BACTERIOLOGICAL ANALYSIS OF SACHET WATER SOLD IN ASOKORO, FEDERAL CAPITAL TERRITORY ABUJA.

BY

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DECLARATION

I hereby declare that this project has been written by me and it is a report of my research work. It has not been presented in any previous application for state diploma or degree. All quotations are indicated and sources of information specially acknowledged by means of references.

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16/02/2021

Date

CERTIFICATION

This is to certify that this project titled "Bacteriological Analysis of Sachet Water Sold in Asokoro, Federal Capital Territory Abuja. Nigeria" meets the regulations governing the award of Postgraduate Diploma in Microbiology, of the School of Postgraduate Studies. Nasarawa State University, Keffi, and is approved for its contribution to knowledge.

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DEDICATION

This research project work is dedicated to the almighty GOD who has always been the source of my strength.

ACKNOWLEDGEMENT

My immeasurable gratitude goes to almighty GOD my healer the one that started this program with me and giving the strength throughout my this program.

My profound gratitude goes to lovely husband Folorunso John oladipo for his love, patient, moral support and funding throughout my program.

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ABSTRACT

Water is a chemical substance that is composed of hydrogen and oxygen. It is vital for all known forms of life and the most abundant natural essential commodity since it covers 71% of the earth's surface. However, cleans drinking water is essential to humans and other forms of life. Good quality water is odourless, colourless, tasteless, and free from faecal pollution.This study aim at determining the bacteriological analysis of sachet vended water sold in Abuja metropolis. A total of 10 sachet water samples of different brands with NAFDAC certification number were bought from different vendors within Abuja and was transported to the microbiology laboratory for examination. Standard microbiological techniques was used in carrying the water analysis. Result from this studies of sachet water from different locations recorded the occurrence of the following bacteria. *Escherichia coli Kebsiella sp and Enterobacter sp* in sachet water samples vended in Abuja. From the isolated organism *E.coli* (42.9%), *Enterobacter spp* (28.6%) and *Klebsiella spp* (28.6 %.). NAFDAC should ensure and enforce strict compliance to the standards as regards the production and sales of packaged sachet water and Residents should be aware of a possible danger of consumption of poorly treated water especially the borehole and well water and the potential health risk associated with such water if consumed.

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

Water is a chemical substance that is composed of hydrogen and oxygen. It is vital for all known forms of life and the most abundant natural essential commodity since it covers 71% of the earth's surface. However, cleans drinking water is essential to humans and other forms of life. Water fit for human consumption is called potable water or drinking water. To function properly, the body requires between one to seven (1-7) litres of water per day to avoid dehydration (Ajayi, 2005). This depends on the level of acidity, temperature, humidity, and amount of alcohol and caffeine intake (Campbell *et al.*, 2006).

The safety of drinking water is a worldwide public health concern. The World Health Organization (WHO) estimated that 1.1 billion of the world"s population does not have access to safe water. In addition, 80 percent of diseases and one-third of deaths in developing countries are due to consumption of contaminated water (WHO, 2011). In Nigeria, it is reported that the incidence of acute watery diarrhoea is approximately 4.9 episodes per year and there are approximately 200,000 diarrhoea related deaths of children aged below five years with an average of 300 deaths per day (United Nations, 2012). Great concern must therefore be given tothe quality of drinking water which is very critical in maintaining good health and for the overall socio-economic development of any society. Due to the scarcity of safe drinking water in Nigeria, communities obtain their potable water in form of sachet water, popularly referred to as „pure water". It is the most common source of drinking water in Nigeria, given that it is relatively cheap,

accessible and generally perceived to be of better quality (Stoler, 2012). Drinking water industries in Nigeria, mostly owned by private institutions obtain water from surface or underground sources and since both types of water can become contaminated by biological and chemical pollutants originating from point and nonpoint sources, it is usually followed by various treatment processes. Bacterial growth in drinking water depends on concentration of assimilated organic carbon, limiting nutrients, disinfectant concentration, type of pipe material, packaging nylons and a number of other factors such as; pH, temperature, hardness, redox potential that controls the growth of microorganisms on pipe surfaces (Lehtola *et al.,* 2002; Ollos *et al.*, 2003). Many of the bacteria isolated in water distribution systems are opportunistic pathogens. The presence of high numbers of opportunistic pathogens in drinking water is of concern because these microorganisms can lead to infection of certain segments of the population (newborn babies, the sick, and the elderly) (Bitton, 2005). According to the guideline set by the World Health Organisation, quality drinking water must not contain *Escherichia coli* or thermotolerant coliform bacteria, giardia worms, viruses, *Cryptosporidium* spp, *Legionella pneumophila, Entamoeba hystolitical* and other opportunistic pathogens such as *Clostridium* sp., *Klebsiella* sp. and *Pseudomonas* (WHO, 2011). The guideline further stated that the water should be tested against the presence of highly virulent pathogens such as *Salmonella typhi, Shigella dysenteriae* and *Vibrio cholerae* that are responsible for typhoid, bacillary dysentery and cholera diseases respectively which arises due to high level of organic decay and fermentation on tropical waters. All these bacteria must not exist in water that are meant for drinking, hence, sources of water for packaged water are subjected to laboratory test by public analyst in which any of the bacteria must not be

found or detected in any 100ml water sample. Various studies carried out on sachet water from different cities have revealed the lack of purity of sachet drinking water in Nigeria (Oladipo *et al.*, 2009; Afiukwa *et al.*, 2010; Edema *et al.*, 2011; Akpoborie, and Ehwarimo, 2012; Onilude *et al.*, 2013). This is attributed to sharp practices, poor hygiene of vendors, polluted environment, and non-adherence to WHO/NAFDAC regulations (Omalu *et al.*, 2010). It is therefore imperative that the quality of sachet water vended be continuously assessed for the protection of public health.

1.2 Statement of Problem

A reliable supply of clean wholesome water is highly essential in a bid to promote healthy living among the inhabitants of defined geological region in addition to being key to sustainable development, health, food production and poverty reduction (Balogun *et al.*, 2014). In many developing countries however, availability of quality water has become a crucial and urgent problem and has become a matter of concern to families and communities (Maduka *et al.*, 2014). It is estimated that 1.2 billion people around the world or one fifth of world's population lack access to safe water either because of unavailability or inadequacy This could probably be due to increase in human and animal population (Meduka *et al.*, 2014; Adegoke *et al.*, 2012) or because potable water now attracts increased rates and fees which many cannot afford due to poverty or because of rapid industrialization, urbanization and new uses of treated water (Mojekeh and Eze, 2011; Gangil *et al.*, 2013). Many therefore result to taking unsafe water which places them at risk of different diseases resulting in public health challenges (Maduka *et al.*, 2014).

1.3 Justification of the Studies

The safety of drinking water is a worldwide public health concern. In Nigeria, it is reported that the incidence of acute watery diarrhoea is approximately 4.9 episodes per year and there are approximately 200,000 diarrhoea related deaths of children aged below five years with an average of 300 deaths per day (WHO 2011). However, Great concern must therefore be given to the quality of drinking water which is very critical in maintaining good health and for the overall socio-economic development of any society through accessing the microbiological standard of those sachet vended waters.

1.4 Aim and objectives

Aim

This study aim at determining the bacteriological analysis of sachet vended water sold in Abuja metropolis.

Objectives

1.5 The specific objectives of this study are to,

Isolate and identify microorganism (bacteria) associated with sachet water vended in Abuja.

Determine the bacteriological analysis of the sachet water vended in Abuja.

CHAPTER TWO

LITERATURE REVIEW

2.1 Study Background

Good quality water is odourless, colourless, tasteless, and free from faecal pollution. 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 (Ezeugwunne *et al.*, 2009). Safe and potable water supplies in urban centers 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 (Ajayi *et al.*, 2013). 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. 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 (CAMON 2007). 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 (Oladipo *et al.*, 2009). 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. (CAMON 2007). 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 endproduct, 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.

Water is a vital substance for the survival of all lives (Thliza *et al.*, 2015). It is absolutely necessary for most life driven processes. It is one of the indispensable resources needed for the continued existence of all living things including man (Gangil *et al.*, 2013). It is also one of the most important and most abundant compound on earth, vital to any organism's survival. Water is one of the most important needs of all forms of life and is unavoidable in man's daily life, constituting a sizeable percentage of man's daily food intake because human bodies do not have reserve supply. It is also an essential requirement of life for drinking, domestic, industrial and agricultural uses. Quality water is colorless, tasteless, odourless, as well as free from faecal contamination (Opara and Nnodim, 2014). Water is an essential part of human nutrition and is required for maintenance of personal hygiene, food production and prevention of diseases (Thliza *et*

al., 2015) It is the most abundant substance in nature and occupies about 70% of the earth's crust (Anyamene and Ojiagu, 2014; Thliza *et al.*, 2015). Due to its natural abundance and also because the protoplasm of many living cells contain about 80% water and most biochemical reactions which occur in the metabolism and growth of living cells involves water medium it is considered a universal solvent (Nwosu and Ogueke, 2004; Okechukwu and Chikezie, 2014). Water is a biological medium which exists as solid, liquid and gas (Thliza *et al.*, 2015).

2.2 Sachet Water

Access to safe drinking water is important to health and development (Adetunde *et al.*, 2014), but because of its inadequacy and government's inability to provide enough, a number of small scale water producing industries are packaging and marketing factory filled sachet drinking water (Thliza *et al.*, 2015). These are small nylon sachets containing 0.5L of water which are electrically heated and sealed at both ends (Adegoke *et al.*, 2012). The sale and consumption of packaged water continue to grow astronomically and rapidly in most countries of the world (Mgbakor *et al.*, 2011; Oyedeji *et al.*, 2010; Gangil *et al.*, 2013). The quest for cheap and readily available source of potable water has led to the emergence of sachet water (Anyamene and Ojiagu, 2014) which is a locally sourced low cost alternative drinking water scheme providing sustainable access to safe water in rural and semi urban settings of developing nations (USEPA, 2012; Balogun *et al.*, 2014). This is thought to be cheaper and more affordable than bottled water and also safer, more hygienic and better than hand filled, hand tied packaged polythene bag water initially popularly sold (Oyedeji *et al.*, 2010; Akinde *et al.*, 2011). Consequently sachet water has gradually become the most consumed liquid for

both the rich and poor (Akinde *et al.*, 2011). Current trends however unfortunately suggest that sachet water could be a route of transmission of enteric pathogens which raises issues of the problem of its purity and health concern (Oladapo *et al.*, 2009; Mgbakor *et al.*, 2011).

2.3 Package Water Contamination

Packaged water, no matter their sources, are susceptible to microbial, toxic organic and inorganic contamination (Anyamene and Ojiagu, 2014; Sudhakar and Manatha, 2004; Gangil *et al.*, 2013). The presence of coliforms in potable water is used as indicator of water contamination (Opara and Nnodim, 2014). Coliform bacteria describe a group of enteric bacteria that include *E. coli*, *Klebsiella* and *Enterobacter* species. They are rod shaped, gram negative organisms which ferment lactose with the production of acid and gas when incubated at 37°C for 48 hours. They are broad class bacteria that live in the digestive tract of humans and animals (Opara and Nnodim 2014). Although coliforms are generally not harmful, they indicate the presence of pathogenic bacteria, viruses and protozoa (Anyamene and Ojiagu, 2014; Goel, 2006).

Escherichia coli is used as indicator of possible recent sewage/ faecal contamination (Anyamene and Ojiagu, 2014; Onuh and Isaac, 2009; Opara and Nnodim, 2004) because this is one of the first bacteria present in water when contamination occurs and will present in larger quantities than some other pathogenic microbes. Other microbial indicators of possible faecal, soil and natural water contaminations are faecal *Enterococci* especially *Enterobacter faecalis*, *Clostridium perfringens* spores, *Clostridium sporanges*, *Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *Klebsiella* spp. *Aeromonas* spp., *Mycobacterium* spp., *Alcaligens*, *Actinetobacter*,

Chromobacterium, *Serratia spp*, *Flavobacterium spp.*, *Proteus* spp., *Bacillus subtilis*, *B. mycoides*, *Enterobacter cloaca*, *Enterobacter aeronenes*, *Nostocida fexibacter* and *Norcardia* spp. (WHO, 2011; Edema *et al.*, 2001). These could cause different disease conditions and clinical signs such as giardiasis, cryptosporidiosis, gastroenteritis, diarrhea, typhoid fever, cholera, bacillary dysentery, hepatitis, shigellosis *etc.* (Akinde *et al.*, 2011; Thliza *et al.*, 2015; Oyedeji *et al.*, 2010; Isikwue and Chikezie, 2014; Aroh *et al.*, 2013; Hughes and Koplan, 2005). Water borne diseases are reported to account for 80% of illnesses in developing world, killing a child every 8 seconds. This is a global public health threat (Hughes and Koplan, 2005).

2.4 Predisposing factor to package water contamination

Various factors predispose packaged water to contamination. These include contaminated sources of water ranging from rain water, shallow well water, rusty unwashed tanker to other contaminated sources (Dibua *et al.*, 2007). Some contaminants enter packaged water through seepage of sewage and rainfall runoffs into well water and exposed boreholes (Defives *et al.*, 1999; Adegoke *et al.*, 2012). Introduction of contamination during the process of production due to contaminated materials or external introduction of contamination from vending machines etc. (Omalu *et al.*, 2010). Prolonged storage of packaged water at favorable environmental conditions aiding total aerobic heterotrophic bacteria to grow to level that may be harmful to humans. The unhygienic conditions and filthy environments in which some of the water packaging companies operate especially in developing countries could introduce contamination to packaged water. These companies are mostly not registered and are engaged in sharp, questionable practices which are encouraged by the irregular and ineffective monitoring of authorities that

enforce standard. They therefore, do not follow international quality standards nor appropriate methods of water treatment (Oyedeji *et al.*, 2010; Ofori, 2011; 2011; Mensah, 2011; Danso-Boateng and Falegan *et al.*, 2014; Isikwue and Chikezie, 2014). Improper handling by hawkers could be another reason why packaged water is contaminated (Adegoke *et al.*, 2012). Absence of sterilization procedures such as pasteurization and thermal sterilization for the treatment of packaged water also increase susceptibility to contamination (Anyamene and Ojiagu, 2014). Ignorance on the implication of carelessness in the production line by producers with sole aim of making profit thereby disregarding safety and quality in producing water for the public (Dodoo *et al.*, 2006; Ackah-Arthur *et al.*, 2012). Use of non feed grade sachets for water production and the release of packaged water to the public without production and expiry date markings could also enhance sachet water contamination.

2.5 Typical Package Water Production Process

Typical production process of packaged water (Adetunde *et al.*, 2014).

Source:

bore-hole / deep well water with submersible pumps

↓

Raw water tank storage (usually PVC)

↓

Industrial modules (consisting of sand bed filter and activated carbon

↓

Water tank (PVC)

↓

Micro-filters 1, 2, 3(5μm -2μm-0.5μm, respectively)

↓

UV sterilizer (attached to sachet water machine)

↓

Packaging (automatic filling machine and heat sealing of polythene sachets)

↓

Sachets stocked in bigger bags ready for distribution

Fig. 1- Flow chart of water production process

There are various water treatment processes, the major procedures are aeration, coagulation, flocculation, sedimentation, slow sand filtration, rapid filtration and disinfection (Ibemesim, 2014). Small scale package water production factories usually employ boiling, sedimentation, coagulation and filtration. Water is brought to a good

rolling boiling point for about 15-20 minutes to destroy microorganisms present, drive out dissolved gases and give a flat taste (Lorch, 2007). Multiple candle pressure filter which employ active carbon filter beds are commonly used in packaged water production to remove contaminants like sand, rust, metal, sediments, algal films and bacteria while sealing is done using heating sealing machines.

2.6 Public health implication of microbial pollution of commercially sold package water

Water has always been associated with disease. The first reported case of polluted drinking water was that of Broad street water pump, London after which many other cases have been reported in different parts of the world (Gangil *et al.*, 2013). Conditions resulting from microbial contamination of drinking water is said to affect a large part of the world's population (Hughes and Koplan, 2005) and continue to be one of the major health problems globally (Boubetra *et al.*, 2011). It accounts for 80% of illnesses in developing world (Isirimah, 2003).

Microbial pollution of packaged water particularly in developing countries has grave implications on public health (Warren and Mark, 1998). It threatens the population's existence causing diseases such as cholera (Njoku and Osondu, 2007; Ackah-Arthur *et al.*, 2012), gastroenteritis, hepatitis, typhoid fever, shigellosis Banu and Menakuru, 2010; Anyamene and Ojiagu, 2014) and giardiasis (Opara and Nnadim, 2014). It could also result in symptoms such as bacillary diarrhea. WHO estimates that 88% of diarrhea cases is caused by unsafe water (CAWST, 2009; WHO, 2004). Mortalities due to water associated diseases and symptoms now exceeds 5 million people per year (Cabral, 2010). Studies carried out on packaged water around the world revealed high level contamination of microorganisms which indicated that most of the brands were unfit for

human consumption. They were found to be of doubtful quality and did not meet the WHO drinking water standards. Consumption of these packaged water brands could result in public health hazard. This, therefore, requires concerted effort on the part of all stake holders to correct the anomaly.

2.7 Acceptable microbial load in water

Microbial limit in portable water (SON 2007; CSF 2014)

Microbiological characteristic parameter	Maximum permitted level
Total coliform count	10cfu/ml
Faecal coliform count	0cfu/ml

2.8 Physical Examination:

Previous studies on sachet water phenomenon in Nigeria have shown that factors responsible for its contamination range from sharp practices, poor hygiene of vendors, polluted environment, and non-adherence to WHO/NAFDAC regulations. This examination involves external features such as label which include product information, specific odour, appearance which includes colour, turbidity, and presence of floating particles or ex-traneous materials. Dada4 in his study on sachet water contamination physically examined samples of 'pure water' from the Nigerian market and recorded that none of the identified brands met the compliance levels set by the regulatory authorities in terms of label requirements such as registration number and batch numbers,

manufacturing and expiry dates, nutritional information, net volumes and sometimes producers names and contact addresses (Cabral, 2010)

2.9 Challenges of safe water supply

A reliable supply of clean wholesome water is highly essential in a bid to promote healthy living among the inhabitants of defined geological region in addition to being key to sustainable development, health, food production and poverty reduction (Balogun *et al.*, 2014). In many developing countries however, availability of quality water has become a crucial and urgent problem and has become a matter of concern to families and communities (Maduka *et al.*, 2014).

It is estimated that 1.2 billion people around the world or one fifth of world's population lack access to safe water either because of unavailability or inadequacy (Oyelude and Ahenkorah, 2011; Akinde *et al.*, 2011; Adebayo *et al.*, 2012). This could probably be due to increase in human and animal population (Meduka *et al.*, 2014; Adegoke *et al.*, 2012) or because potable water now attracts increased rates and fees which many cannot afford due to poverty (Oyelude and Ahenkorah, 2011; Adewoye *et al.*, 2013; Balogun *et al.*, 2014) or because of rapid industrialization, urbanization and new uses of treated water (Mojekeh and Eze, 2011; Gangil *et al.*, 2013). Many therefore result to taking unsafe water which places them at risk of different diseases resulting in public health challenges (Maduka *et al.*, 2014).

2.10 Portable Water

Potable water is that which one will not mind to put in his pot, water that is safe for cooking and drinking, containing less than 1000 ml per liter dissolved solids, does not contain chemical substances and microorganisms in amounts that could cause hazard to

health. It should be clear, without disagreeable taste, color or odor therefore fit for human and animal consumption. It is water that has been treated, cleaned or filtered to meet established drinking standards (Aroh *et al.*, 2013; Isikwue and Chikezie, 2014). It can be sourced from surface water such as river, streams or the ground water such as spring, well and borehole (Aroh *et al.*, 2013).Water quality is the physical, chemical, radiological and biological characteristics of water in relation to the requirements of human and animal need (Johnson *et al.*, 1997; Diersing, 2009; Danso-Boateng and Frimpong, 2013). Agencies that regulate water qualities worldwide rely on the World Health Organization (WHO) standards and guidelines for drinking water quality (WHO, 1993, 1996, 2001 and 2002). So as to ensure the highest quality of potable water in order to safe guard public health (Anunobi *et al.*, 2006; Isikwue and Chikezie, 2014).

2.11 Water Born Diseases

Quite a number of human pathogens find their way into a susceptible host through contaminated water. These pathogens often called waterborne pathogens, have the ability to survive at least for a short period in water and thus water may act as a route of transmission for them. Waterborne diseases are posing a serious threat to health since the potential of contaminated water to transmit disease is very high. Often they lead to epidemic. According to a WHO survey about 30,000 people die from water-related diseases every day. About 80% of all illness in developing countries is water related (Jan and Robert, 2002). Water plays an essential role in the spread of many communicable diseases and epidemics. Diarrhoeal diseases, mostly caused by poor hygiene and lack of safe water, are a major cause of morbidity and mortality among refugee and displaced populations. Large scale and severe outbreaks have occurred frequently, particularly in

the initial phase of a refugee crisis situation. The most striking example is that reported among Rwandan refugees in Goma (Zaire) during the 1994 Genocide, where extremely high mortality rates were associated with explosive epidemics of Cholera and shigellosis (Medecins Sans Frontieres, 2008).

Acute microbial diarrheal diseases are a major public health problem in developing countries. People affected by diarrheal diseases are those with the lowest financial resources and poorest hygienic facilities. Children under five, primarily in Asian and African countries, are the most affected by microbial diseases transmitted through water (Seas *et al.*, 2000). Microbial waterborne diseases also affect developed countries. In the USA, it has been estimated that each year 560,000 people suffer from severe waterborne diseases, and 7.1 million suffer from a mild to moderate infections, resulting in estimated 12,000 deaths a year (Medema *et al.*, 2003).

2.12 Common Water Born Diseases Associated with Drinking Water

Table 2

Disease	Causal Bacterial Agent
Cholera	*Vibrio cholerae*, serovarieties O1 and O139
Gastroenteritis	Mainly *Vibrio parahaemolyticus*
Typhoid fever and other serious salmonellosis	*Salmonella enterica* subsp. *Enteric* serovar Paratyphi
	Salmonella enterica subsp. *Enterica* serovar Typhi
	Salmonella enterica subsp. *Enteric* serovarTyphimurium
Bacillary dysentery or shigellosis	*Shigella dysenteriae*
	Shigella flexneri
	Shigell aboydii
	Shigella sonnei
Acute diarrhoeas and gastroenteritis	*Escherichiacoli*, particularly serotypes such as O148, O157 and O124

(Medema *et al.*, 2003).

2.13 Important Water Quality Parameters and their Associated WHO Standards

2.13.1 pH

Dodoo *et al.*, (2006) stated that in chemistry, pH is the measure of the acidity or basicity of an aqueous solution. Solutions with a pH less than 7 are said to be acidic and solutions with a pH greater than 7 are basic or alkaline. Drinking water has a pH of 6.5 - 9.5 (WHO, 2011).

2.13.2 Turbidity

Turbidity is a principal physical characteristic of water and is an expression of the optical property that causes light to be scattered and absorbed by particles and molecules rather than transmitted in straight lines through a water sample. It is caused by suspended matter or impurities that interfere with the clarity of water (WHO, 2003).

Excessive turbidity is aesthetically unappealing, and may represent a health concern. If not removed, turbidity can promote regrowth of pathogens in the distribution system leading to water borne diseases outbreak which have caused significant cases of gastroenteritis throughout the United States and the world (WHO 2003, 2011).

2.13.3 Total Hardness

Hardness in water is caused by dissolving calcium and, to lesser extent, magnesium. It is usually expressed as the equivalent quantity of calcium carbonate. Depending on pH and alkalinity, hardness above 200 mg/L can result in scale deposition, especially on heating. Soft water hardness of less than 100 mg/L have a low buffering capacity and tends to be corrosive (WHO, 2011). According to WHO (2003) hardness in excess of 500 mg/L is tolerable.

2.13.4 Conductivity

Conductivity is the measure of water's ability to conduct an electric current. The conductivity of water depends on the concentration of dissolved solids in the water. Conductivity can be used as a diagnostic tool for interpreting other water quality information. Water with high conductivity indicates high concentration of dissolved solids. High concentration of dissolved solids of a drinking water gives it an unpalatable taste and can cause gastrointestinal distress (WHO, 2011).

2.13.5 Colour

Clean water should be clear with no noticeable colour deposits. Colour in drinking water may be due to the presence of organic matter such as humid substances, metals such as iron and manganese, or highly coloured industrial waste (Obiri-Danso *et al.*, 2003). Experience has shown that consumers may turn to alternative, perhaps unsafe sources when their water displays aesthetically displeasing levels of colour typically exceeding 15ptco. Drinking water should ideally be colourless (WHO, 2011).

2.13.6 Microbial load (Total Coliform)

The microbiological quality of drinking water is a concern to consumers, water suppliers, regulators and public health authorities. The number of disease outbreaks that have been reported throughout the world demonstrates that transmission of pathogens by drinking water remains a significant cause of illness. However, estimates of illness based solely on detected outbreaks are likely to underestimate the problem (Dufour *et al*, .2003)

According to WHO (2011), the objective of zero coliform (total and faecal coliforms) count per 100ml of water is the goal for all water supplies and should be the target even in emergencies.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was design to covered some areas of Abuja. Abuja is the capital city of Nigeria located in the centre of the country within the federal capital territory (FCT 2015)

3.2 Collection of samples

A total of 10 sachet water samples of different brands with NAFDAC certification number were bought from different vendors within Abuja and was transported to the microbiology laboratory of nasarawa state University for analysis.

3.3 Culture Media

The culture media use was Nutrient agar to determine the total viable bacterial count, Eosin Methylene Blue agar to enumerate *Escherichia coli,* and MacConkey agar for Coliform count.

3.4 Media Preparation

Culture media were prepared according to the respective Manufacturers' specification and sterilized in an autoclave at 121^0 for 15 minutes.

3.5 Isolation of Bacteria

Bacterial was isolated using standard microbiological techniques.

3.6 Determination of Total Coliform Bacteria

This was determined using the most Probable Number (MPN) approach according to (APHA, 1998) 10ml of MacConkey broth was poured in 15 test tubes using sterile syringe, with 5 from the 15 test tubes containing double strength of MacConkey broth. 10ml of water sample was inoculated in the first five test tubes containing double strength

of MacConkey broth, 1ml of water was inoculated in the second five test tubes while 0.1ml of water was inoculated in the last five test tubes. The test tubes were kept in an incubator and observed at the end of 24- 48 hours. The number of positive bottles, was indicated by a colour change and gas formation in each row and was recorded and bacterial load determined from the Most Probable Number.

3.7 Identification of Isolates

A loopful of broth from the positive tubes on the presumptive test was streaked onto MacConkey agar plates and incubated at 37^0c for 18-24 hours. Isolates were further identified by colonial morphological characteristics, gram stain, motility and biochemical tests which include; indole, methyl red, citrate, oxidase, urease, catalase and voges-proskauer test.

3.8 Gram staining

Briefly, a drop of distil water was placed on a glass slide and colonies of the suspected organism from the cultured plates were emulsified. The slide was then be allowed to dry by air and then passed through a Bunsen flame twice so as to fix the smear properly. The slide was then flooded and rinsed thereafter with slow running tap water. The rinsed slide was further flooded with Lugols iodine for 30seconds and immediately decolorized with acetones and rinsed with running tap water the slide was thereafter counter stained with safranin and rinsed on slow running tap water again. The rinsed slide was carefully bloated and examined under microscope using oil immersion objective (×100). Gram negative bacteria appear pinkish or reddish, while Gram positive bacteria appear purple or blue.

3.9 Biochemical Identification

Biochemical Test were performed for re- identification and these test include:

3.9.1 Catalase

This test demonstrates the presence of enzyme catalase in the organism.

Procedure

- The work bench was disinfected with 70% alcohol
- A stand alone colony of the organism was emulsified in 3% hydrogen peroxide on clean grease-free slide. Positive organism showed effervescence.
- The same procedure was applied to other isolates.

3.12.2 Citrate

The principle of this test is that the test organisms is cultured in a medium which contains sodium citrate, an ammonium salt and indicator bromothymol blue.

Procedure

- Inoculum was streaked over the slant of Simmon's citrate agar in a tube.

The tube was incubated for 24-48 hrs.

Result Interpretation

Growth on the slant and change in colour to blue of the medium indicates positive result.

3.9.3 Indole

Indole test was performed to determine the ability of the organism to split tryptophan molecule into indole.

Procedure

- Tryptone broth was inoculated with the test organism
- It was incubated for 18-24 hours at 37^0C
- 15 drops of Kovac's reagent was added down the inner wall of the tube.

Result Interpretation

Development of bright red color at the interface of the reagent and the broth within secon ds after adding the reagent is indicative of the presence of indole and is a positive test.

3.9.4 Methyl Red

To test the ability of the organism to produce and maintain stable acid end products from glucose fermentation and to overcome the buffering capacity of the system.

Procedure

- The MR/VP broth was inoculated with a pure culture of the test organisms and was incubated at 35^0C for 48-72 hours.
- 5 drops of MR reagent was added to the broth.

Result Interpretation

Positive result is red (indicating pH below 6). Negative result is yellow (indicating no acid production).

3.12.5 Voges proskeur

To determine the ability of the organisms to produce neutral end product acetyl methyl ca rbonyl (acetoin) from glucose fermentation.

Procedure

- Pure culture of the organism was inoculated into MR/VP broth and was incubated for 24 hours at 37^0 C.
- 1ml of the broth was transferred to a sterile test tube.
- 0.6ml of VP (A) was added followed by 0.2ml of VP (B)
- The tube was shaked gently to expose the medium to atmospheric oxygen and was allowed for 10-15 minutes.

Result Interpretation

Positive result shows pinkish colour at the surface of the medium and negative result shows yellow colour at the surface of the medium.

3.9.6 Oxidase Test

Oxidase test was used to determine the presence of bacterial cytochrome oxidase enzymes

Procedure

- A filter paper was soaked with the substrate tetramethyl-p-phenylenediamine
- The paper was moisten with a sterile distilled water
- The colony of the organism to be tested was picked with a sterile planium loop and a smear was made on the filter paper
- The inoculated area of the paper was observed for colour change to deep blue or purple within 10-30 seconds

Result Interpretation

Positive result shows purple colour and negative shows no colour

CHAPTER FOUR

RESULT

4.1 Isolation of microorganism

Table 4.1 result from isolation of microorganism from sachet water

Sample	brand	media used	presumptive organism
Sample 1	A_1	EMB/Mac	*E. coli*
Sample 2	A_2	EMB/ Mac	*Enterobacter spp*
Sample 3	A_3	EMB /Mac	*E. coli*
Sample 4	A_4	EMB/ Mac	*klebsiella spp*
Sample 5	A_6	EMB /Mac	*E. coli*
sample 6	A_7	EMB / Mac	*Enterobacter spp*
sample 7	A_8	EMB/ /Mac	*Klebsila spp*
Sample 8	A	EMB/Mac	No growth
Sample 9	A	EMB/Mac	No growth
Sample 10	A	EMB/Mac	No growth

key

EMB = Eosine methylene blue

Mac = MacConky

E.coli = Escherichia coli

It was also found from this study that result from isolation of microorganism from sachet water comprise some of the following organism *E. coli*, *Enterobacter spp, klebsiella spp,* and *Klebsila spp.*

4.2 Percentage rate

Table 2 percentage occurrence of the organism isolated

Sample	*enterobacter spp %*	*E.coli %*	*Kebsiella spp %*
Sample 1	0(0.0)	2(28.6)	0(0.0)
Sample 2	1(14.3)	0(0.0)	0(0.0)
Sample 3	0(0.0)	1(14.3)	0(0.0)
Sample 4	0(0.0)	0(0.0)	1(14.3)
Sample 5	0(0.0)	1(14.3)	0(0.0)
Sample 6	1(14.3)	0(0.0)	0(0.0)
Sample 7	0(0.0)	0(0.0)	1(14.3)
Sample 8	0(0.0)	0(0.0)	0(0.0)
Sample 9	0(0.0)	0(0.0)	0(0.0)
Sample 10	0(0.0)	0(0.0)	0(0.0)
Total	2(28.6)	4(57.2)	2(28.6)

Key *E.coli* = *Escherichia coli.*

The percentage occurrence of the organism isolated shows that sample has the highest occurrence of 2 with 28 percentage while the leas occurrence is 1 with 14 percentage.

Table 4.3 Cultural, Morphological and Bochemical identification of organism isolated from boys and girls hostel water tank

Table 4.3.1: Cultural, Morphological and Biochemical Identification of Organisms

RESULTS

Cultural Characteristics	Morphological Characteristics		Biochemical Tests						Inference Isolate
	Gram Stain	Shape	IND	MR	VP	CT	OXD	CAT	
1. Pinkish colouration on MAC and greenish metallic sheen on EMB	-	rods	+	+	-	-	-	-	*Escherichia Coli*
2. Grows as yellow mucoid colonies on and increase in size after 48hrs	+	rods	-	-	+	+	-	-	*Klebsiella sp*
3. pinkish mucoid colonies on MAC	+	rods	-	-	+	+	-	-	*Enterobacter spp*

key IND = Indole , MR = Methyl red, VG = Voges proskeur, CT = Citrate, OXD = Oxidase, CAT = Catalase.

Mac =Macconky

EMB = Eosine methylene blue .

4.4 Total viable count (CFU/ml)

Table 4 total viable count (cfu/ml)

Sample	brand	TVC (cfu /ml)
Sample 1	A_1	$1.3x10^6$
Sample 2	A_2	$1.2x10^6$
Sample 3	A_3	$1.1x10^6$
Sample 4	A_4	$1.4x10^6$
Sample 5	A_6	$1.9x10^6$
Sample 6	A_7	$1.2x10^6$
Sample 7	A_8	$1.6x10^6$

Total viable count (cfu/ml, sample 5 has the highest viable count while Sample 3 has the least viable count.

CHAPTER FIVE

DISCUSSION CONCLUSION AND RECOMMENDATON

5.1 Discussion

The steady upward trend in urbanization in Abuja has led to an annual growth rate in the population this has consequently led to crowding of people in unplanned squatter settlements where provision of basic amenities such as adequate and quality water is costly managed. The current study was therefore designed with the objectives of assessing bacteriological quality of sachet water sold in Abuja.Occurrence of bacteria was recorded in eleven of the sachet-water samples. The total bacterial counts were higher than what is stipulated to be acceptable for portable water (1.0×10^1 cfu/ml). (NAFDAC, 2004). This study also showed the presence of pathogenic bacteria in 70 % of the sachet water samples that were analyzed. High load of coliforms and *E. coli* were found in some of the sachet-waters. WHO standard stated that there must be no pathogenic bacteria in portable water (NAFDAC, 2004). This is an indication that the sachet-water samples are contaminated,

Especially with feacal materials and unsafe for drinking. The presence of relative heavy load of bacteria in water packaged for drinking purposes has been previously documented in literature (Onifade and Ilori, 2008; Oladipo *et al.*, 2009; Oyedeji *et al.*, 2010; Onilude *et al.*, 2013). Microbial contamination of treated and packaged water is due to several factors that contribute to failure of treatment process. They include poor hygienic conditions of the handlers, presence of biofilms on processing surfaces due to poor cleaning and sanitation and packaging of treated water under unhygienic conditions

(Ollos *et al.*, 2003). The bacteria characterized and identified from the sachet water samples were found mostly to be opportunistic pathogens which are usually isolated from unhygienic environments or materials (Bitton, 2005). Reports from this studies of sachet water from different locations recorded the occurrence of these bacteria. *Escherichia coli Kebsiella sp and Enterobacter sp* in sachet water samples vended in Abuja. From the isolated organism *Ecoli* have the highest percentage occurrence of 42.9% followed by *Enterobacter sp* 28.6% and *Klebsiella sp* with the percentage occurrence of 28.6 %. These microorganisms are versatile in their nutrient requirements and can survive with limited nutrient availability. Most of these bacteria are indigenous to aquatic environments (Berger and Oshiro, 2002). The ingestion of these bacteria with contaminated water constitute public health risks to the immune compromised members of the population, especially newborn babies, elderly and sick people. Out of all the faecal coliforms, *E.coli* is always present in the faeces of human, animals, and birds in large numbers. *E.coli* bacteria are rapidly found in water that has been contaminated with faecal matter. Therefore, *E.coli* is an essential indicator of water pollution by faecal material of human and animal origins. In this study, the presence of pathogenic bacteria in sachet water can be attributed to a number of factors such as the raw water source used, hygienic practices observed during production, lack of protective measures due to the common treatment methods used (ozonation and chlorination) against bacterial growth, malfunctioning of equipments and poor quality control systems.personal and domestic hygiene, effective disposal of human excreta and waste water are quite significant in combating waterborne diseases in this study.

5.2 Conclusion

In conclusion, waterborne diseases pose very serious threats to society. Seven of the ten sachet water types studied did not meet WHO standards for drinking water, hence, routine monitoring of producers of sachet water should be enforced to ensure adherence to drinking water standard.

After assessing sachet water phenomenon in Nigeria, more attention to intervention rather than suppressing packaged water in a bid to protect public health is advocated as residents may revert to poorer sources which could lead to more grievous conditions.

5.3 Recommendation

NAFDAC should ensure and enforce strict compliance to the standards as regards the production and sales of packaged sachet water.

Residents should be aware of a possible danger of consumption of poorly treated water especially the borehole and well water and the potential health risk associated with such water if consumed.

Standard Organisation of Nigeria (SON) should be actively involved in the regulation of the quality of packages used in packaging water.

Sachet water manufacturers should adequately make necessary investigation to identify the point of entry of contaminants and get it rectified.

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