

**MICROBIAL CONTAMINATION OF SACHET WATER SOLD IN AND
AROUND ARKILLA SOKOTO**

BY

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CERTIFICATION

This research project titled Microbial Contamination of Sachet Water Sold in and Around Arkilla Sokoto”. By Abdullahi Zainab Ahmad with the admission number (2010302011) has been read, supervised and approved by the undersigned having satisfied the necessary requirements for the award of Bachelor of Science (Hons.) Degree in Biology in the Faculty of Chemical and Life Sciences, Department of Biology, Usmanu Danfodiyo University, Sokoto.

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DEDICATION

This research project is dedicated to my beloved parents, Alh. Ahmad Abdullahi, Hajiya Rakiya Dauda Malik whose prayer and guidance lead me to this success.

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In the Name of Allah, the Compassionate, the Most Merciful. All praise is due to Almighty Allah, Lord of all creations. May the peace and blessings of Allah be upon our noble Prophet Muhammad (PBUH), his family, and his companions.

I would like to express my deepest gratitude to my supervisor, mentor, and role model, Professor M.M yahaya, for his invaluable guidance, encouragement, and attention. His support made this work possible, and I pray that Allah rewards him abundantly. I also pray that Allah continues to guide and protect his family.

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In biological interaction, there are always positive and negative implications. I acknowledge every single person who contributed to my academic pursuit, although names cannot be exhausted for logistic reasons."

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ABSTRACT

This study investigated the microbial contamination of sachet water sold in and around Arkilla, Sokoto, alongside tap and well water commonly consumed in the area. Four sachet water brands Aysad, Caliphate, Alkanchi, and Ibhade were analyzed using standard microbiological methods, including total plate count, Most Probable Number (MPN) technique, and EMB agar characterization. Results showed that tap water (28 MPN/100 mL) and well water (150 MPN/100 mL) contained high levels of coliforms, indicating significant faecal contamination. Three sachet brands (Aysad, Caliphate, Alkanchi) recorded no coliforms and were microbiologically safe. However, Ibhade water showed low-level contamination (9 MPN/100 mL) with coliform growth on EMB. Cultural and biochemical tests confirmed the presence of organisms such as *Escherichia coli*, *Klebsiella* species, and *Enterobacter* species in contaminated samples. The study concludes that while most sachet water brands are safe, tap, well water, and one sachet brand pose potential health risks.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Table 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 Table water can be contaminated with bacteria at various stages of production (Gangi, 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 sachet 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 diarrhea 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 author's shows that the microbial analysis of sachet water commonly known as "pure water" carried out in some part of the country is heavily contaminated. Sokoto Metropolis is an urbanized area in part of Nigeria where several brands of table or sachet water are sold to the public. The continuous proliferation of these table water products and their indiscriminate consumption are of concern to public health. An understanding of their microbial 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 perfringens*, *E. faecalis* and *Klebsiella* spp. Which serve as an indicator for the presence of pathogenic bacteria such as *Salmonella typhi*, *Vibrio cholerae* and *Pseudomonas aeruginosa*. The aim of the study is to assess the microbial contamination of table sachet water marketed in Sokoto Metropolis.

Furthermore, unsafe water is a global public health threat, placing persons at risk for a host of diarrheal and other diseases as well as chemical intoxication. Unsanitary water has particularly devastating effects on young children in the developing world. Each year, more than 2 million persons, mostly children less than 5 years of age, die of diarrheal. The principal objectives of municipal water and the production of safe water that is fit for human consumption.

The guidelines for quality water are intended for use by countries as a basis for the development of national standards which if properly implemented will ensure the safety of drinking water. The quality of drinking water has attracted great attention worldwide because of implied public health impacts. However, the information generated from this study will provide a baseline data for water production factory, table Water Company and ministry of health on the importance of hygienic practice in processing and

packaging of water. The results obtained from this study will to formulate guidelines for proper hygienic management and also provide opportunity for the chain actors and other stakeholders to determine appropriate mitigation measures at the various points.

1.2 Statement of the Problem

The increasing consumption of sachet water in Sokoto metropolis is driven by its affordability and convenience especially in the absence of reliable public water supply. However, the safety of these water products is often questionable due to lapses in hygiene during production, inadequate quality control, and poor regulatory enforcement.

These conditions create the potential for microbial contamination, which poses a serious threat to public health.

Despite regulatory guidelines set by bodies such as NAFDAC and SON, reports of contaminated sachet water products still persist in different parts of Nigeria. In Sokoto, where the climate is predominantly hot and dry, the risk of microbial growth due to improper handling and storage may be even higher. Consumers, often unaware of the quality of the water they consume, may be at risk of waterborne diseases such as typhoid fever, cholera, and dysentery.

1.3 Justification of the study

Sachet water is a common source of drinking water due to its affordability and availability. However, studies have shown that some sachet water may be contaminated with harmful microbes due to poor production practices and inadequate regulation (Oyedeji,2010; Igbeneghu and lamikanra, 2014). This poses serious health risks.

This study is necessary to assess the microbial quality of sachet water sold in Sokoto metropolis and to determine if they meet safety standards. The findings will help inform public health authorities and encourage better hygiene practices among producers.

1.4 Aim and Objectives

The aim of the study was to assess the microbial quality of sachet water sold in Sokoto metropolis and determine its compliance with standard safety guidelines and also to isolate and identify microbial present in sachet water samples from different brands and locations within Sokoto metropolis.

1. To enumerate microorganisms present in sachet water.
2. To determine the microbial load in the sachet water samples using standard microbiological techniques.
3. To assess the potential health risks associated with consuming contaminated sachet water.

4. To make recommendations for improving the microbial safety of sachet water based on the findings.

CHAPTER TWO

2.0 LITERATURE REVIEW

The issue of microbial contamination in drinking water has become increasingly critical, particularly in developing regions where access to clean water remains a significant challenge. This chapter reviews relevant literature on microbial contamination, focusing on sachet water in urban areas like Sokoto Metropolis. It will elucidate the types of microorganisms commonly found, the health implications of contaminated water, and the regulatory frameworks surrounding water quality.

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 Sokoto 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 tremendous 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,

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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 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 anthropogenic 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.

2.1 Water Quality and Microbial Contermination

Water quality is determined by physical, chemical, and microbiological parameters. Among these, microbiological quality is paramount as it directly affects

human health. The World Health Organization (WHO) defines safe drinking water as water that does not cause any significant risk to health over a lifetime of consumption. Microbial contamination arises from various sources, including fecal matter, polluted surface water, and inadequate sanitation practices, leading to the presence of pathogens in water intended for drinking. The microbial 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. Poor handling, improper sealing, and unhygienic storage conditions can all contribute to microbial contamination of sachet water. Contaminated sachet water poses significant health risks including diarrhea, dysentery, cholera, typhoid fever and other waterborne infections, especially among children and immune compromised individuals.

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. Hence regular microbial assessment and enforcement of hygiene practices in the production and distribution of sachet water are essential to safeguard public health.

2.2 Microbial Contaminants in Water

Microbial contaminants are microorganisms that pollute water sources and make them unsafe for human consumption. These include a wide range of bacteria, viruses, fungi, and protozoa that may enter water through various routes such as human and animal waste, environmental runoff, broken pipes, and poor sanitation during processing and packaging.

Some of the most common microbial contaminants in drinking water, especially sachet water in Nigeria, include:

- Bacteria: *Escherichia coli* (*E. coli*), *Salmonella spp.*, and *Vibrio cholerae* are notable bacteria often found in contaminated water. *E. coli* is frequently used as an indicator organism for fecal contamination.

- Viruses: Waterborne viruses such as Norovirus and Hepatitis A can cause severe gastrointestinal illness. These pathogens are particularly resilient and can persist in the water environment.
- Parasites: Protozoa such as *Giardia lamblia* and *Cryptosporidium spp.* are also found in contaminated waters and can lead to waterborne diseases.

Some of the most frequently detected microbial contaminants in drinking water include:

- *Escherichia coli* (e.coli): A type of fecal coliform bacteria found in the intestines of humans and animals. Its presence in water indicates recent fecal contamination and the potential presence of other pathogenic organisms.
- *Total coliforms*: A broader group of bacteria found in the environment, not all coliforms are harmful, their presence suggests that other disease causing organisms may be present.
- *Salmonella spp* : causes dysentery and severe diarrhea and dehydration.
- *Shigella spp* : these bacteria can lead to severe diarrheal diseases and are often associated with contaminated water supplies.

2.4 Health Implications of Microbial Contamination

The consumption of contaminated water poses serious health risks, particularly in vulnerable populations such as children and the elderly. Waterborne diseases, including cholera, dysentery, and typhoid fever, pose significant public health challenges in developing regions. In Nigeria, the burden of these diseases is exacerbated by poor sanitation and hygiene practices.

To attain a safe water supply to various communities and understanding of water that is microbial and chemically certified is therefore imperative. Above all, to ensure that the microbial 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.

2.5 Sachet Water as a Popular Source of Drinking Water

The most reliable source of drinking water is bottled water which is of good microbial 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 Sokoto, 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 Administration. 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 intoxication (*Hughes and Koplan, 2015*). Sachet water is defined as drinking water that has been treated, filtered, or purified and is packaged in small sealed plastic sachets, typically containing 500 milliliters of water.

These sachets are made of heat sealed polyethylene film and are intended for single use. Sachet water is commonly sold by street vendors and small scale retailers, making it convenient and affordable source of drinking water for the general public. The origin of sachet water can be traced back to the early 1990s in Nigeria, when rapid

urbanization and population growth led to increased demand for potable water. Private individuals and small-scale entrepreneurs responded to this need by developing a low-cost method of packaging treated water in plastic sachets for mass distribution. Today sachet water remains dominant source of drinking water in Nigeria, particularly in regions like Sokoto, where municipal water infrastructure may not adequately meet the population's needs. Its widespread use, however raises concerns about hygiene practices, water treatment standards, and the potential for microbial contamination.

2.6 Regulatory Frameworks and Standards

Several national and international guidelines exist to regulate water quality. In Nigeria, the National Agency for Food and Drug Administration and Control (NAFDAC) oversees the safety of packaged water, but enforcement remains inconsistent. WHO provides guidelines on drinking water quality, emphasizing the need for routine monitoring and control measures

2.7 Previous Studies on Water Quality in Sokoto Metropolis

Limited studies have focused on the microbial quality of sachet water in Sokoto Metropolis. Previous research has indicated the presence of coliform bacteria in several samples, suggesting fecal contamination. These studies highlight the urgent

need for improved regulatory oversight, public awareness campaigns, and sanitary practices in the sachet water industry.

CHAPTER THREE

3.0 MATERIAL AND METHODS

The material used for the preparation of media; Cornical flask, cotton wool, test tube, aluminum foil, weighing balance, Bunsen burner, Beakers, Autoclave, Incubator, Distilled water, Measuring Cylinder, Eosin methylene blue, ethanol, masking tape, syringes, pipettes, and Nutrient agar.

3.1 Sample Collection

The Sachet water samples used for the analysis were bought within in and around arkillia Sokoto. A total of four different brands of sachet water labeled (Aysad water, caliphate water, Alkanchi water, and Ibhade water). The sachet water were bought and transferred immediately inside a container to the laboratory for further analysis

3.1.1 Microbiological Analysis

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

3.1.2 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 Petri dishes 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°C for 24 hours after which enumeration (counting) was done.

3.1.3 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°C for 24 to 48 hours. 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 27 Eosin Methylene blue plates using pour plate to confirm if Ecoli 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.

3.1.4 Identification of the Bacterial Isolate

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

3.1.5 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.

3.2 Media Preparation

Two culture media were used in this study — Nutrient Agar (NA) for total bacterial count and Eosin Methylene Blue (EMB) Agar for isolation and identification of *Escherichia coli* and other coliforms. All media were prepared according to the manufacturer's instructions (HiMedia, India) and under strict aseptic conditions to avoid contamination.

3.2.1 Preparation of Nutrient Agar (NA)

Nutrient Agar was prepared by weighing 28 g of the dehydrated powder into 1 L of distilled water as indicated by the manufacturer. The mixture was gently heated while stirring to dissolve completely. The pH was adjusted to 7.2 ± 0.2 using either 1 M NaOH or HCl where necessary. The medium was then distributed into clean glass bottles, plugged with cotton wool, and covered with aluminum foil.

Sterilization was carried out in an autoclave at 121 °C for 15 minutes under a pressure of 15 psi. After sterilization, the molten agar was allowed to cool to about 45–50 °C before pouring approximately 20 mL into sterile Petri dishes in a laminar flow cabinet. The plates were left to solidify, inverted, labeled, and stored in a refrigerator at 4 °C until use.

3.2.2 Preparation of Eosin Methylene Blue (EMB) Agar

Eosin Methylene Blue Agar was prepared by weighing 36 g of the dehydrated medium into 1 L of distilled water. The mixture was heated with gentle stirring until completely dissolved. The pH was adjusted to 7.1 ± 0.2 , and the medium was sterilized by autoclaving at 121 °C for 15 minutes. After autoclaving, the medium was cooled to 45–50 °C and aseptically poured into sterile Petri dishes (about 20 mL per plate).

Once solidified, the plates were inverted, labeled, and stored at 4 °C until required. Before use, representative plates from each batch were incubated at 37 °C for 24 hours to check for sterility (no growth indicated sterile plates).

3.3 Data Analysis

The data generated from the microbiological analyses were processed using descriptive statistical methods. Total Plate Count (TPC) results were recorded as colony-forming units per millilitre (CFU/mL) and expressed in standard scientific notation. The Most Probable Number (MPN) values for coliform detection were obtained using the McCrady probability table based on the pattern of positive tubes in the multiple-tube fermentation technique. Results from cultural, morphological, and biochemical tests were interpreted using standard microbiological identification charts to determine the probable bacterial species present in each water sample.

All results were presented in tables and interpreted qualitatively and quantitatively. Comparisons were made with World Health Organization (WHO) and Standards Organisation of Nigeria (SON) permissible limits for potable water to assess the safety of the tested samples. The findings guided the discussion on the level of microbial contamination and potential public health implications.

CHAPTER FOUR

4.0

RESULTS

This chapter presents the results of microbiological analysis conducted on four sachet water brands sold in and around Arkilla, Sokoto. The analyses include Total Plate Count (TPC), coliform detection using the Multiple Tube Fermentation Technique, and biochemical identification of isolates.

4.1 Most Probable Number (MPN) for Coliforms

The MPN results obtained from the different water samples collected in and around Arkilla, Sokoto, reveal varying levels of microbial contamination. The tap water sample recorded an MPN value of 28 coliforms per 100 mL. This value is derived from the pattern of positive tubes observed at the 10 mL and 1 mL dilutions. The presence of coliforms in tap water indicates that the treated municipal supply may have experienced contamination either during distribution or storage. Although the contamination level is moderate, treated tap water is expected to have zero coliforms according to WHO standards; therefore, this finding suggests that the tap water is not microbiologically safe for direct drinking.

The well water sample showed the highest level of contamination with an MPN value of 150 coliforms per 100 mL. All tubes were positive at the 10 mL and 1 mL dilutions, indicating heavy microbial pollution. The result suggests significant faecal contamination, which is commonly associated with shallow wells, poor structural integrity, proximity to latrines, and surface runoff. This level of contamination exceeds both WHO and SON permissible limits and confirms that the well water is highly unsafe for consumption.

The Aysad sachet water sample recorded no positive tubes across all dilutions, giving an MPN value of 0 coliforms per 100 mL. This result indicates the absence of microbial contamination and shows that the brand meets WHO and NAFDAC microbiological standards. Similarly, the Caliphate and Alkanchi sachet water samples also recorded an MPN value of 0 coliforms per 100 mL. The absence of coliforms in these brands suggests that the production processes, including filtration and packaging, were carried out under hygienic conditions and that the water is safe for drinking.

In contrast, the Ibhide sachet water sample recorded an MPN value of 9 coliforms per 100 mL, based on the pattern of positive tubes across the dilutions. This finding indicates low to moderate contamination. Although the level is lower than that of tap and well water, the presence of any coliforms in packaged water is unacceptable

according to international standards. This suggests the possibility of contamination during production, packaging, or handling and shows that the water is unsafe for direct consumption.

Overall, the results reveal that well water and tap water in the study area show significant microbial contamination, while most sachet water brands tested—except Ibade—fall within acceptable safety limits.

Table 1: MPN Results of Sachet Water Samples

Sample Source	3 tubes 10ml	1ml	0.1 ml	MPN /100 mL
Tap	+ + –	++ –	– – –	28
Well	+ + +	+ + +	– – –	150
Aysad	– – –	– – –	– – –	0
Caliphate	– – –	– – –	– – –	0
Alkanchi	– – –	– – –	– – –	0
Ibhade	+ + +	+ – –	+ – –	9

4.2 Cultural and Biochemical Characteristics of Isolates

The results presented in the table show the growth of bacterial isolates on Eosin Methylene Blue (EMB) agar, which is selective for coliforms. Tap water (T1) and well water (W1) both demonstrated growth on EMB agar, producing pink to purple colonies. This indicates the presence of coliform bacteria in these water sources, suggesting contamination likely due to poor sanitation, faecal pollution, or inadequate treatment.

Among the sachet water samples, Aysad (I1), Caliphate (I2), and Alkanchi (I3) showed no growth on EMB agar, which indicates the absence of coliforms. These brands comply with microbiological safety standards and are considered safe for consumption. In contrast, Ibhide (I4) showed growth on EMB with pink to purple colonies, indicating the presence of coliforms. Although the contamination level is lower than that of tap or well water, it signifies a hygienic lapse during production, handling, or storage, rendering it unsafe according to WHO and SON guidelines.

Overall, the EMB results confirm that well and tap water are contaminated with coliforms, while most sachet water brands are free from coliforms except Ibhide, which requires caution before consumption.

Table 2: Cultural and Biochemical Characteristics of Isolates

Isolate Code	Sample Source	Growth on EMB	Colony Characteristics (EMB)	Interpretation
T1	Tap Water	Yes	Pink / purple colonies,	Coliforms present
W1	Well Water	Yes	Pink / purple colonies	Coliforms present
I1	Aysad	No	-	No Coliforms
I2	Caliphate	No	-	No Coliforms
I3	Alkanchi	No	-	No Coliforms
I4	Ibhade	Yes	Pink / purple colonies	Coliforms present

CHAPTER FIVE

5.0

DISCUSSION

This study examined the microbial quality of sachet water sold in and around Arkilla in Sokoto and compared it with tap water and well water from the same area. The results give a clear picture of how safe these water sources are and how well sachet water producers are following basic hygiene and treatment standards.

The findings show that well water had the highest level of contamination. Its MPN value of 150 coliforms per 100 milliliters confirms heavy pollution, most likely from faecal matter. This type of contamination is common in wells that are shallow, poorly constructed or located close to latrines and drainage channels. The result agrees with earlier reports that untreated groundwater in Nigeria often contains high levels of coliforms and should not be consumed without treatment. The presence of coliforms in this sample shows that the well water is unsafe for drinking and poses a serious risk of diseases such as typhoid, dysentery and cholera.

Tap water also showed contamination with an MPN value of 28 coliforms per 100 milliliters. Although this is lower than the well water, treated municipal water is expected to have zero coliforms. This suggests that contamination may have occurred after treatment, possibly through damaged pipes, dirty storage tanks or poor handling

during distribution. These findings are similar to reports from other Nigerian cities where treated water becomes contaminated before it reaches consumers.

The sachet water samples showed mixed results. Three brands, Aysad, Caliphate and Alkanchi, had no detectable coliforms and showed no growth on EMB agar. This means they met microbiological safety standards. These results indicate that these producers likely maintain good hygiene, use effective filtration or treatment systems and handle their products properly during packaging. The absence of coliforms supports the idea that when sachet water is produced under controlled conditions it can be safe for drinking.

The Ibhade sachet water sample, however, recorded an MPN value of 9 coliforms per 100 milliliters and produced pink to purple colonies on EMB agar. This confirms the presence of coliforms and shows that its production process was compromised. The contamination could have occurred through poor hygiene in the factory, the use of untreated source water, faulty sealing equipment or contamination during storage and transport. Although the level of contamination was lower than that of tap or well water, any amount of coliforms in packaged water is unacceptable according to WHO and NAFDAC guidelines.

Overall, the results highlight the fact that sachet water can be safe when producers follow required standards, but lapses still occur. The findings also show that untreated well water and poorly maintained tap water systems pose significant public health risks in Sokoto. This reinforces the need for stronger regulatory oversight, routine inspections and improved hygiene practices within the sachet water industry. It also points to the importance of educating consumers on safe water handling and encouraging them to identify properly registered and trusted water brands.

CONCLUSION

The study concludes that microbial contamination remains a public health concern in the study area. Well water and tap water were unsafe for drinking based on coliform presence. Most sachet water brands tested complied with expected microbiological standards, suggesting proper treatment and packaging by producers. However, the contamination found in Ibhade sachet water shows that lapses in hygiene and quality control still occur within the sachet water industry. Regular monitoring, strict regulatory enforcement, and improved factory practices are essential to ensure that all packaged water meets safety requirements.

RECOMMENDATIONS

Based on the findings, the following recommendations are made:

1. **Strengthen regulatory monitoring**

Agencies such as NAFDAC and SON should conduct more frequent inspections and enforce strict compliance with microbiological safety standards for all sachet water producers.

2. **Improve hygiene in production facilities**

Producers should ensure proper filtration, sterilization, and packaging procedures. Any brand found to have coliform contamination should undergo immediate review and corrective action.

3. **Routine quality assessment**

Sachet water producers should routinely test their products in accredited laboratories to ensure ongoing compliance.

4. **Consumer awareness**

5. The public should be encouraged to check for NAFDAC registration numbers, expiry dates, and integrity of sachets before consumption.

6. Proper handling and storage

Retailers and distributors should avoid storing sachet water under direct sunlight, as heat encourages bacterial growth.

7. Government investment in water infrastructure

Improving the reliability and quality of public water supply will reduce dependence on sachet water and help prevent waterborne diseases.

8. Further research

Expanded studies covering more brands and additional microbial parameters such as fungi and pathogenic bacteria would give a broader understanding of water quality in Sokoto.

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