



Bacteriological Analysis of Drinking Water in Zamfara North Senatorial District, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author AYF designed the study, performed the experiments and wrote the first draft of the manuscript. Author BM managed the analyses of the study and carried out statistical analysis. Author IAK managed the literature searches, wrote the protocols and performed the experiment. All authors read and approved the final manuscript.

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ABSTRACT

Provision of portable drinking water is of public health concern especially in developing countries where this is unavailable. Microbes are ubiquitous and are known to contaminate materials including food and water. Microbial contamination cannot be detected by sight, smell or taste. A basic laboratory test is the best way to tell if coliform organisms are present as they can be there with no appearance or taste difference. The microbiological quality of drinking water (DW) in Zamfara North Senatorial Zone was examined. A total of 16, two each from each of eight brands of sachet water were bought from water vendors, and were examined for total bacteria load, total coliform and presence of bacteria species using standard microbiological techniques. The result showed that the total viable count of bacteria in all the samples ranged from 6.0×10^2 CFU/ml to 4.0×10^8 CFU/ml. Total coliform was 1.8×10^7 MPN/100 ml for all the four tested samples (D, G, I, J). The organisms isolated were *Pseudomonas maltophila*, *Escherichia coli*, *Citrobacter freundii*, *Pseudomonas pseudomollia*, *Salmonella typhi*, *Shigella* species, and *Pseudomonas dimineta*. Prevalence of

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different isolates revealed that *Pseudomonas*, *C. freundii*, *S. typhi* and *E. coli* were predominant in comparison to *Shigella species*. The present study revealed that the microbial quality often exceed World Health Organization (WHO) and Food and Agricultural Organization (FAO) allowable limit of 1.0×10^2 CFU/ml for potable water and Standards Organization of Nigeria (SON) maximum permissible level of 10 CFU/ml (total coliform) and 0 CFU/100ml. The high microbial isolates and load may have contaminated the water from the environment. These microbes found in the drinking water sources are known to cause several diseases. Present study indicate that water testing would ensure the supply and availability of contamination-free drinking water; and awareness amongst people towards sanitation and hygienic conditions for storage of drinking water is needed to keep away the use of contaminated water. The present study suggests that drinking water sources should be properly treated prior to consumption using appropriate methods; so as to reduce the occurrence of waterborne diseases.

Keywords: *Bacteria; drinking water quality (DW), faecal; contaminants; coliforms; MPN.*

1. INTRODUCTION

Water is one of the most important as well as one of the most abundant compounds on earth, and is vital to the survival of any organism [1]. Water in nature is seldom totally pure. Rainfall is contaminated as it falls to earth [2]. The combustion of fossil fuel put sulphur compounds as being responsible for pollution of rain water by precipitation [3]. However, water that moves below the ground surface undergoes natural filtration that removes most organisms [4]. For this reason, water from springs and deep wells are generally of better quality than flowing water.

Production and sale of sachet water, popularly called "pure water" in Nigeria is presently a lucrative business as a result of which many people are involved in production and marketing of the products [4]. For this reason, water in sachets is readily available and affordable but there are concerns about their purity. Safe and portable water supplies in urban centers in Nigeria are still inadequate despite four decades of independence and several efforts from various governments [5]. The integrity of the hygienic environment and the conditions where the majority of the water in sachets are produced has also been questioned [6]. Although nationally documented evidence is rare, there are claims of past outbreaks of water borne illnesses that ensued from consumption of polluted water in sachets [6].

Disease-causing microbes transmitted via drinking water are predominantly of faecal origin and are referred to as enteric pathogens [7,8]. The World Health Organization (WHO) standards state that drinking water should not contain any microorganism known to be pathogenic or any bacteria indicative of faecal pollution [9]; since

the presence of these microorganisms has been traditionally seen as an indicator of faecal contamination, tests are useful for monitoring the microbiological quality of water used for consumption. Recognition that water is source of pathogenic microorganisms dates back to 1800 A. D. [10]. Because it is very expensive and time consuming to test for all pathogens, it is suggested that a single group of microorganisms that come from the same source as human pathogens can be used to indicate the presence of pathogens [11]. An understanding of the microbiological quality and safety of drinking water are therefore imperative and should be a cause of concern to consumers, water suppliers, regulators and public health authorities [12]. The Nigerian 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. Availability and access to safe drinking water is essential to health and of great concern to families and communities especially in developing countries where provision of safe drinking water is not available [13]. The quality of drinking water is a powerful environmental determinant of health. It is a basic human right and a component of effective policy for health protection [14].

Lack of safe drinking water is a threat to public health and wellbeing of the people and exposes them to risk of water borne diseases such as diarrheal, cholera, typhoid, dysentery, gastroenteritis as well as chemical intoxication [15,16]. According to the reports of WHO [17] and Hughes and Koplan [18], an estimated 1.1 and 2.6 billion persons lack access to clean water and adequate sanitation respectively. Also,

reports have indicated the effect of unsafe water on young children especially in the developing countries. More than 2 million persons, mostly children, die of diarrheal disease [19,20]. In 1997, the World Health Organization reported that 40% of deaths in developing countries occur due to infection from water related diseases and estimated 500 million cases of diarrhea occurs every year in children below 5 years in parts of Asia, Africa and Latin America [21]. Therefore drinking water contaminated from any source is of primary importance due to the danger and risk of water borne diseases [16]. An adequate, safe and accessible supply must be available to all [21].

Diseases related to contamination of drinking water constitute a major burden on human health, interventions to improve the quality of drinking water provides significant health benefits. Recently, the United Nations General Assembly declared the period from 2005 to 2015 as the International decade for action, 'Water for life' [21]. The association between water, sanitation, hygiene and health are well known. Many diseases are associated with contaminated water which man consume directly or indirectly through cooking, washing utensils, bathing, etc. Such circumstances results in various infections and diarrheal diseases.

The aim of this research was to evaluate the microbiological quality of drinking water (Sachet water) sold in Zamfara North senatorial zones to find out the degree to which they meet the standards set out by the world Health Organization, and evaluates the awareness of people for maintaining cleanliness and hygiene conditions for storage of drinking water.

2. MATERIALS AND METHODS

2.1 Study Area

Zamfara state was carved out of Sokoto state in 1996. It is situated towards the extreme North-West geo-political zone of Nigeria between longitudes 7°2' East around Tsafe towards its North-Eastern border to latitude 11°24' North around Dansadau towards the south boundary to latitude 12°40' North around shinkafi towards its North-Eastern border. It has a landmark of 38,418 Sq. Km representing about 4% of the landmass of Nigeria; with a population 3,259,846 people with 51% being females as at 2006. The estimated population for the year 2013 is

4,064,012. Access to water supply in the state is over 45%, access to quality safe drinking water (defined as water from pipe born, hand pump boreholes and protected wells) is lower at about 20%. The primary occupation of the populace is farming. Another economic activity is livestock production: sheep, cattle, and goat are reared on a large scale. The main languages are Fulani, Dakarkari, and Kamaku.

2.2 Sample Collection

The brands of sachet water being sold in Birnin Magaji, Kauran Namoda, Shinkafi, and Zurmi senatorial zones of Zamfara North are numerous in numbers. Out of these, eight most popular brands, two most popular sachet water brands from each of the towns in the senatorial zone were identified and selected based on patronage by consumers and distributors. A total of sixteen (16), two each from each of the eight brands of sachet water were bought from water vendors for 2 days (21st to 22nd march, 2017). These samples were labeled and transported in ice packs to Microbiology laboratory of Usmanu Danfodiyo University, Sokoto and were examined for turbidity, colour, odour, taste, pH, NAFDAC registration number, manufacturing and expiry date, and analyzed within two hours of collection.

2.3 Sample Processing

Prior to analysis, the water in each sachet was thoroughly mixed and a portion of the sachet was wiped with cotton wool soaked in ethanol before piercing with sterile needle and syringe. Sterile syringe was used to pour nine millilitres (9 mls) of distilled water into four test tubes each per sample and autoclaved at 121°C for 15 minutes. One ml (1 ml) of each sample was transferred into sterilized tubes to obtain dilution factors (10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶ and 10⁻⁷) for all samples.

2.4 Isolation and Preservation of Isolated Organisms

Two media were used for the segregation of bacteria from water samples. These were: Nutrient Agar (NA)/ Nutrient Broth (NB), and MacConkey Agar (MA)/MacConkey Broth (MB). The serial dilution plating procedure was applied for isolation of microorganisms from water samples.

2.5 Determination of Total Bacterial Count

Total bacterial count of samples was determined as described by Onilude et al. [4]. Aliquots from final dilution factor of each sample were aseptically inoculated onto sterile nutrient agar plates by spread plate method using sterile bend glass rod, and the plates were incubated at 37°C for 24 hours. Distinct colonies were counted after 24 hours period of incubation using bacterial colony counter. The results were expressed as Count (CFU/ml) = Number of colonies X Dilution factor.

2.6 Morphological Characterization

2.6.1 Colony morphology

Isolated microorganisms exhibited different types of colonies on agar surface which were later identified morphologically by observing their shapes (circular, dot like, irregular etc).

2.6.2 Gram staining

Further identification of isolated bacteria colonies was done by Gram staining as gram positive and gram negative.

2.7 Biochemical Characterization

Biochemical test were performed and their characterization for isolated microorganisms through sugar fermentation, indole production, MR-VP test, and citrate utilization was carried out. In sugar fermentation tests (glucose, lactose, sucrose), three culture tubes filled with three different kinds of broth containing glucose, lactose, and sucrose were selected. Each contained 0.5% sugar along with sufficient amount of beef extract and peptone. pH indicator (bromothymol blue) was added for acid detection. Each culture tube was inoculated with the test bacterial culture and incubated at 37°C for 24 hours. Three different sugar broths were inoculated by the test bacteria and observed for colour change, gas and acid production in test tubes. In indole production, tryptone broth was inoculated with bacterial culture and incubated at 37±1°C for 48 hours. After the bacterial growth, 0.5 ml of Kovac's reagent was added. Red colour band appears at the junction media. Further tests, MRVP broth was prepared and it was taken in test tubes. It was inoculated with isolated microorganisms and slants were

incubated at 37±1°C for 48 hours. After incubation period of 48 hours, the change in colour was recorded in streaked slants. For citrate utilization, citrate agar slants were inoculated by streaking method of inoculation. An un-inoculated slant was kept as control. The slants were cultivated at 37°C for 48 hours.

2.8 Detection of Coliforms (MPN Technique)

The technique used for the detection of coliform bacteria was the multiple fermentation technique, now referred to as most probable number, MPN [22]. The technique has been used for the analysis of drinking water for many years with satisfactory results. It is the only procedure that can be used if water samples are very turbid or if semi-solids such as sediments or sludge are to be analysed. It is customary to report the results of multiple fermentation tube test for coliforms as a Most Probable Number (MPN) index. This is an index of the number of coliform bacteria that, more probably than any other number, would give the results shown by the test. It is not a count of the actual number of indicator bacteria present in the sample. MPN technique involves three successive steps (presumptive, confirmatory and completed test).

The primary isolation was carried out using MacConkey broth, a total amount of 100 ml water sample was taken from each sachet as (five 10 ml and one 50 ml amount). Sterile syringes were used to inoculate 10 ml, 1.0 ml and 0.1 ml of the samples into three sets of three test tubes (a double strength, and single strengths) respectively, each tube containing MacConkey broth of the same amount each containing an inverted Durham's tube for gas collection and detection. The tubes were closed tightly and shaken to distribute the sample throughout the medium. These were incubated aerobically at 37°C for 18 to 24 hours. Acid production was evidenced by a change in the colour of the MacConkey broth from purple to yellow and gas production was evidenced by entrapment of gas in the Durhams tube. The total numbers of tubes showing evidence of acid and gas production were recorded and corresponding MPN of coliforms from the 100 ml was estimated by reference from the probability table. This is called Presumptive Test.

In the confirmatory test for faecal coliform, a loopful of broth from the positive tubes in the

presumptive test was transferred into sterile petri dishes containing Eosine Methylene Blue (EMB) broth and incubated at 37°C for 24 hours. After incubation, colonies were observed and production of gas in the tube after hours is positive.

The completed test was performed by inoculating a tube of MacConkey broth with a colony from positive confirmatory test. Simultaneously, a loop of organism was streaked onto a slant of nutrient agar. The tubes were incubated at 37°C for 48 hours.

2.9 Data Analysis

The data was analyzed using Statistical Package of Social Sciences (SPSS) version 25.0. Total bacteria count was carried out using bacteria colony counter, and faecal coliform were measured using Most Probable Number (MPN) technique.

3. RESULTS

3.1 Colony Morphology

Isolated colonies were small sized, opaque, frequently white pigmented often leathery and appeared dry. Colonies were partially submerged in Nutrient agar medium plates and MacConkey agar plates.

3.2 Gram Staining

The cells morphology was determined by Grams staining technique, and revealed characteristics negative rods.

3.3 Biochemical Tests

It is obvious from the perusal of information given in Table 3 that isolated organisms were gram negative rods while confirmation of isolated organisms revealed their identity as *Pseudomonas maltophilia*, *Escherichia coli*, *Citrobacter freundii*, *Pseudomonas peodomollia*, *Salmonella typhi*, *Shigella* species, and *Pseudomonas dimineta* on the basis of sugar fermentation, indole production, Methyl red and Voges-Proskauer and citrate utilization tests as given in Table 4.

Table 1 shows total bacterial count (CFU/ml) of isolates.

Table 2 shows presumptive coliforms counts (MPN/100 ml) of isolates.

Table 3 shows gram reaction of isolates.

Table 4 shows biochemical characterization of isolates.

Table 1. Total bacterial count (CFU/ml) of isolates

S/No.	Samples	Total bacterial count (CFU/ml)
1.	A	3.3×10^6
2.	B	3.0×10^6
3.	C	4.0×10^6
4.	D	1.5×10^6
5.	E	1.4×10^6
6.	F	35.0×10^7
7.	G	1.8×10^6
8.	H	40.0×10^7
9.	I	35.0×10^7
10.	J	0.0×10^5
11.	K	3.4×10^6
12.	L	20.0×10^7
13.	M	40.0×10^7
14.	N	0.6×10^3
15.	O	3.5×10^6
16.	P	0.7×10^6

CFU/ml = Colony Forming Unit per Milliliter

4. DISCUSSION

Humans need regular and accessible quality water supply, which constitute a significant part of the protoplasm and provides vital necessity for physiological and biochemical processes [23]. Drinking water is meant for human consumption, hence should be free of pathogens and other objectionable materials including colour, tastes etc. The microbial quality of drinking water is a concern to consumers, water suppliers, regulators and public health authorities; this is because water is a vehicle through which waterborne pathogens that could cause diseases are transmitted [24]. Water testing is a significant approach to ensure the supply and availability of contamination-free drinking water. Contamination of water sources have been reported by several authors as a medium of disease outbreak and spread in developing countries and rural areas [25,26]. This study was aimed to investigate the microbiological quality of drinking water in Zamfara North senatorial zone.

The result of the present study showed that total viable bacterial count in the drinking water

samples ranged from 6.0×10^2 CFU/ml to 4.0×10^8 CFU/ml; this was high and exceeded the recommended limit of <500 CFU/ml. This result is similar to the work of Ngwa and Chrysanthus [27] and Agwaranze et al. [28] who reported viable count of bacteria in the range of 2.0×10^3 CFU/ml to 7.3×10^4 CFU/ml and 8.6×10^3 CFU/ml to 3.04×10^4 CFU/ml respectively. The high bacteria count is an indication that the various sources of the drinking water samples are highly contaminated which could be of public health concern. The high values could be attributed to run-off water that enters some of the water sources during raining seasons and particles from the environment which gain access into the processing plant from time to time. Modification in drinking water quality can be caused by pollution from both point sources such as industrial and treated water sewage releases and diffuse source such as storm-water runoff from agricultural and urban areas [29].

Total coliform count of the drinking water samples in the present study was 1.8×10^7 MPN/100ml for all the four tested samples (D, G, I, J). These values are high when compared with the permissible MPN index by world Health Organization of 10 coliforms/100ml of water sample. This observation is consistent with the reports of many researchers [16,27,30] who reported high coliform counts in water analyzed.

The high number of total coliforms could be due to inadequate maintenance of the drinking water. It can also be attributed to percolation of sewage into the ground water sources [16]. The presence of coliforms (water quality indicator) suggests the occurrence of entero pathogens in such water [31]. *E. coli* is considered to be more closely associated with faecal contamination; it is the best organism for assessing drinking water quality [32].

The result of the present study also revealed that the drinking water samples were highly contaminated with one or more of the following isolates, *Pseudomonas*, *C. freundii*, *S. typhi* and *E. coli* and *Shigella* species. Most of these organisms are potential pathogens of the coliform group. Similar findings have been recorded by Ngwa and Chrysanthus [27] who reported the presence of *Escherichia coli*, and *Salmonella* species from water samples. Also similar organisms have been reported by Idowu et al. [33] on water samples from Shagamu. According to the reports of Bonde [34] and Akeredolu [35], any water used for drinking or cleaning purposes should not contain bacteria of faecal origin. Therefore, the presence of coliform bacteria such as *Escherichia coli* and the rests in some of the water samples make them unfit for drinking or human consumption [25].

Table 2. Presumptive coliforms count (MPN/100 ml) of isolates

S/No.	Samples	No. of tubes showing positive presumptive test			Coliform count (MPN/100 ml)
		3 of 10 ml	3 of 1.0 ml	3 of 0.1 ml	
1.	D	0	0	1	1.8
2.	G	0	1	0	1.8
3.	I	0	1	0	1.8
4.	J	0	0	1	1.8

Table 3. Gram reaction of isolates

S/N	Sample	Media	Gram reaction		
1.	A	EMB	Gram negative	Short – rod	In clusters
2.	A	EMB	Gram negative	Short – rod	In pairs
3.	B	EMB	Gram negative	Short – rod	In pairs
4.	C	EMB	Gram negative	Short – rod	In clusters
5.	D	EMB	Gram negative	Short – rod	In clusters
6.	F	EMB	Gram negative	Short – rod	In pairs
7.	G	EMB	Gram negative	Short – rod	In pairs
8.	J	SS	Gram negative	Short – rod	In clusters
9.	C	SS	Gram negative	Short – rod	In clusters
10.	M	SS	Gram negative	Short – rod	In clusters
11.	N	SS	Gram negative	Short – rod	In pairs
12.	E	EMB	Gram negative	Short – rod	In clusters

Table 4. Biochemical characterization of isolates

S/N	Samples	Media	Catalase	Urease	MR	VP	Indole	Glucose	Lactose	Sucrose	Oxidase	Citrate	H ₂ S	Gas	Mobility	Organisms
1.	A	EMB	+	+	+	-	-	+	+	+	+	+	+	+	+	<i>Pseudomonas maltophilia</i>
2.	A	EMB	+	+	+	-	+	+	+	-	-	-	+	-	-	<i>Escherichia coli</i>
3.	B	EMB	+	+	+	-	-	+	+	-	-	+	-	+	+	<i>Citrobacter freundii</i>
4.	C	EMB	+	+	+	-	+	+	+	+	-	+	+	-	+	<i>Citrobacter freundii</i>
5.	D	EMB	+	+	+	-	-	+	+	-	-	+	-	+	+	<i>Citrobacter freundii</i>
6.	F	EMB	+	+	+	-	+	+	+	-	-	-	+	-	-	<i>Escherichia coli</i>
7.	G	EMB	+	+	+	-	-	+	+	+	+	+	-	+	+	<i>Pseudomonas peodomollia</i>
8.	J	SS	+	-	+	-	-	+	-	-	-	-	-	-	+	<i>Salmonella typhi</i>
9.	C	SS	+	-	+	-	-	+	-	-	-	-	-	-	+	<i>Salmonella typhi</i>
10.	M	SS	+	-	+	-	-	+	-	-	-	-	-	-	+	<i>Salmonella typhi</i>
11.	N	SS	+	-	+	-	-	+	-	-	-	-	-	-	-	<i>Shigella species</i>
12.	E	EMB	+	-	-	+	-	-	-	-	-	+	-	-	-	<i>Pseudomonas dimineta</i>

The percentage occurrence of organisms isolated from the drinking water samples in the present study showed that *Pseudomonas*, *C. freundii*, *S. typhi* and *E. coli* were predominant in comparison to *Shigella* species. Sohani et al. [36] carried out microbiological analysis of surface water in Indore (Madhya Pradesh, India) by using samples from variable sources of different regions wherein they observed a high prevalence of coliform group of bacteria namely *E. coli*, *Klebsiella*, *Salmonella* and *Shigella* on EMB agar.

Therefore the result of this study corroborates their studies that *E. coli*, *Salmonella*, and *Pseudomonas* are the frequent waterborne microbes. The presence of these organisms in some of the drinking water samples under study are of public health importance. This may be due to improper hygienic practices around the area. It could also be attributed to drainage and flooding from contaminated surface water into the water sources [33]. Also, the presence of these organisms may be due to openness of the water sources which allows easy entrance of particles from the surroundings or drawing water from the sources with contaminated equipments as noted by Ngwa and Chrysanthus [27].

The implication of bacterial diversity found in the drinking water samples in this study is that these organisms are associated with infectious diseases including gastroenteritis, typhoid and paratyphoid fevers, bacillary dysentery, cholera [23] and urinary tract infections etc. Specifically, faecal coliform could cause typhoid fever, hepatitis, gastroenteritis, dysentery, cholera, hepatitis A and poliomyelitis [37]. *Salmonella* species is the causative agent of typhoid fever. Typhoid fever is one of the enteric diseases that are transmitted through water [23]. *Citrobacter* species causes meningitis and brain abscess [24]. Different species of *Pseudomonas* are pathogenic. For instance, *Pseudomonas aeruginosa* can cause perirectal infections, pediatric diarrhea, gastroenteritis and enterocolitis and giving port of entry for septicemia and bacteremia; skin and soft tissue, urinary tract, bone and joint, respiratory infections, Bacteremia and septicemia [38,39].

About 80% of all medical issues in the world are due to inadequate sanitation and polluted water [40]. Waterborne illnesses are global public health problem and faecal contamination of potable water imparts an important role in these

outbreaks. Water related diseases present a human tragedy thus killing millions of peoples each year [41].

5. CONCLUSION

Drinking water is supposed to be free from objectionable (colour, taste, turbidity) and microbial contaminants. Sachet water popularly known as "Pure water" is patronized by Nigerians probably due to their convenience to quench taste. This packaged water are vended in several locations including several public places including motor parks, garage, markets, streets, along express ways, outskirts of schools and hospitals. The microbial density of the various drinking water samples in this study often exceed the World Health Organization/Food and Agricultural Organization allowable limit of 1.0×10^2 CFU/ml for potable water and Standards Organization of Nigeria maximum permissible level of 10 CFU/ml (total coliform) and 0 CFU/100ml (Thermo tolerant Coliform or *E. coli*, Faecal streptococcus and *Clostridium perfringens* spore), and this makes the water unsuitable for drinking. Also, bacteria isolated from the various samples are mostly enteric organisms which are potential pathogens of public health concern. To reduce the incidence of microbial waterborne diseases that could result due to the organisms encountered in this study, particularly in Zamfara North senatorial zones and Nigeria as a whole, we suggest that drinking water should be adequately treated before consumption. There is also need for drinking water maintenance, environmental sanitation around the water processing areas, hygienic practices by households and individuals. There should also be strategic monitoring and surveillance approach; which need to be encouraged to ensure compliance by water vendors. Chlorination plays vital role as an important factor in removal of bacteria from water supplies; it kills microorganisms by cleaving the chemical bonds in their molecules.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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