BACTERIOLOGICAL ANALYSIS OF SACHET WATER SOLD IN AUCHI.

BY

SILAS BERTHA
AST/2382060182

DECEMBER, 2022.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCE LABORATORY TECHNOLOGY, SCHOOL OF APPLIED SCIENCE AND TECHNOLOGY, AUCHI POLYTECHNIC, AUCHI.
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF HIGHER NATIONAL DIPLOMA (HND) IN MICROBIOLOGY.

DECEMBER, 2022.

CERTIFICATION

I certify that this project **BACTERIOLOGICAL ANALYSIS OF SACHET WATER SOLD IN AUCHI** was carried out by **SILAS BERTHA, AST/2382060182** of the Department of Biological Science Laboratory Technology, Auchi Polytechnic, Auchi. In partial fulfillment of the requirement of the award of Higher National Diploma (HND) in Microbiology.

MRS. YAKUBU, PATIENCE

PROJECT SUPERVISOR

DATE----------

MR. ANTHONY OHIMAI

HEAD OF DEPARTMENT

DATE----------

DEDICATION

This project work is dedicated to God Almighty for his grace and mercies and to my parents MR AND MRS UDUAKOBONG SILAS.

ACKNOWLEDGEMENT

I wish to express my profound gratitude to my maker, the Lord God Almighty for giving me the grace to write this book.

I also would not fail to thank my project supervisor, Mrs. Yakubu P. for her wonderful contribution, advice and guidance rendered to me during the course of carrying out this study.

To my parents. Thank you for your support show of love and encouragement in the course my study.

I gratefully acknowledge all articles, owners of website from which some materials were downloaded in this study.

To my siblings and friends, I am grateful for your support.

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ABSTRACT

Bacteriological analysis of five samples of sachet drinking water sold in Auchi was carried out to determine the portability of the sampled water. Pourplate technique was used for estimation of total bacterial count and.. Mean heterotrophic bacterial counts (cfu/ml) ranged from $7.0x10^5$ *to* $1.0x10^4$*. The bacteria isolated were Staphylococcus aureus, Streptococcus spp, Bacillus sp. Most of the sachet water brands fell below drinking water standard and are therefore, of doubtful quality. There is need for strict attention and routine monitoring by regulatory agencies of these water sources to avert outbreak of epidemic disease and also to raise standards of quality of sachet water produced and sold in Auchi.*

CHAPTER ONE

INTRODUCTION

Packaged water has ever been implicated as a source of outbreaks of cholera and typhoid fever as well as traveler's disease in countries such as Portugal and Spain (Bardalo and Machado 2014). Several studies have shown that packaged water can be contaminated with bacteria at various stages of production (Gangil *et al*, 2013). Under improper or prolonged storage of bottled water, bacteria can grow to levels that may be harmful to human health (Fisher *et al*, 2015).The demand for good quality water for drinking and other purposes is no doubt exceeding supply especially in some regions of developing countries where drought has claimed thousands of lives and lifted economic and social damage (Frederiksen, 2016). The provision of an adequate supply of a safe drinking water was one of the eight components of primary health care identified by the international conference on primary health care in 1978 (Dufour *et al.*, 2012). Unsafe water is a global public health threat, placing persons at risk of diarrhea and a host of other diseases as well as chemical intoxication (Hughes and Koplan, 2015).

Sale of packaged water has exploded all over the world in recent years, largely as a result of public perception that it is safe, tastes better, and has a better quality compared to raw tap water (Fisher *et al,* 2015). The increment in consumption globally could also be due to a result of an increase in per capita use as well as population growth. Even in countries where tap water quality is considered excellent, demand was so high, making packaged water the fastest growing product of the nonalcoholic beverage market worldwide (Stoler, 2014). Access to safe drinking water is still one of the major challenges of the 21st century (Cabral, 2020). Unsafe water is a global public health threat, placing persons at risk for a host of diarrhoeal diseases as well as chemical intoxication. Although disease outbreaks due to contaminated packaged water are not common, any possible contamination may lead to widespread epidemic because of the high demand and coverage. Therefore access to an adequate and safe water supply is very important and can result in significant benefits to health. Studies done by previous authors shows that the bacteriological analysis of sachet water commonly known as "pure water" carried out in some part of the country is heavily contaminated. Auchi Metropolis is an urbanized area in part of Nigeria where several

brands of bottled and sachet water are sold to the public. The continuous proliferation of these packaged water products and their indiscriminate consumption are of concern to public health. An understanding of their microbiological quality and safety are therefore pertinent. Microbial contamination by human or animal excreta is the most common reason for water to be considered unsafe for drinking because of the high probability of presence of pathogenic organisms. Some of these indicator organisms include *E. coli*, *Clostridium perfringes*, *E. faecalis* and *Klebsiella* spp. Which serve as an indicator for the presence of pathogenic bacteria such as *Salmonella typhi*, *Vibrio chlolerea* and *Pseudomonas aeruginosa.* The aim of the study is to examine the microbiological quality of sachet water marketed in Auchi Metropolis.

AIMS

The aim of the study was to ascertain if sachet water, popularly called pure water, was actually indeed pure or poor after the flood disaster.

OBJECTIVES

The specific objectives were:

1) To isolate microorganisms present in sachet water;
2) To enumerate microorganisms present in sachet water;
3) To characterize isolates obtained using biochemical parameters;
4) To determine some physicochemical properties of sachet water

SCOPE OF STUDY

Due to improper disposal of sewage and handling of food and water collecting or storage utensils water can be contaminated by coliform organisms such as *Escherichia coli*. With the discovering of *Escherichia coli* in water, bacteriologist and other scientists have developed method of checking the quality of public drinking water sources. Presence of *E.coli* shows fecal contamination of water.

PURPOSE OF STUDY

The purpose of this work is to isolate organisms from Sachet water in Auchi to ascertain the quality level of the water.

SIGNIFICANCE OF STUDY

This research work is aimed at solving or at least reducing problems from drinking contaminated water. Also it will serve as base for other researchers to attempt solving problems regarding water pollution cases that had be accepted as way of life.

LIMITATION OF STUDY

The inadequacy of materials as chemicals and other laboratory equipments as a problem. Instability of light was also a constrain encountered. Lack of venue for the practical work was also a limiting factor.

CHAPTER TWO

LITERATURE REVIEW

Water from most sources is unfit for immediate consumption without some sort of treatment (APHA, 2022). The consequences of water-borne bacteria and viral infections such as polio, hepatitis, cholera, typhoid, diarrhea, stomach cramps, etc., have been well established. In 2006, water-borne diseases were estimated to cause 1.8 million deaths worldwide each year, while about 1.1 billion people lacked safe drinking water. Consequent to the realization of the potential health hazards that may result from contaminated drinking water, contamination of drinking water from any source is therefore of primary importance because of the danger and risk of water-borne diseases . To live in good health, people need to have access to good quality water in adequate quantity. A report in 2014 (Westrell, 2014) showed that out of the total urban households in Ekiti state (Nigeria) only 53.3% have access to pipe-borne water and only 43.3% of the households in the southwestern part of the country have such access. Rapid urban growth often puts tre-mendous pressure on water resources.

In many parts of Nigeria and several other African countries, piped water supply is either unavailable or irregular, especially in the small-sized communities and towns. Even in most Nigerian cities, the supply of water for domestic purposes has several accompanying inadequacies. According to Sangodoyin (2013) reasons given for these inadequacies include an enormous socio-economic rate of development, a growing industrial base, poor planning, insufficient funding, haphazard implementation of programs, and a lack of maintenance culture as well as technically deficient personnel.

Potable water is defined as water that is free from disease-producing microorganisms and chemical substances deleterious to health (Smith, 2022). Water can be obtained from a number of sources, among which are streams, lakes, rivers, ponds, springs and wells. Unfortunately, clean, pure and safe water only exists briefly in Nature and is immediately polluted by prevailing environmental factors and human activities. It is often assumed that natural, uncontaminated water from deep wells is clean and healthy, and this is usually true with regard to bacteriological composition. However, bacterial pollution of water sources may occur and is mostly derived from watershed corrosion as well as drainage from sewage,

swamps or soil with high humus content. This type of hazard exists particularly in lime-stone areas where underground chambers or fissures may permit water to flow into the freely moving streams without caution for human drinking purposes because of the inherent risks. In August, 2011, a devastating flood hit the city of Ibadan, Nigeria leading to loss of several lives. After the flood there was subsequent outbreak of cholera that claimed 18 lives and left several hospitalized. Prior to the cholera incidence was the general knowledge that sachet water sources were was not sterile and that they may contain bacteria from naturally occurring sources as well as those introduced during manufacturing and consumer handling (Cabral and Fernandez, 2012).

Water is an essential requirement of life for drinking, domestic, industrial and agricultural uses. Its quality and quantity which vary over space and time are important components in the integral development of any area. Any change in the natural quality of water may disturb the equilibrium system and it would become unfit for designated uses. (Gleick, 2016) reported that the protoplasm of many living cells contain about 80 percent water and most of the biochemical reactions which occur in metabolism and

growth of living cells involve water medium, hence it is referred to as universal solvent. Introduction of anthropogent chemicals, that have impact on health when present in trace amounts, has become a problem. A reliable supply of wholesome water is highly essential to promote healthy living amongst the inhabitants of any geological region. The world industrialized standard model for delivery of safe drinking water and sanitation technology is not affordable in much of the developing world. Flussche *et al*, 2015 remarked, that water in sachets is readily available and affordable more than bottled water, but there are concerns about its purity. The need to define the quality of water has developed with the increasing demand. The available must have specific characteristics, signifying its quality. There has been a tremendous increase in the demand for fresh water due to rapid growth of population and the accelerated pace of industrialization.

Contaminated water still threatens the wellbeing of the population, particularly in under-developed and developing countries. Water quality and quantity are inextricably linked, but quality deserves special attention because of its implication on health and life. Every year, thousands of cholera cases causing many human fatalities are said to occur in Nigeria and

recently it was experienced in Bauchi State. Studies by Riemann *et al,* 2013 has confirmed that in Nigeria water related diarrhea is the most prevalent disease among the populace after malaria.

The microbiological quality of drinking water is a concern to consumers, water suppliers, regulators and public health authorities. The potential of drinking water to transmit microbial pathogens to great number of people causing subsequent illness is well documented in many countries at all levels of economic development (Dufour *et al,* 2013). The number of outbreaks that have been reported throughout the world demonstrates that transmission of pathogens by drinking water remains a significant cause of illness. However, estimate of illness based solely on detected outbreaks is likely to underestimate the problem. A significant proportion of water-borne illnesses are likely to go undetected by the communicable diseases surveillance reporting systems. The symptoms of gastrointestinal illness (nausea, diarrhea, vomiting and abdominal pain) are usually mild and generally last a few days to a week and only a small percentage of those affected will visit a health facility. In Ghana most consumers get water supply from sources other than the Ghana Water Company Limited (GWCL)

via their taps because only 40% of the total urban population is directly covered by the GWCL's networks. The safety of the water obtained from sources other than the GWCL cannot be ascertained hence the water is mostly used for other household activities rather than for direct consumption. The most reliable source of drinking water is bottled water which is of good bacteriological quality (Obiri *et al,* 2013) but it is expensive and thus only within the means of the affluent in the society.

As an alternative, small-scale industries have come up with sachet water popularly known as "PURE WATER". This product is 500ml of water in clear nylon square sachets which have been electrically heated and sealed at both ends and widely patronized by both low and middle income earners. The production of sachet water has increased tremendously with over 300 registered producers and 600 unregistered in Ghana. According to the Food and Drugs Board of Ghana, majority of sachet water are produced under questionable hygienic environmental conditions and they have had cause to impose a ban on some producers. Besides, some products do not bear the stamp of approval of the Food and Drugs Board. Even those who have registered do not always meet the standard required of them. Regardless of

all these problems, the production of sachet water enjoys a high patronage because apart from affordability, it is considered wholesome for drinking purposes as compared to tap or well water.

In the era of colonialism in Nigeria, water was supplied to the public free by the government. But Nigeria has moved from mixed economy to capitalist economy. In cities and towns today, water now attracts rates and fees (Adelegan, 2014). There are several rules and regulations for drinking water. In Nigeria, such regulations are monitored by the National Agency for Food and Drug Administration and Control (NAFDAC), which was established as a parastatal of the Federal Ministry of Health by Decree No. 15 of 1993.Unsafe water is a global public health threat, placing persons at risk for a host of diarrhea and other disease as well as chemical lintoxication (Hughes and Koplan, 2015). To attain a safe water supply to various communities and understanding of water that is microbiologically and chemically certified is therefore imperative. Above all, to ensure that the microbiological characteristic of drinking water is safe for human consumption, the Nigeria based National Agency for Food and Drugs Administration Control (NAFDAC) in association with the World Health

Organization (WHO), recommended that potable water for human consumption should not contain any microorganism that is known to be pathogenic and the coliform number per 100 ml of water must be zero, although , it may contain three coliform per 100 ml of water sample in occasional samples (WHO, 2014). The most reliable source of drinking water is bottled water which is of good bacteriological quality (Obiri *et al*, 2013) but it is expensive and thus only within the means of the affluent in the society. As an alternative, small-scale industries have come up with sachet water popularly known as "PURE WATER". This product is 500ml of water in clear nylon square sachets which have been electrically heated and sealed at both ends and widely patronized by both low and middle income earners.

The production of sachet water has increased tremendously in Nigeria. The integrity of these sachet waters is doubtful, in fact, unconfirmed report abounds that most of the vendors do not treat their sachet waters before selling to the public. This become a concern for public health workers and any right thinking individual when one consider the fact that public including nursing mothers patronize these vendors to procure water for their small children (Shear *et al*., 2015). Many people in rural and urban

communities rely on sachet water and/ or borehole water as the source(s) of their drinking water supply. It has been discovered that some sachet water does not bear the stamp of approval of NAFDAC. Even those who have registered do not always meet the standard required of them. Regardless of all these problems, the production of sachet water enjoys a high patronage because apart from affordability, it is considered wholesome for drinking purposes as compared to tap or well water. Surveillance carried out by NAFDAC between 2004 and 2005 revealed that some producers of packaged water indulge in sharp practices such as packaging of untreated water, production under unhygienic conditions, illegal production of unregistered water in unapproved premises, use of non-food grade sachets and release of packaged water for distribution and sale without date marking. These malpractices compelled the agency to formulate guidelines for the production of wholesome packaged water. However, despite the policies formulated by public and international agencies to address this problem, the situation in Nigeria seems degenerating and therefore demands increased attention. In order to effectively solve the problem however, there is a need to fully assess the extent of the problem and its causes. Drinking water

regulations require that potable water for human consumption be free from human-disease-causing bacteria and specific indicator bacteria that are indicative of the presence of these pathogens (Lisle, 2019). Potential health problems may exist due to the microbial content of sachet water since water is one of the vehicles for the transmission of pathogenic organisms (Brock, 2021; Prescott *et al.*, 2015). However, the type of organisms present in sachet water depends on a number of factors such as the type of soil over which the water flows, contamination by animals' sewage and agricultural waste (Hunter, 2013). Waterborne diseases are associated with improper provision of water and sanitary services and the effect of these diseases vary in severity from stomach upsets to even death. Most of the victims are young children especially from the developing world, an estimated number of more than 34 million people die as a result of these water related diseases making it the leading cause of diseases and death around the world (WHO, 2015). The bacteriological quality of drinking water is of paramount importance and monitoring must be given highest priority, this is so because studies have attributed several disease outbreaks to untreated or poorly treated water containing bacteria pathogen that have been isolated from sachet water. The

microbiological quality of drinking water is a concern to consumers, water suppliers, regulators and public health authorities. The potential of drinking water to transmit microbial pathogens to great number of people causing subsequent illness is well documented in many countries at all levels of economic development.

Most people living in the major cities of Nigeria do not have access to pipe borne water, probably due to unavailability or inadequacy where obtainable (Omalu *et al*., 2020). People, therefore, resort to the costlier alternative of buying water from vendors; sachet or bottled water became a major source of drinking water.

Sachet water, a brand of packaged water has, therefore, gradually become the most widely consumed liquid for both the rich and the poor in Nigeria. It is the brand of choice to everyone because it is a cheaper alternative to the bottled brand, considered to be the refreshment of the affluent. Hygiene, purity, tastes, and, most importantly, safety is probably amongst various reasons for sachet water consumption. Unfortunately, the problems of its purity and health concerns have begun to manifest (Oladipo *et al*., 2019).

Sachet water is regulated as a food product in Nigeria by National Agency for Foods Drugs Administration and Control (NAFDAC). The agency relies on World Health Organization (WHO) standards for the product regulation, registration and certification. There has been a tremendous improvement in sachet water regulations by NAFDAC as the number of illegal producers has drastically reduced and most brands on sale now have NAFDAC registration.

Sachet water is not completely sterile; it may not be entirely free of all infectious microorganisms. The potential danger associated with sachet water is contamination, which is a factor of the source of the water itself, treatment, packaging materials, dispensing into packaging materials and closure (Omalu *et al*., 2020). Under prolonged storage of packaged water at favorable environmental conditions, total aerobic heterotrophic bacteria can grow to levels that may be harmful to humans (Warburton *et al*., 2022). Total aerobic heterotrophic bacterial counts are sensitive and practical indicators of water treatment efficiency as well as after-growth and biofilm formation. Some of the total aerobic heterotrophic bacteria have been identified as opportunistic pathogens. These microorganisms can be found in source

waters and in treated drinking water. Thus, consumption of water containing large numbers of total aerobic heterotrophic bacteria can lead to diseases such as gastroenteritis and mucous membrane infections particularly in persons whose immune systems are compromised by AIDS, organ transplantation or chemotherapy (Grabow, 2016).

Good quality water is odourless, colourless, tasteless, and free from faecal pollution (Ezeugwunne *et al,* 2019). A reliable supply of clean wholesome water is highly essential in a bid to promoting healthy living among the inhabitants of a defined geographical region. Safe and potable water supplies in urban centres in Nigeria are still inadequate in spite of four decades of independence and several efforts from various governments. The standard industrialized world model for delivering of safe drinking water and sanitation technology is, however, not affordable in most of the developing world. Consequently, given the renewed global commitments towards the Millennium Development Goals (MDG) marked for 2015, the importance and contribution of locally sourced low-cost alternative drinking water schemes to sustainable access in rural and urban settings of developing nations cannot be overemphasized (Ajayi *et al,* 2018). One of such local

intervention in Nigeria where public drinking water supply is unreliable is drinking water sold in polythene sachets. Water in sachets is readily available and affordable, but there are concerns about its purity. The integrity of the hygienic environment and conditions where the majority of the water in sachets are produced has been questioned. Apart from environmental contaminants, contamination from improper vendor handling also poses threats to the health of the ignorant consumers who drink often times without any proper cleaning of the sachets. Previous studies have identified handling as the source of infection in food and water-borne diseases in several countries. Water related diseases continued to be one the major health problems globally. The National Agency for Food and Drug Administration and Control (NAFDAC) is mandated to enforce compliance with internationally defined drinking water guidelines, but regulation of the packaged water industry aimed at good quality assurance has remained a challenge to the agency. To control this menace of contaminated water in sachets, NAFDAC declared a possible 'gradual' nationwide ban on sachet water to allow manufacturers of sachet water to start winding down or change to bottle packaging. Successful implementation of this ban has

remained far from reality as the sachet water market is witnessing tremendous growth, especially among the poor and middle social classes. Few studies conducted in recent years on the quality of packaged water in Nigeria focused primarily on the end-product, leaving out the processes that determine the fate of the packaged water, and the people (various stakeholders involved) in whose hands lie the will and power to effect the desired change (Oladipo *et al,* 2019). (Yusuf *et al,* 2017). Inspite of its potentials for good groundwater storage, there is incessant water shortage in the Urban Auchi Area, because the distribution and supply system of treated water are very poor. This has made over 90% of the population reliant on water provision from sources other than the Auchi water scheme. Many people depend on water vendors for provision of water for domestic and daily needs and this has led to the advent of locally sourced low cost alternatives sachet water called 'Pure water' becoming a major source of drinking water. The production, marketing and consumption of sachet water has increased tremendously with several brands being marketed in Nigeria and other developing nations (Ogan, 2022). (Feng, *et al,* 2022). (Adelegan, 2014). There are several rules and regulations for drinking water. In Nigeria,

such regulations are monitored by the National Agency for Food and Drug Administration and Control (NAFDAC), which was established as a parastatal of the Federal Ministry of Health by Decree No. 15 of 1993. Surveillance carried out by NAFDAC between 2004 and 2005 revealed that some producers of packaged water indulge in sharp practices such as packaging of untreated water, production under unhygienic conditions, illegal production of unregistered water in unapproved premises, use of non-food grade sachets and release of packaged water for distribution and sale without date marking. These malpractices compelled the agency to formulate guidelines for the production of wholesome packaged water. However, despite the policies formulated by public and international agencies to address this problem, the situation in Nigeria seems degenerating and therefore demands increased attention. In order to effectively solve the problem however, there is a need to fully assess the extent of the problem and its causes. Drinking water regulations require that potable water for human consumption be free from human-disease-causing bacteria and specific indicator bacteria that are indicative of the presence of these pathogens (Lisle, 2019). This does not mean that drinking water should be

sterile. There are some bacteria that have a greater probability of causing disease in humans. These bacteria are classified as pathogens. Examples of bacterial pathogens and their related diseases are *Salmonella typhi* (typhoid fever), *Shigella dysenteriae* (dysentery), and *Legionella pneumophilia* (Legionnaire's Disease).

Water related diseases continue to be one of the major health problems globally. The high prevalence of diarrhea among children and infants can be traced to the use of unsafe water and unhygienic practices (Tortora *et al*., 2022). (Sofola and Lawal, 2013). In many developing countries, availability of water has become a critical and urgent problem and it is a matter of great concern to families and communities depending on non-public water supply system (Okonko *et al*., 2018). Increase in human population exerts an enormous pressure on the provision of safe drinking water especially in developing countries (Okonko *et al*., 2018). Unsafe water is a global public health threat, placing persons at risk for a host of diarrhea and other disease as well as chemical intoxication (Hughes and Koplan, 2015). Unsanitary water has particularly developing effects on young children in the developing world. Each year greater than 2 million persons,

mostly children less than 5 years of age, die of diarrhea disease (Hughes and Koplan, 2015). For children in this age group, diarrhea disease accounted for 17% of all death from 2000 to 2003 (WHO, 2015), ranking third among causes of death, after neonatal causes and acute respiration infections (WHO, 2015). Water in nature is seldom totally pure. Rainfall is contaminated as it falls to earth, the combustion of fossil fuel put sulphur compound responsible for acid precipitation in the air. Water that moves below the ground's surface undergoes filtration that removes most organisms. For this reason, water from springs and deep wells are generally of good quality. The most dangerous form of water pollution occurs when feacal contaminant like *Escherichia coli* enter the water supply. Contaminants ingested into water supply cause many diseases. Examples of such pathogens are *Salmonella* spp, *Shigella* spp, *Vibrio cholerae* and *E. coli* (Tortora *et al*., 2012).

Industrial and agricultural chemicals leached from the land, enter water in a great amount and they could be resistant to biodegradation. Apart from this, rural water often has excessive amount of nitrite from microbial action on agricultural fertilizers (Lellan *et al*., 2021). When ingested nitrite competes for oxygen in the blood (Lellan *et al*., 2021). To attain a safe water

supply to various communities, an understanding of water that is microbiologically and chemically certified is therefore imperative. Above all, to ensure that the microbiological characteristic of drinking water is safe for human consumption, the Nigeria based National Agency for Food and Drugs Administration Control (NAFDAC) in association with the World Health Organization (WHO), recommended that potable water for human consumption should not contain any microorganism that is known to be pathogenic and the coliform number per 100 ml of water must be zero (WHO, 2014). (Shear *et al*., 2015). (Czachor, 2022). (Tibetts, 2000). (Obiri-Danso *et al*., 2013). (Steiner *et al*., 2017).

CHAPTER THREE

MATERIAL AND METHODS

The material used for the preparation of media; Cotton flask,glassslide, cotton wool, test tube, aluminum foil, weighing balance, Bunsen burner, Methylated spirit, Beakers, Autoclave, Incubator, Distilled water, Measuring Cylinder, Nutrient agar, Eosin methylene blue, syringes, pipettes, and Nutrient agar. Gram stain reagents such as crystal violet, lugol iodine, ethanol, safranine. Cover slip testtube, testtube rack, conical flask etc.

SAMPLE COLLECTION

The Sachet water samples used for the analysis were bought within Auchi. A total of five different brands of sachet water labeled A-E (Miclyn water, Makabanu water, De Standard water, Aqua-Efua water and Aksel water respectively). The sachet water were bought and transferred immediately inside a container to the laboratory for further analysis

MICROBIOLOGICAL ANALYSIS

The microbiological analysis carried out were total plate count for enumeration of microorganism in the sachet water samples.

ENUMERATION OF MICROORGANISMS

This was carried out by first diluting each of the water samples serially from 10^{-1} to 10^{-4} dilution series. 10ml each from the samples was aseptically transferred to presterilized test tube (10^{-1}) containing 9ml of sterile distilled water using sterile pipettes. This was mixed properly and thereafter was transferred into the next test tube (10^{-2}). Serial dilution was carried out until the last tube (10^{-6}). 1ml of aliquots from each of the test tubes containing serially diluted samples was transferred into sterile Petridishes labeled with each dilution factor.Media used were prepared according to manufacturer instructions. Thereafter sterile molten nutrient agar was poured into these plates aseptically, allowed to solidify and then incubated at 37^{o}C for 24 hours after which enumeration (counting) was done.

MULTIPLE TUBE TECHNIQUE

The multiple tube technique was used for the detection of the presence of *Escherichia coli*. This involves the presumptive, confirmed and the completed tests.

In the presumptive test, three sets of tube were used. The first set 'A' contained double strength lactose broth was inoculated with 10ml of water sample for each samples tested. The second set of three test tubes 'B' contained lactose broth single strength which was inoculated with 1ml of each of the water sample. For all samples tested. The third set of test tube also contained single strength lactose broth was inoculated with 0.1ml of the water samples for all the samples tested. All the sets of tested was thereafter incubated at 37^{o}C for 24 to 48 hour. The presence of gas which is shown by the presence of air in Durham tubes is indicative of positive or doubtful result. The absence of gas bubbles in the Durham tube indicates negative result.

In the confirmed test, the positive presumptive tube was plated on Eosin Methylene blue (EMB) plates. 1ml of each positive inoculated on

Eosin Methylene blue plates using pour plate to confirm if *E.coli* was present.

In completed test, Lactose broth was inoculated with aid of a sterile wire loop. Inoculums was taken from the doubtful tubes from the confirmed test and incubated for 24 hours. The presence of acid and gas production in the lactose broth constitute a completed test, positive test and its absence indicate a negative completed test.

IDENTIFICATION OF THE BACTERIAL ISOLATE

The bacteria isolated was characterized and identified on the basis of its cultural morphological and biochemical characteristics.

CULTURAL CHARACTERIZATION

This involves the growth requirements as well as appearance of culture to the naked eye. So therefore, colonies surface, size, shape and edges/margin were noticed.

MORPHOLOGICAL CHARACTERIZATION

Morphological characterization through gram staining was observed. The procedure is as follows:

Gram staining techniques: The Gram staining techniques is the most important and most frequently used stain in bacteriology. It is used to differentiate bacteria into two groups designated as gram positive (Blue to purple reaction) and gram negative (Pink to red reaction)

A moderately thin smear of the culture (to be identified) was made on a clean grease free slide. The slide was air dried and the reversed side was quickly passed three times over a flame. This was to allow the organism stick to the slide so as to avoid wash off Suring subsequent process of flooding. The slide was stained with Methyl violet for 60 seconds and then washed away with water. The slide was then flooded with lugol's iodine for 60 seconds, washed with water and decolourized with acetone for one minutes. It was further flushed with water and then counter stained with safranin for 30 seconds. The slide was finally washed and blotted with filter paper and examined under oil immersion objective of the microscope.

BIOCHEMICAL CHARACTERIZATION

An aliquot (3mls) of each sterilized sugar solution glucose, lactose, mannitol, sucrose, maltose) were dispensed into ach bijou bottle, phenol red was added as an indicator to each of the sugar solutions. The test organisms were then inoculated aseptically into the different sugar solution. They were incubated at 37^{o}C over night they were observed for colour change which indicates a positive test.

MOTILITY TEST

The hanging drop method was used. A sterile wire loop was used to pick a discrete colony into 5ml of sterile peptone water in a bottle and incubated for about 3hours. At the end of incubation, a drop of the culture in peptone water was put into cover slip. Plastacin was used to make a circle on a clean slide, the slide was then inverted over the cover slip so that the drop of peptone water will be in the centre of the plastacine circle and was viewed under the microscope using the x40 objective. When observed, motile organisms were seen in steamy movement.

OXIDASE TEST

Some drop of 1% solution of tetramethyl-phenylene thiamine was poured on a whatman filter paper. Pour colony of the test organism was picked using the edge of a clean slide and smear on the filter paper. Organisms which produce the enzymes cytochrome oxidase turn the colour to the filter paper to intense purple when held for 5 to 20 seconds which indicates a positive test.

INDOLE TEST

This test is used to know the ability of bacteria to release indole from the splitting of the amino acid tryptophan. The test organism was inoculated into 6ml sterile peptone water and incubated for 3hours at 37^{o}C kovac's reagent about 0.2-0.3ml dimethylaminobenzaladehyde was added to the culture after incubation the bottles were shaken and allow to stand when later observed in reddish brown ring colouration at the top of the culture indicates a positive results.

CATALASE TEST

This test is used to identify organism that can produced the enzymes catalse. This was done by placing a drop of distilled water clean slide an inoculums from the pure culture was emulsified into it, a few drops of hydrogen peroxide was added to the suspension of the organisms production of bubbles indicates a positive test.

UREASE TEST

This is used to ascertain the ability of some bacteria to split to compound urea to carbondioxide and ammonia. The organisms for the test were inoculated into the surface of urea agar. Slope and incubated at $37^{o}C$ any positive reaction changes to medium t red.

CITRATE UTILIZATION TEST

This is used to differentiate citrate utilizing organisms from those that do not suspected organisms were streaked lightly into simmon citrate agar slopes in tube and incubated for 48 hours. Positive reaction is observed when the medium change from green to bright red.

CHAPTER FOUR

RESULTS

The results obtained for the total viable plate counts for all the samples of sachet water (A-E) analyzed are shown in Table 1 below.

A. Miclyn water

B. Makabanu water

C. De Standard water

D. Aqua-Efua water

E. Aksel water,

TABLE 1: SAMPLE (A-E) with NAFDAC NUMBER AND ADDRESS.

SAMPLES	NAMES	NAFDAC NO.	BATCH NO.	QUANTITY	ADDRESS
A	Miclyn water	A1-6711L	Nil	60cl	Aigbona, Auchi.
B	Makabanu water	E1-2798L	Nil	60cl	Mabishak global venture
C	De Standard water	A1-3310L	Nil	60cl	Standard management services
D	Aqua-Efua water	E1-6708L	Nil	50cl	Qualfables enterprises
E	Aksel water	D1-18057L	Nil	60cl	Prime natural spring water.

TABLE 2: AVERAGE TOTAL VIABLE PLATE COUNT (CFU/ML) OF SAMPLE (A-E).

WATER SAMPLES	BACTERIAL COUNT (CFU/ML)
A	$1.0x10^4$
B	2.0×10^4
C	$2.2X10^4$
D	$7.0X10^5$
E	$1.4 X10^4$

TABLE 3: OCCURRENCE OF BACTERIA IN SACHET WATER SAMPLES IN AUCHI.

WATER SAMPLES	BACTERIAL ISOLATE
A	*Staphylococcus spp*
B	*Bacillus spp, Staphylococcus spp*
C	*Streptococcus spp, Bacillus spp*
D	*Streptococcus spp, Bacillus spp*
E	*Bacillus spp, Staphylococcus spp*

Table 4: CULTURAL, MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF BACTERIA.

TABLE	ISOLATES	ISOLATE 1	ISOLATE 2	ISOLATE 3
	cultural characteristics	Light brown, flat colonies with irregular edges and dull surface, forms pellide on broth and rapidly spread on wet surface of nutrient agar.	Slightly pink circular colonies	Non-pigmented dull circular smooth and viscous colonies
Morphological characteristics	Red-shaped cells, occurring singly with central spores	Short rod in short chains	Straight rods occurring angry	Straight rods occurring singly
Biochemical test	Gram stain	+	+	+
	Spore	+	-	-
	Catalase	+	+	+
	Indole	+	-	-
	Starch hydrolysis	+	+	-
Carbohydrate utilization	Glucose	A	A/G	A/G
	Lactose	-	A/G	A/G
	Fructose	A	A/G	A
	Galactose	-	A/G	A
	Mannose	-	A/G	A
	Maltose	-	A/G	A
	Arabinose	A	A/G	A
	Mannitol	A	-	A
	Probable identity	*Bacillus subtilis*	*Staphylococcus aureuus*	*Streptococcus sp*

\+ Positive
\- Negative A Acid, AG Acid and Ga

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

The presence of *Bacillus* species in the sachet water could be as a result of contamination from poor staff handling during processing of the water samples (Obiri-Danso *et al.*, 2013). *Bacillus* spp., produces enterotoxin which could be deadly when ingested into the body (Okonko *et al*., 2018). The pH values of these bacteria are within the range reported by Okonko *et al*. (2018). Most microbes grow best at pH value of between 6.6-7.5, while few grow below pH of 4. The optimum pH required by bacteria varies and sensitive to changes, thus a fluctuation in optimum pH may lead to a change in the metabolism of the bacteria (Steiner *et al*., 2017). The hunt for quick money has resulted in "pure water" business and the associated inability to follow specified treatment process. Sachet water, inspite of being sealed, is thus observed to contribute to health risk to consumers due to the presence of pathogens often associated with food poisoning and intoxication, in the water.

CONCLUSION.

Conclusively, the presence of bacteria in this study may be as a result of improper handling, processing and purification procedures, unhygienic handling after production.

Water with such bacteria are not safe for human consumption hence, the water source should be reexamined by the NAFDAC (Tortora *et al*., 2012). Also microbiologically water is required for drinking, recreation and industrial uses as

stipulated by the WHO and NAFDAC drinking water standards. Okonko *et al*. (2016) suggested that 99.8% of death in developing countries is due to unhygienic water and sanitation. Besides, the sources of untreated drinking water could be veritable reservoir of several other opportunistic pathogens of human and chemical poisoning.

RECOMMENDATION

The following recommendation can help the existing water packaging factories to improve on their production and to put clean contaminant free water on the table of consumers.The management should routinely educate their staff on high hygiene standard. These sachet water business should be critically reviewed by NAFDAC to ensure that producer comply with standards at every stage of production and distribution processes. NAFDAC should also organize campaigns to educate producers as well as consumers on the health hazards associated with contaminated or untreated water. Defaulters should be banned and fake NAFDAC numbers should be banned.

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