et al.,* 2020). The principle micro organism pathogens that have been shown to cause human intestinal sickness related to drinking water are: *Salmonella typhi*, Typhoid fever; *Salmonella paratyph*i-A, paratyphoid fever; different *Salmonella* species, salmonellosis, enteric fever; *Shigella dysenteriae*, *S. Flexneri*, and *S. sonnei*, bacillary dysentery; Vibrio cholerae, cholera; *Leptospira* sp., leptospirosis; *Yersinia enterocolitica*, gastroenteritis; *Francisella tularensis*, tularemia; *Escherichia coli*, gastroenteritis; and *Pseudomonas aeruginosa*, diverse infections (Ateba and Maribeng, 2021). The infecting dose varies with the pressure in addition to the age and trendy health of the host. Babies, the elderly and the immunocompromised can be especially inclined.

2.3 Viral Pathogens

The major viral pathogens that can be transmitted by way of consuming water from sewage or wastewater are the enteric viruses (acid stable picornaviruses), reoviruses, adenoviruses, and hepatitis A. humans infected by means of those ingested viruses do not always emerge as ill, however a ailment involving the gastrointestinal system, principal nervous machine, pores and skin, and coronary heart is feasible (Kamanula *et al.,* 2024).

2.4 Protozoa Pathogens

The principle protozoa pathogens that can be transmitted by means of drinking water from sewage or wastewater are *Entamoeba histolytica, Giardia lamblia*, and *Cryptosporidium*.

Entamoeba histolytica is substantially extra time-honored in tropical and subtropical regions. *Cryptosporidium* and *G. lamblia* are most frequently visible in rural and decrease socioeconomic areas, but these protozoa may be located in ingesting water that has been infected with the aid of sewage (Aydin, 2017). Human beings inflamed by means of these protozoa may not have any signs. Symptomatic sufferers might also complain of stomach cramps and diarrhea.

2.5 Intestinal Worms

The precept intestinal worms that are transmitted in ingesting water include *Ascaris lumbricoides*, the round bug; *Trichuris trichiura*, the whipworm; *Ancylostoma duodenale* and *Necator americanus*, the hookworms; and *Strongyloides stercoralis*, the threadworm. It's far not going that the transmission of helminthic infections into consuming water is good sized (Ballester and Sunyer, 2020).

Chemical disinfection making use of a few form of chlorine stays the selection for disinfecting private wells. Chlorine has been shown to be an powerful disinfectant for bacteria and viruses, however fashionable knowledge of the vulnerability of protozoa and helminths to chlorine is incomplete. It's miles recognised that protozoa and helminths are greater proof against chlorine than bacteria. The presence of coliforms or *E. coli* could warrant chlorination because of the ability chance for contamination (Bezuidenhout *et al.,* 2022). The modern-day NC groundwater nice preferred for the coliform organisms is 1 in line with a hundred milliliters (15NCAC 2L.0202), and a brand new fashionable is proposed at 0. The EPA maximum contaminant aim (MCGL) for Cryptosporidium, Giardia lamblia, viruses, and total coliforms is zero mg/L.

There's a set of nuisance organisms together detailed as iron, sulfur micro organism, and sulfate-decreasing micro organism that may be observed in consuming water. These bacteria aren't

pathogenic and are evidently found in the soil (Ikeme *et al.,* 2024). Those nuisance microorganisms are responsible for numerous changes of iron normally in the form of slime and often purpose a terrible odor and/or taste in the water. Further, iron bacteria may additionally purpose plugging of well and a reddish tinge to the water. Sulfur and sulfate reducing micro organism may additionally cause rusty water and corrosion of pipes.

2.6 Iron Bacteria

Iron micro organism are maximum in all likelihood to appear in water with surprisingly excessive iron concentrations (10 to 30 mg/L), but may expand in water with low iron concentrations (0.1 to zero.3 mg/L) if the delivery of iron is constantly renewed (Chidnma *et al.,* 2016). Iron microorganism oxidizes ferrous iron to ferric iron and purpose the precipitation of ferric hydrate. The precipitation of ferric hydrate reasons a nasty scent and taste and reddish tinge to the water.

2.7 Sulfur Bacteria

Sulfur bacteria oxidize sulfur to sulfate at the same time as sulfate-reducing bacteria lessen sulfate to hydrogen sulfide. Those merchandise motive a bad odor and taste to the water. Sulfur bacteria may additionally convey massive quantities of iron into answer underneath conditions favorable for its improvement (Adogo *et al.,* 2016). The hydrogen sulfide made from the sulfate-reducing bacteria reacts with dissolved iron to shape black insoluble iron sulfide. Chlorination is used to manipulate those nuisance organisms in addition to lowering the iron, sulfide, and/or sulfur content material for the water.

2.8 Health Risk Associated with Contaminated Water

Water-borne illnesses (i.e., diarrhea, gastrointestinal contamination) due to diverse bacteria, viruses, and protozoa were the reasons of many outbreaks. In growing international locations, along with those in Africa, water-borne illnesses infect thousands and thousands. In keeping with world health business enterprise, every yr 3.4 million humans, in the main children, die from water-associated sicknesses (Ehiowemwenguan *et al.,* 2024). In keeping with United International locations kids's Fund (UNICEF) assessment, 4000 youngsters die every day because of contaminated water. WHO reports that over 2.6 billion humans lack get right of entry to to clean water, that's responsible for approximately 2.2 million deaths annually, of which 1.four million are in kids. improving water exceptional can lessen the global ailment burden by way of approximately 4 %.

Despite the fact that water-associated sicknesses in developing international locations are frequent, they're also a extreme assignment in advanced countries, who compiled records of outbreaks in the U.S. (1986 – 2000), mentioned 5,905 cases and ninety five outbreaks associated with recreational water. Gastrointestinal illness (GI) caused by range of various microbes and germs, which causes symptoms, such as diarrhea, nausea, vomiting, fever, abdominal ache, changed into liable for about 29.53% instances (Ajayi *et al.,* 2018). More than 27% of instances had been as a result of *Shigella* spp. similarly, 10.99%, 10.08%, and 6.59% of the cases were because of Cryptosporidium parvum, Adenovirus three, and Leptospira, respectively. Almost 23% and 21% of the outbreaks had been due to GI and Shigella spp, respectively. Similarly, 16.84 %, 12.63%, and 7.37% of the outbreaks had been due to *Naegleria fowleri*, *E. coli* 0157:H7, and *Schistosoma* spp., respectively. Except acute gastroenteritis, major etiological dealers inclusive of Giarida, *Cryptosporadium*, *E. coli* 0157:H7, V. cholera, and *Salmonella* were

the sellers accountable for many outbreaks (Bharath *et al.,* 2023). all through the same length 437,082 cases and 48 outbreaks have been due to infected drinking water, of which approximately ninety five.89% of the cases have been due to *Cryptosporidium parvum*. Nearly 42% and 31% of the outbreaks have been as a result of Giardia lamblia and GI, respectively. Reporting data on water-borne outbreaks within the U.S., Dada (2023) found that at least 1870 outbreaks (23 according to year) passed off among 1920 and 2002. Those pronounced outbreaks and their mentioned prevalence of ailments are possibly to be a sarcasm of real numbers because of nonreported instances and lacking publicity information. To defend public health, the U.S. EPA's country wide number one consuming Water rules (NPDWRs) contain requirements describing the maximum Contaminant degree (MCL) the very best stage of a contaminate allowable in ingesting water (Dibua *et al.,* 2017). The U.S. EPA has defined the MCL of various microorganisms, along with *Cryptosporidium, Giardia lamblia, Legionella*, and total Coliforms (together with fecal coliform and E. coli), and viruses. The maximum Contaminant degree intention (MCLG) the extent of a contaminant in ingesting water below which there is no recognized danger to public fitness has also been proposed with the aid of the U.S. EPA. The MCLC ranges for *Cryptosporidium, Giardia lamblia, Legionella*, and overall Coliforms are zero. The EPA calls for ninety nine% removal of Cryptosporidium in drinking water, and the elimination possibilities of *Giardia lamblia* and viruses are ninety nine (99.99%), respectively (Franco and Cantusio, 2020). Although there's no restriction for Legionella, EPA believes that if *Giardia lamblia* and viruses are eliminated/inactivated, then drinking water possibly to be freed from *Legionella*. The U.S. EPA calls for routine sampling of consuming water for checking out total coliform and E. coli, and if a routine sample is tremendous, then repeat samples are required. If, in any repeat sample, total coliform or *E. coli* is detected then the ingesting water has an acute

MCL violation. For a drinking water device that collects fewer than 40 recurring samples in line with month, no multiple sample may be overall coliform-effective in keeping with month. For a system that collects greater than 40 routine samples, no more than five% of samples total coliform-tremendous in a month is authorized (Obiri –Danso *et al.,* 2023).

Each 12 months about 42,000 cases of salmonellosis are mentioned within the U.S. *Schistosomiasis* isn't always mentioned in the U.S. as it is not endemic; but, 2 hundred million humans are infected global. In 2011, about 1,060 instances of Guinea computer virus disease, because of the parasite *Dracunculus medinensis*, have been suggested in lots of faraway components of Africa that don't have secure ingesting water (Olayemi, 2019). Malaria, a protozoal sickness of the Genus Plasmodium transmitted by mosquitos breeding in contaminated water, impacts three hundred–500 million humans, and causes over 1,000,000 deaths each year (greater than 90% of deaths in Africa). typical the morbidity and mortality because of contaminated water are huge and want to be managed by using enhancing the safety of secure water (i.e., recreational as well as drinking water) in each developing and evolved countries (Chandra *et al.,* 2022).

CHAPTER THREE

MATERIALS AND METHODS

2.1 Collection of Samples

Five (5) different brands of sachet water (Uniben Table Water, Faith Mark, Olivia, Uzama and Notre Dame) were brought roundly in the afternoon (2:00pm) within the University of Benin, Ugbowo campus. The sachet water samples were taken to the laboratory for bacteria isolation and identification.

2.2 Preparation of Culture Media

All media were prepared according to manufacturers instruction. The media used in this study include nutrient agar.

2.2.1 Nutrient Agar

Twenty-eight grammes (28 g) of nutrient powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminium foil paper. It was mixed thoroughly and sterilized by autoclaving at 121 ^{o}C for 15 min. The medium was cooled to 45 ^{o} - 50 ^{o}C and then dispensed aseptically into steril Petri dishes.

2.3 Enumeration of Microorganisms

Serial dilutions from the resulting growth from the nutrient broth medium were pour-plated on Nutrient agar for isolation of bacteria. The nutrient agar plates were incubated for 24 h at 37 °C under aerobic condition. Discrete bacteria colonies were sub-cultured onto nutrient agar plates and incubated at 37 °C for 24 h.

2.4 Identification of Organisms

Pure isolated colonies were differentiated using Gram's staining technique followed by biochemical identification using indole, catalase, citrate, oxidase, coagulase, and urease test. Isolates were identified and named based on the cultural, morphological, and the biochemical characteristics following.

2.5 Morphological Test

2.5.1 Gram Staining

Smears of the isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Gram's iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95 % alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with saffranin solution for one minute. Finally, the slides were washed off with distilled water, air dried and observed under oil immersion objective.

2.5.2 Motility Test

Selected colonies were inoculated into the mobility medium with a straight sterilized needle; the needle was used to stab about one-half of the length of the medium. The tubes were incubated at 37 ^{o}C for 18 - 24 h with their caps loosened. Fuzzy growth away from the line of inoculation denotes motility of the organism.

2.6 Biochemical Test

2.6.1 Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns 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 positive catalase enzyme.

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

2.6.2 Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1 %) solution of oxidase reagent (tetramethyl-phenylenediamine- 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.

2.6.3 Coagulase Test

Coagulase is a protein enzyme produced by microorganisms, among them is *S. aureus.* The enzyme protease converts fibrinogen to fibrin resulting to blood clotting. The Slide method was used. In slide test, clean slide was divided into two sections, to one section of the slide the test organism was smeared on it using a sterile wire loop while a drop of distilled water was added to the other section which serves as control. Then human plasma was added to both sections and the slide was rock gently for some minutes. A clumping/agglutination of the plasma was used to indicate the presence of coagulase.

2.6.4 Urease test

The bacterial isolates were inoculated into slants of urea medium and incubated at 37 ^{o}C for 24-48 h. Urease positive cultures produced a red-pink colour due to changes in the colour of the indicator.

$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$

2.6.5 Indole Test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in buffered peptone water. The test is usually used as an aid in the differentiation of Gram negative bacilli especially those of the enterobacteriaceae. Peptone water was prepared and about 3 ml of it was dispensed in Bijou tubes using a sterile pipette. Then, fresh sterile loops were used to pick a well-isolated colony of bacteria and inoculated into bijou tubes, thereafter, the tubes were incubated at 37 ºC for 48 h. After incubation period, 0.5 ml of Kovac's Indole Reagent was added to the inoculated bijou tubes. The tubes were subjected to gentle shaking and examined for red colour on the surface layer within 10 min. A red ring on top of the tube indicated indole positive reaction.

2.6.5 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 ^{o}C for 24 - 48 h. The development of deep blue colour after incubation indicates a positive result.

2.6.6 Sugar Fermentation Test

Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. Since most bacteria especially Gram negative bacteria utilize different sugars as source of carbon and energy with the production of either acid and gas or acid only, the test is used as an aid in their differentiation. The growth medium used was peptone water and the peptone water was prepared in a conical flask and the indicators; phenol red was added. The mixture was dispensed into test tubes containing Durhams tubes. The tubes with their content were sterilized by autoclaving at 12 ^{o}C for 15 min. 1 % solution of the sugar was prepared and sterilized separately at 115 ^{o}C for 10 min. This was then asceptically dispensed in 5 ml volume into the tubes containing the peptone water and indicator. The tubes were inoculated with young culture of the isolates and incubated at 37 ^{o}C. Acid and gas production or acid only were observed after about 24 h of incubation. Acid production was indicated by the change of the medium from light green to yellow colour while gas production was indicated by the presence of gas in the Durham's tubes.

2.7 Pathogenicity Testing

Pathogenicity testing was done to observe the disease causing ability of the organisms. These tests included: Gelatin liquefaction test, DNase test, Spirit blue test and Haemolysin test.

2.7.1 Gelatin Liquefaction Test

The gelatin liquefaction/hydrolysis test is used to detect the ability of bacteria to produce gelatinase. The production of gelatinase is used as a presumptive test for the various organisms including; *Staphylococcus* species, Enterobactericeae, and some gram positive bacilli. Six test tubes were washed and autoclaved. The gelatin agar was prepared by following the

manufacturers instructions accurately after which it were poured into the test tubes and allowed to cool. Colonies from the samples were inoculated into the test tubes accordingly and Incubated for 24 hr at 37 ^{0}C. After which the test tubes were removed and placed in ice block for 30 minutes. The solidification of the contents of the test tubes indicated a negative result to the gelatin test while failure of the test tubes contents to solidify indicated a positive test.

2.7.2 DNase Test

DNase Test Agar w/Methyl Green is a solid medium used to detect the deoxyribonuclease (DNase) activity in microorganisms. DNase test agar was prepared and poured into petri dishes and allowed to solidify. Colonies from the samples was streaked onto the agar in the various plates and Incubated at 37 ^{0}C for 24 hr. Unchanging color of the green colonies gave a negative result while changing of the green color to blue indicated a positive result to the test.

2.7.3 Spirit Blue Test

Spirit blue agar was prepared according to the manufacturers instructions. It was cooled and 1ml of sterile olive oil were added to the agar and poured into petri dishes in a sterile environment. Colonies from the samples were streaked onto the plates and inoculated at 37 ^{0}C for 24 hr. Becteria that produced lipase hydrolyzed the olive oil and produced a ‘halo' around the zone of growth indicated a positive result while the colonies that didn’t produce a halo around the region of streaking indicated negative results. This test is used to identify organisms that are capable of producing lipase.

2.7.4 Haemolysin Test

The hemolysin agar was prepared by adding 1ml of blood to nutrient agar after which it was cooled and distributed into the appropriate sterile petri dishes. Colonies from the samples was inoculated onto the different plates and Incubated at 37 ^{0}C for 24 hr after which results was taken. If the medium is discolored or darkened after growth, the organism has demonstrated alpha-hemolysis. If the medium has been cleared under growth, the organism is beta-hemolytic. No discernible change in the color of the medium constitutes gamma-hemolysis.

2.8 Antibiotic Susceptibility Testing

The agar diffusion technique as described by Bauer *et al.* (1996) was used. Using a sterile loop, an inoculum was picked from the surface of each selected colony and transferred into a sterile test tube containing 5 ml of nutrient broth and incubated at 37 ºC for 24 h. Thereafter, bacterial suspension was streaked on the surface of Muller Hinton Agar. The appropriate multi-disc depending on whether the test organism plated was a Gram-negative or Gram-positive organism was then placed firmly onto the surface of the dried plates, using sterile forceps. The plates were left at room temperature for one hour to allow diffusion of the different antibiotics from the disc into the medium. The plates were then incubated at 37 °C for 18 - 24 h. Interpretation of results was done using the zone sizes. Zone of inhibition of greater than 10 mm were considered sensitive, 5 - 10 mm moderate sensitive and no zone of inhibition resistant.

2.9 Data Analysis

Analysis of variance (ANOVA) and Dunnet's method were employed for data evaluation; $p<0.05$ was taken as statistically significant. The software package, Graph pad prism 5 was used for data analysis.

CHAPTER FOUR

RESULTS

Table 1 shows the bacterial count of different brands of sachet water samples within the University of Benin, Ugbowo campus. Uniben table water had a count of 3.2±0.72 x10^4 cfu/mL, Faith mark (2.0±0.91 x10^4 cfu/mL), Olivia (1.9±0.67 x10^4 cfu/mL), Uzama (3.3±1.51 x10^4 cfu/mL) and Notre dame (1.1±0.96 x10^4 cfu/mL). However Uzama sachet water had the highest bacterial count, while Notre dame had the lowest.

Table 2 shows the cultural, morphological and biochemical characteristics of the bacteria isolates from different brands of sachet water samples within the University of Benin, Ugbowo campus. The identified bacteria isolates were *Bacillus subtilis, Staphylococcus aureus, Micrococcus letus* and *Escherichia coli* respectively.

Figure 1 show frequency of distribution of bacterial isolates from different brands of sachet water samples within the University of Benin, Ugbowo campus. *Staphylococcus aureus* had the highest frequency of 5, *Bacillus subtilis* (4), Micrococcus letus (4), while Escherichia coli had the lowest of 3.

Table 3 shows the phenotypic virulence determinants of bacteria isolates from different brands of sachet water samples within the University of Benin, Ugbowo campus. The pathogenicity test proved that all isolates were pathogenic as they tested positive to Gelatin liquefaction test, DNase test, Spirit blue test and Haemolysin test.

Table 3 shows the antibiotics susceptibility pattern of bacteria isolates from different brands of sachet water samples within the University of Benin, Ugbowo campus. Bacteria isolates such as *Bacillus subtilis, Staphylococcus aureus, Micrococcus letus* and *Escherichia coli* were sensitive

gentamycin and ciprofloxacin and resistant to Pefloxacin, Ampiciox, Zinnacef, Amoxicillin, Rocephin and Septrin.

Figure 2 shows the multi antibiotic resistance index (MARI) value of bacteria isolates different brands of sachet water samples within the University of Benin, Ugbowo campus. *Staphylococcus aureus* had highest value of 0.7, *Bacillus subtilis* and *Escherichia coli* has 0.6 each, while *Micrococcus letus* had the lowest value of 0.4.

Table 1: Bacterial count of different brands of sachet water samples within the University of Benin, Ugbowo campus.

Sachet Water Samples	**Bacterial count x10^4 cfu/mL**
Uniben Table Water	3.2±0.72
Faith Mark	2.0±0.91
Olivia	1.9±0.67
Uzama	3.3±1.51
Notre Dame	1.1±0.96

Values are presented as mean ± SEM; n=3.

Table 2: Cultural, morphological and biochemical characteristics of the bacteria isolates from different brands of sachet water samples within the University of Benin, Ugbowo campus.

Characteristics	**1**	**2**	**3**	**4**
Cultural				
Elevation	Flat	Convex	Convex	Low convex
Margin	Entire	Entire	Smooth	Entire
Colour	Cream	Yellow	White	Cream
Shape	Circular	Circular	Circular	Circular
Size	Large	Medium	Small	Medium
Morphological				
Gram staining	+	+	+	-
Cell type	Rod	Cocci	Cocci	Rod
Cell arrangement	Chains	Cluster	Chains	Single
Spore staining	+	-	-	-
Biochemical				
Catalase	+	+	-	+
Oxidase	-	-	-	+
Coagulase	+	+	-	-
Citrate	+	+	+	+
Urease	+	+	+	-
Indole	-	-	-	-
Glucose	+	+	+	+
Isolates	*Bacillus subtilis*	*Staphylococcus aureus*	*Micrococcus letus*	*Escherichia coli*

Key: + positive, - negative

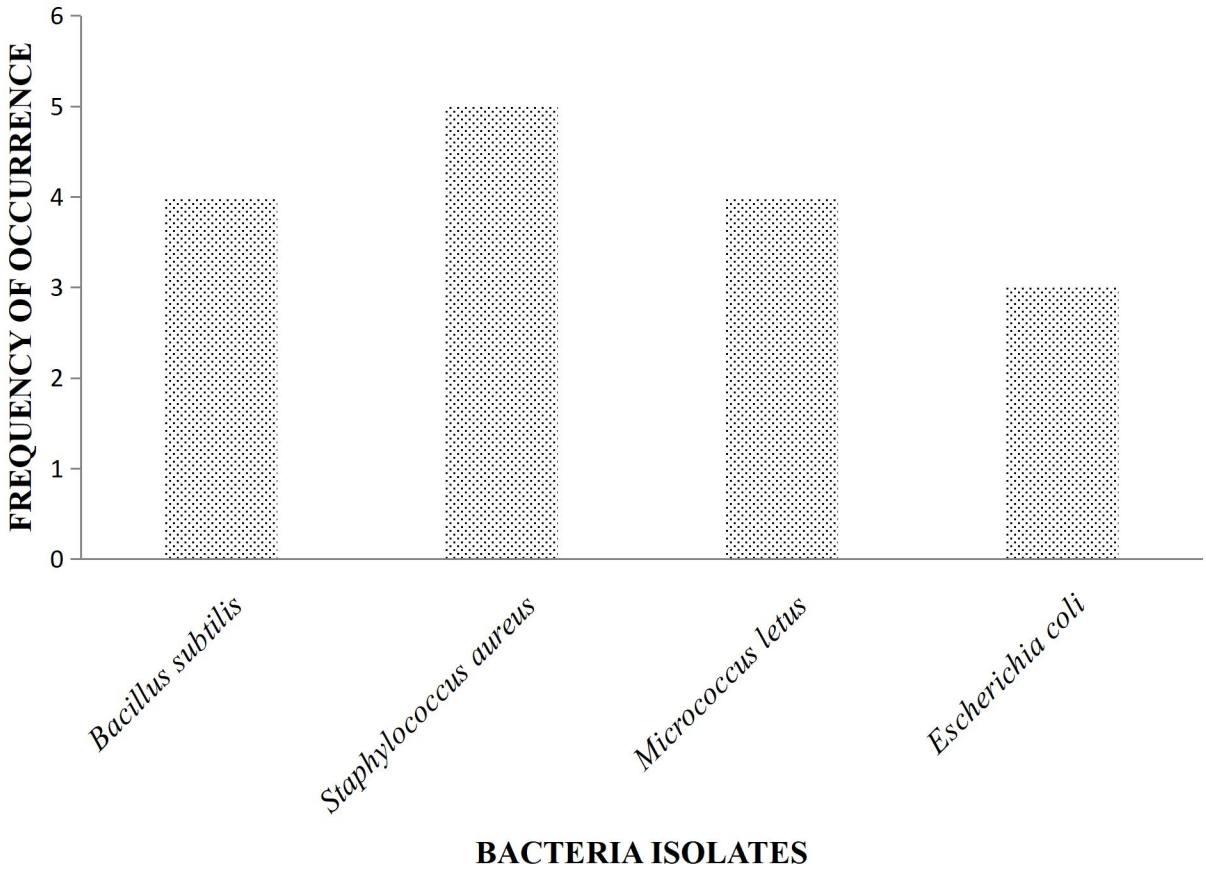

Figure 1: Frequency of distribution of bacterial isolates from different brands of sachet water samples within the University of Benin, Ugbowo campus.

Table 3: Phenotypic virulence determinants of bacteria isolates from different brands of sachet water samples within the University of Benin, Ugbowo campus

Isolates	**Hemolysin**	**DNAse**	**Gelatinase**	**Lipase**
Staphylococcus aureus	β 0(0)	5(100)	5(100)	5(100)
Escherichia coli	β 3(100)	3(100)	0(0)	3(100)
Micrococcus letus	β 14(100)	4(100)	0(0)	4(100)
Bacillus subtilis	β 4(100)	0(0)	4(100)	4(100)

Table 4: Antibiotics susceptibility pattern of bacteria isolates from different brands of sachet water samples within the University of Benin, Ugbowo campus.

	Antibiotic zone of inhibition (mm)									
Bacterial isolates	**PEF**	**CN**	**APX**	**Z**	**AM**	**R**	**CPX**	**S**	**SXT**	**E**
Bacillus subtilis	25 (S)	20 (S)	12 (R)	10 (R)	10 (R)	10 (R)	23 (S)	16 (R)	18(R)	19(S)
Staphylococcus aureus	10 (R)	21 (R)	10 (R)	10 (R)	10 (R)	10 (R)	20 (S)	11 (R)	18 (R)	20(S)
Micrococcus letus	24 (S)	22 (R)	10 (R)	10 (R)	10 (R)	10 (R)	20 (S)	20 (S)	20 (S)	20 (S)
Escherichia coli	23 (S)	20 (R)	12 (R)	10 (R)	10 (R)	10 (R)	20 (S)	20 (S)	18 (R)	20(S)

Key:-

Antibiotic	**Disc code**	**Resistant < or = (mm)**	**Intermediate (mm)**	**Susceptible > or = (mm)**
Pefloxacin	PEF	14	15-19	20
Gentamycin	CN	14	15-19	20
Ampiciox	APX	12	13-17	18
Zinnacef	Z	15	16-20	21
Amoxicillin	AM	12	13-18	19
Rocephin	R	13	14-19	20
Ciprofloxacin	CPX	14	15-18	19
Streptomycin	S	11	12-18	19
Septrin	SXT	13	12-19	20
Erythromycin	E	13	12-18	19

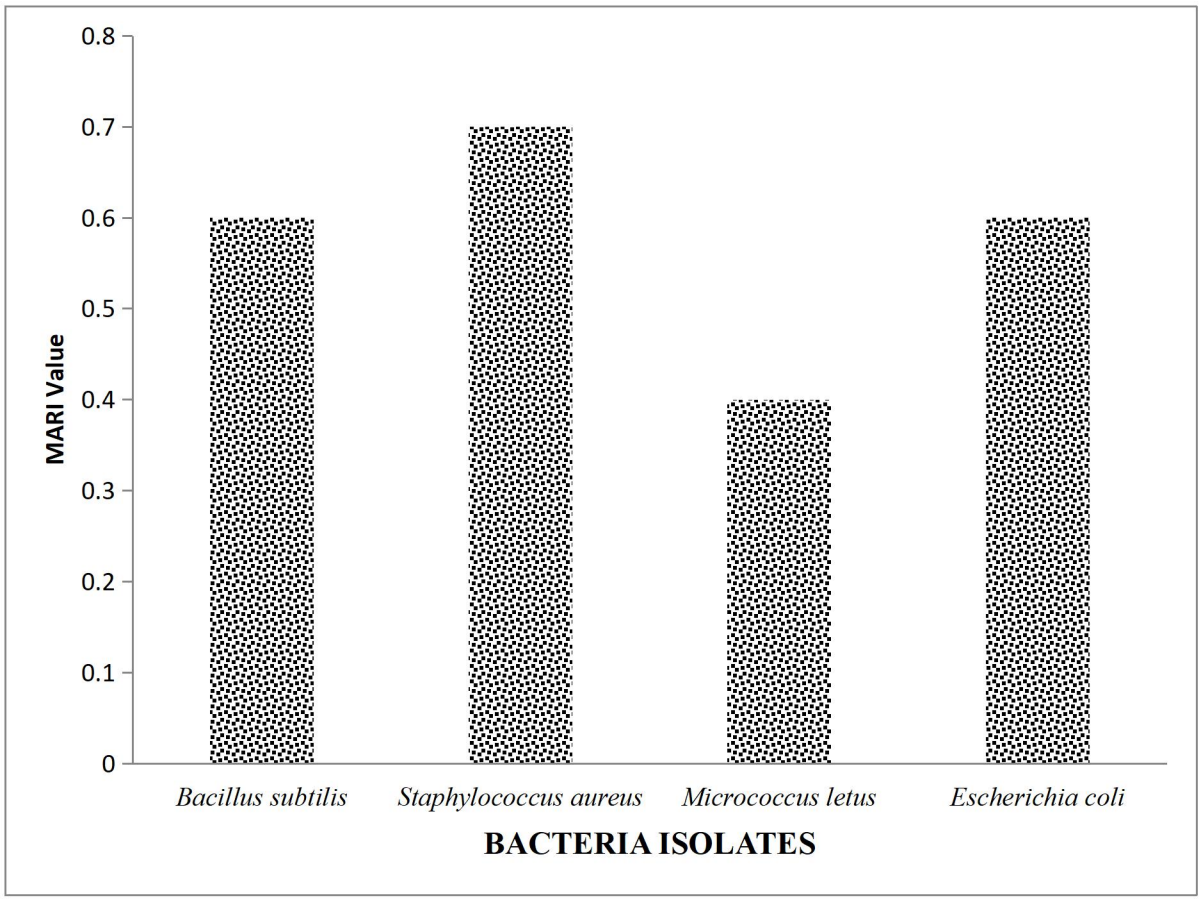

Figure 2: Multi antibiotic resistance index (MARI) value of bacteria isolates different brands of sachet water samples within the University of Benin, Ugbowo campus.

CHAPTER FIVE

DISCUSSION

The quality of drinking water is a critical public health concern, particularly in environments such as university campuses where students are often reliant on packaged water sources. This study investigates the bacterial count of various sachet water brands available within the University of Benin, Ugbowo Campus, focusing on Uniben Table Water, Faith Mark, Olivia, Uzama, and Notre Dame. The findings reveal significant variations in bacterial counts among these brands, underscoring the need for rigorous quality control measures to ensure safe drinking water for consumers. In the analysis conducted, Uniben Table Water exhibited a bacterial count of 3.2±0.72 X 104 Cfu/mL (colony-forming units per milliliter), indicating a moderate level of microbial presence. This finding is noteworthy considering that Uniben Table Water is often perceived as a reliable source due to its association with the university itself. However, it remains essential to interpret this result within the context of acceptable safety standards for drinking water as established by health authorities (Bharath *et al.,* 2023).

Faith Mark and Olivia sachet waters presented bacterial counts of 2.0±0.91 X 10^4 Cfu/mL and 1.9±0.67 X 10^4 Cfu/mL respectively. These values suggest that while both brands maintain relatively lower levels of bacteria compared to Uniben Table Water, they still fall short of ideal microbial safety thresholds set by regulatory bodies such as the World Health Organization ($1.0x10^2$) (Okonkwo *et al.,* 2019). Continuous monitoring and assessment are crucial to ensure these brands do not pose health risks to consumers. Uzama sachet water, with a bacterial count of 3.3±1.51 x104 Cfu/mL, raises concerns due to its notably high microbial load, indicating potential lapses in production or packaging processes that could compromise consumer safety Notre Dame sachet water, on the other hand, stood out with the lowest bacterial count of

1.1±0.96 x10^4 CFU/mL, suggesting that it adheres more closely to recommended safety standards and exemplifies a commitment to maintaining high-quality control measures in its production and distribution processes (Olowe *et al.,* 2015). Regular testing and quality assurance protocols should be prioritized by all manufacturers to safeguard public health and ensure consumer confidence in the safety of sachet water products available on campus.

This result also highlights the presence and prevalence of specific bacterial species, notably *Staphylococcus aureus, Bacillus subtilis, Micrococcus letus*, and *Escherichia coli*. *Staphylococcus aureus* emerged as the predominant isolate with a frequency of five occurrences. This bacterium is known for its pathogenic potential; it can cause a range of infections from minor skin conditions to more severe illnesses such as pneumonia or sepsis (Thivya *et al.,* 2024). The high frequency of *Staphylococcus aureus* in sachet water raises serious concerns about hygiene practices during production and packaging processes. It underscores the urgent need for regulatory oversight and improved sanitation measures among producers to mitigate health risks associated with contaminated drinking water (Govindarajan and Senthilnathan, 2024).

Following *Staphylococcus aureus* in prevalence were Bacillus subtilis and Micrococcus letus, each recorded four occurrences. *Bacillus subtilis* is generally considered non-pathogenic; however, its presence indicates possible issues related to environmental contamination during production or handling processes (Ikeme *et al.,* 2024). On the other hand, *Micrococcus letus* is typically regarded as part of normal skin flora but can still signify unsatisfactory sanitation standards if found in potable water sources. *Escherichia coli,* although detected with the lowest frequency of three occurrences, is a critical indicator of fecal contamination and poses significant health risks if consumed. The presence of *Escherichia coli* in sachet water is particularly alarming, as it suggests a direct breach in sanitary barriers. Immediate corrective actions and

stringent monitoring protocols are imperative to ensure the safety and quality of sachet water distributed within the campus. These findings highlight the necessity for comprehensive microbiological assessments and regular inspections to safeguard public health in university environments (Kamanula *et al.,* 2024).

In this present study, pathogenicity tests were conducted to ascertain the virulence characteristics of bacterial isolates. The results confirmed that all tested isolates exhibited pathogenic behavior as indicated by their positive responses to several key assays: Gelatin Liquefaction Test, DNase Test, Spirit Blue Test, and Haemolysin Test. Each test serves as a vital indicator of specific virulence factors; for instance, gelatin liquefaction demonstrates a bacterium's ability to hydrolyze gelatin into amino acids, thereby facilitating tissue invasion and nutrient acquisition (Seth *et al.,* 2024). Similarly, DNase production indicates a bacterium's capability to degrade nucleic acids, which may aid in evading host immune responses. The Spirit Blue Test evaluates lipolytic activity; positive results suggest that the bacteria can utilize lipids as a carbon source while potentially contributing to tissue damage in infected hosts. Furthermore, hemolysin production signifies a pathogen's ability to lyse red blood cells, releasing hemoglobin and providing additional nutrients necessary for bacterial growth (Bello *et al.,* 2023). Collectively, these tests illustrate that the bacterial isolates possess multiple mechanisms through which they can inflict harm on human hosts. This comprehensive understanding of the virulence determinants underscores the critical need for regular monitoring and assessment of sachet water quality to prevent potential outbreaks and safeguard public health within the campus community (Obiri –Danso *et al.,* 2023). The presence of these virulent bacteria in sachet water samples raises significant concerns regarding the safety and hygiene practices employed in the production and distribution of these widely consumed products. Given the widespread reliance on sachet

water as a primary source of drinking water for many students and faculty, addressing these safety issues becomes an urgent priority to ensure the well-being of the entire university population (Vaishali and Punita, 2023).

The results of the present study also revealed significant findings regarding the susceptibility patterns of bacterial isolates such as *Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus,* and *Escherichia coli*. The results indicated that these bacteria were sensitive to gentamicin and ciprofloxacin but exhibited resistance to several commonly used antibiotics including pefloxacin, Ampiciox, Zinnacef, amoxicillin, rocephin, and septrin. This pattern raises critical questions about the implications for public health and the efficacy of current treatment protocols for infections caused by these pathogens (Bezuidenhout *et al.,* 2022). The observed sensitivity of the bacterial isolates to gentamicin and ciprofloxacin suggests that these antibiotics could be effective therapeutic options for treating infections associated with contaminated sachet water. Gentamicin is an aminoglycoside antibiotic known for its broad-spectrum activity against Gram-negative bacteria as well as some Gram-positive bacteria (Chandra *et al.,* 2022). Ciprofloxacin belongs to the fluoroquinolone class and is widely utilized due to its potent bactericidal effects against a variety of pathogens. The findings highlight the importance of selecting appropriate antibiotics based on susceptibility patterns in clinical settings to ensure effective treatment outcomes (Erah *et al.,* 2022). Conversely, the resistance demonstrated towards pefloxacin, Ampiciox (a combination antibiotic), Zinnacef (cefuroxime axetil), amoxicillin (a penicillin derivative), rocephin (ceftriaxone), and septrin (trimethoprim-sulfamethoxazole) presents a concerning trend that warrants further investigation. This resistance can be attributed to various factors including overuse or misuse of antibiotics in both human

medicine and agriculture, leading to selective pressure on bacterial populations (Ateba, and Maribeng, 2021).

5.1 Conclusion

The results of this study show the bacteriological analysis of five brands of sachet water. Bacteria such as *Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus,* and *Escherichia coli*. were found present in the different brands of sachet water sold within the University of Benin, Ugbowo campus. The total bacterial counts for all water samples were generally high exceeding the limit recommended by both Environmental Protection Agency (EPA) and World Health Organization (WHO) ($1.0x10^2$ cfu/ml), which is the standard limit of heterotrophic count for drinking water. Therefore, appropriate treatment processes should be utilized for production of quality and safe packaged drinking waters.

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