



## BACTERIOLOGICAL EXAMINATION OF DRINKING WATER FROM DIFFERENT SOURCES IN SOKOTO STATE, NIGERIA

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### Abstract

A total of 50 water samples comprising; 20 well water, 15 sachet water, 10 borehole water and 5 river water samples, were obtained from Wamakko, Dange-shuni and Wurno local government areas in Sokoto Central, Sokoto West and Sokoto East, Nigeria respectively between March and May 2015. They were analyzed using membrane filtration and pour plate techniques for faecal coliforms count and total coliform count respectively. The total coliform count for all the sources ranged between 0 and  $3.28 \times 10^2$  with a total mean of  $1.11 \times 10^2$ , that of faecal coliform count was found to range between 0 and 92 with a mean total count of 12.58. Among the bacteria isolated and identified, *Escherichia coli* had the highest total prevalence of 233 (40.31%) and occurring in all the water samples. *Klebsiella spp* had 99 (17.13%) occurring in all the sources, *Salmonella spp* 43 (7.44%) occurring in all the sources except for borehole, *Pseudomonas* species 88 (15.22%) occurring in all the sources, *Staphylococcus aureus* with 115 (19.90%) occurring in all the sources except for borehole. However, the pHs for all the samples were found to be within the range of 6.51 to 8.0 which are within WHO acceptable range of 6.0 to 8.5. From this study, it is obvious that the water sources from the areas under study are bacteriologically unfit for drinking purposes. These samples with high total coliform count are of concern and calls for urgent and stringent measures aimed at ensuring a safer drinking water for the populace.

**Keywords:** Bacterial, Coliform, Filtration, Sokoto, Water

### Introduction

Water is an essential part of human nutrition, both directly as drinking water or indirectly as a constituent of food, in addition to various other applications in daily life. Water is not only essential for life; it also remains an important source of disease transmission, infant mortality in many developing countries, key parameter influencing survival and growth of microorganisms in foods and other microbial environments. Water-borne pathogens are found in different water bodies these include; streams, rivers, lakes, springs, wells, and every known source of water for human consumption including surface and underground waters.<sup>1</sup> Prominent among these pathogens are: *Salmonella typhi* responsible for typhoid fever, *Shigella dysenteriae* implicated as the causative agent of bacillary dysentery, *Escherichia*

*coli* which causes gastroenteritis, *Klebsiella pneumoniae* which causes pneumonia and *Enterobacter cloacae* which causes urinary tract and respiratory tract infections, to mention a few of them.<sup>1</sup> The provision of adequate supply of safe drinking water was one of the eight components of Primary Health Care identified by the international conference on Primary Health Care in 1978.<sup>2</sup> Increasing human population has exerted an enormous pressure on the provision of safe drinking water especially in developing countries.<sup>2</sup> The demand for safe drinking water in Nigeria cannot be overemphasized, considering the inability of the governments to provide adequate pipe-borne water to the populace. Packaged water in bottles or food grade polythene sachets designed for food processing is a ready alternative for the ever-growing population of over 140 million people in Nigeria. However, safe drinking

water is very scarce. The ever-increasing demand for readily available drinking water has led to the concept of sachet water. It is a general perception that packaged water is safe for human consumption. Sachet water in Nigeria is popularly known as 'pure water', normally sold at the rate of ₦5.00 per sachet (less than a dollar).

Potable water is any packaged water that has been processed, sealed and released into the market.<sup>3</sup> Coliforms are rod-shaped Gram negative organisms which ferment lactose with the production of acid and gas when incubated at 37°C. Faecal coliform is a smaller group within the total coliform family; it inhabits the intestine of mammal and has a relatively short life span. This serves as an indication of contamination by sewage. *Escherichia coli* is the most preferred coliform used in analysis of faecal contamination. It does not grow and reproduce in the environment consequently, it is considered to be the species of coliform bacteria that is the best indicator of faecal Sachet water production in the country has of recent been on the increase with Sokoto town, North western Nigeria, having lots of these sachet water manufacturers. The need for potable water is of great public health significance because of water borne infection.<sup>4</sup>

Sachet water is viewed as the latest, low-cost technological incarnation of vended water in developing cities. It is also prevalent in countries contiguous to Nigeria and Ghana, for example Cote d'Ivoire, Burkina Faso, Togo, Benin, Niger and Cameroon.<sup>5</sup> Given the renewed global commitments towards the MDGs in 2015, the contribution of sachet drinking water cannot be overemphasized. There are, however, significant varying levels of contamination of the sachet drinking water which had aimed to provide a low cost and safe alternative source of drinking water.

The use of bad water is restrained by its quality which makes it unhealthy for consumption. Water quality assessment is therefore an important aspect of water resources evaluation.<sup>6</sup>

Portable or drinking water is defined as having acceptable physical, chemical and bacteriological qualities that makes it safe for drinking and cooking. One of the conditions that safe water for human consumption should fulfill is that it should be free from microbiological contamination. Safe drinking water is a fundamental human right-as much a right as clean air. As a matter of fact in most of the African and Asian countries, safe drinking water is not readily

available. Of the 6 billion people on earth, more than 1 billion lack accesses to safe drinking water and about 2.5 billion do not have access to adequate safe drinking water. In addition to these shortcomings, various types of waterborne diseases kill on average more than 6 million children each year i.e., about 16,000 children a day.<sup>7</sup>

This study therefore aims at investigating the prevalence of pathogenic organisms as pathogenic bacteria, most importantly the fecal coliform in packaged drinking water sold in Sokoto state, Nigeria.

## Materials and Methods

### Study area

The study area is Sokoto, the capital of Sokoto state located at latitude 13°N and between longitude 4°E and 6°E in the extreme Northwest of Nigeria. It covers approximately an area of 56,000 square kilometers. The state shares border with Niger Republic to the North, Kebbi state to the south and Zamfara state to the East. Based on the 2006 population census, it has a projected population of about 4,244,399 as at 2009.

Sokoto state is in the dry sahel, surrounded by sandy savannah and isolated hills with an annual average temperature of 28.3°(82.9°F), Sokoto is on the whole a very hot area. However, maximum day time temperatures are for most of the year generally under 40°C (104.0°F).

The rainy season is from June to October with an annual rainfall ranging between 500mm to 1300mm. The major source of portable drinking water is the sachet water probably because of its affordability and a lot of this water is consumed due to hot temperature of the area.

### Study sites

The study areas or sites selected for this research work were Wamakko, Dange-shuni and Wurno local government areas in Sokoto State. Areas from which samples were collected are in Sokoto Central, Sokoto West and Sokoto East respectively.

### Study sample

The study samples for this research had a total of Fifty (50) drinking water samples. Fifteen (15) sachet water were obtained from point of sale retail outlets, twenty (20) well water samples, ten (10) borehole water samples and five (5) pond/river/stream water samples. All samples collected were sum up to the total from aforementioned areas. The choice of fifty (50)

drinking water samples was purposeful to increase the power of the study.

### Collecting samples from various sources

#### Well water samples

A sterile sample bottle was tied on a weighed length of rope using a heavy piece of metal as a weight and the bottle attached just above the weight, the cap was aseptically removed from the bottle, and the bottle lowered down the well until covered with water in the well, the bottle was raised out of the well and the cap carefully replaced, the bottle was labeled with the sample code.

#### From tap outlet of boreholes

External fittings were removed from the tap, the outside nozzle of the tap was carefully cleaned, the tap was turned on and the water was allowed to run and waster for 1 minute, the tap was sterilized using to flame the nozzle until the whole tap is unbearably hot to touch. The tap was allowed to cool by running the water to waste for a few seconds, the sample bottle was filled from a gentle flow of water from the tap, and the cap of the bottle replaced. The sample bottle was labeled with the sample code number using water-proof marker.

#### Pond/river/stream

A sterile cup was used to collect the sample from the pond and then transferred in to the collecting containers via a sterile funnel and the containers were well labeled.

#### Transporting samples to the laboratory

Immediately after collection, the samples were placed in an insulated cold box and transported to the laboratory for testing. Water samples were examined within 6 hours of collection.

#### Test for odour and colour

A 20 mL volume of each water sample was poured into a clean beaker. The beaker was then shaken vigorously to check for any frothing and allowed to settle. The beaker was then observed under bright light for presence of any particulate matter and then brought close to the nose to test for any odour present.<sup>8</sup>

#### Test for taste

Small volumes of each sample was tasted with the tongue and then immediately rinsed with taste free distilled water after each sample, the result recorded accordingly.

#### Enumeration of faecal coliform from water samples

The membrane filtration method was used to process all water samples. For each water sample, 100ml was filtered in duplicate through 0.45µm pore size nitrocellulose membranes. The filters were placed on EMB agar and incubated at 44.5°C for 24 hours, for enumeration of *E.coli* as well as enumeration of other faecal coliforms (FC).

Briefly, 100ml of the water samples were aseptically transferred into sterile filtration units fitted with sterile 0.45µm pore size nitrocellulose membrane filters. This unit was connected to a suction machine which enabled efficient and timely filtration process. After filtration used filters were aseptically transferred onto freshly prepared EMB ager plates and incubated. Counts were made using electronic counting machine (Colony counter), and figures expressed as colony forming unit per millilitre (cfu/ml).

#### The Eijkman test for faecal coli

Although coliforms were easy to detect, their association with fecal contamination was questionable because some coliforms are found naturally in environmental samples.<sup>9</sup> This led to the introduction of the fecal coliforms as an indicator of contamination. Fecal coliforms, first defined based on the works of Eijkman [10] is a subset of total coliforms that grows and ferments lactose at elevated incubation temperature, hence also referred to as thermotolerant coliforms. Fecal coliforms analyses are done at 45.5°C for food testing, except for water, shellfish and shellfish harvest water analyses which uses 44.5°C for 24-48 hours using Eijkman lactose broth media.<sup>11</sup>

**Principle of the test:** This test is used for differentiating *E. coli* from other coliforms based on their ability to liberate gas from lactose of gram negative enteric bacteria. Eijkman lactose broth is used for the detection and differentiation of *Escherichia coli* from other coliform organisms on the basis of their ability to grow and liberate gas from lactose.

### Enumeration of total coliform from water samples

A serial dilution (1/10) of water samples was prepared using sterile distilled water as diluents. From each dilution, 0.1 ml was spread aseptically onto duplicate plates of MacConkey agar and incubated at 37<sup>0</sup> C for 24hrs. Typical lactose fermenting colonies of coliforms organisms were counted and multiplied by the dilution factor to get the correct cfu/ml.

### Temperature and Ph measurement

The temperature was measured using Digital mercury in glass thermometer to the nearest 0.01, while the Greenspan pH 300 pH meter was used to measure the pH. The pH meter was calibrated according to the manufacturer's direction using two buffers (pH 7 and 10) for calibration. The probe was placed in the sample for some time for meter to equilibrate. The pH was read directly from the meter according to the manufacturer's directions to the nearest 0.01.

#### Drinking water guidelines (guidelines for parameters)

Parameters	Maximum permissible limit
pH	6.5-8.5
Temperature	Ambient
TCC	10 cfu per/ml
FCC	0 cfu/100ml
Protozoa-giardia/ <i>Cryptosporidium</i> oocyst	3 log reduction and/or inactivation

Key: TCC = Total coliform count, FCC = Faecal coliform count

Adapted from Nigeria Standard For Drinking Water Quality (2015).

### Identification of various bacteria

#### Colonial morphology

Colonies were examined using the following morphological characteristics as shape, size, topography and color. Gram staining was also done from the colonial growth on the culture media.

#### Simmons citrate agar

**Procedures:** A light suspension of the organism was made in saline, it was stab inoculated in Simmons citrate agar with a straight wire and then incubated at 37<sup>0</sup>C for 24 hours. A blue color growth in Simmons agar indicated positive result.

#### Coagulase test

**Procedures:** A drop of saline was placed on two separate spot on the glass slide, colonies of the organisms was emulsified on each of the drops to make a suspension. A drop of citrated plasma was added to one of the suspension and rocked for few minutes and coarse clumping was checked for, the clumping indicates positive coagulase test.

#### Catalase test

**Procedure:** 3ml of hydrogen peroxide solution was poured into a clean test tube; several colonies of the test organism were removed from the cultured plate using a sterile glass rod and immersed into the hydrogen peroxide solution. Immediately air bubbles were looked for positive results.<sup>12</sup>

#### Indole test

**Procedure:** The test organisms were inoculated in 3ml amount of sterile peptone water and incubated for about 20hours. Kovac's reagent was added to the 20hours peptone water culture, Red ring was observed above the peptone water, which indicates positive indole production.

#### Oxidase test

**Procedure:** A suspected colony was picked with a sterile wire loop and smeared on an oxidase strip (containing 1% Tetramethyl paraphenylene diamine dihydrochloride). A deep purple color appearing within 10 seconds indicates positive oxidase test.

#### Urease test

**Procedure:** Urea slant in the bijou bottle was stab with organism using a straight wire; it was incubated at 37<sup>0</sup>C for 24hours. The development of a bright pink or red color indicated a positive reaction.

#### Kligler Iron agar

**Procedure:** A well isolated colony was picked with a sterile straight wire and stabbed into the butt of KIA tube and then streaked on the surface of the slope. It was incubated at 37<sup>0</sup>C for 24hours. The changes in the medium red/yellow butt or slant were observed.<sup>12</sup>

### Results

A total of fifty (50) water samples; twenty (20) well, fifteen (15) sachet, ten (10) borehole and five (5) river water samples were analyzed for total coliform count, fecal coliform count, temperature and pH and also determination of bacteria in the water samples.

Among isolates from well water, *Escherichia coli* had the highest prevalence 65 (11.24%) followed by *Staphylococcus aureus* 49 (8.47%), *Salmonella spp* with 26 (4.49%), while *Pseudomonas spp* had 19 (3.28%) and *Klebsiella spp* had the least prevalence. Samples from river had the second number of isolates with total isolates of 151 (26.10%), followed by samples from sachet water with the total number of isolates of 143 (25.58%) and borehole water had the least number of isolates of 174 (30.07%) including *E.coli* 68(11.76%), *Klebsiella spp* 25 (4.32%) and *Pseudomonas spp* 12 (2.07%) (Table 1).



The total coliform count for the well water sources ranged between 7 and  $3.24 \times 10^2$  with a mean of  $1.46 \times 10^2$  and fecal coliform count ranged between 1 and 34 with a mean of 9.15, the mean pH and temperature were found to be 6.79 and 28.75 respectively (Table 2).

The mean values of total coliform and faecal coliform counts for sachet water were higher than that of the drinking water guidelines.<sup>13</sup> The pH which ranged between 6.50 and 7.85 were within the range of the drinking water guidelines (Table 3). The mean values of total coliform count and fecal coliform counts for borehole water source were also above the drinking water standards, that of total coliform and fecal coliform counts which ranged between 41 and  $1.68 \times 10^2$  and 3 and 56 respectively, while the is within the range of the drinking water guidelines (Table 4).

The result of the total coliform count for the river sources ranged between 64 and  $3.28 \times 10^2$  with a mean of  $1.89 \times 10^2$  and that of fecal coliform counts ranged between 9 and 92 with a mean of 34. The pH was also found to be within the range of the drinking water guidelines (Table 5).

The mean total coliform counts were generally high with river water, followed by well water, followed by borehole water and sachet water samples had the least mean total coliform count as shown in (Table 6).

The comparison of the mean values for faecal coliform counts between the different sources shows that samples from river had the highest faecal coliform count, followed by borehole water and then by well water, while sachet water had the least mean faecal coliform count (Table 7).

The comparison of both the mean values for the total coliform and faecal coliform counts from different sampling locations, shows that in both the counts, samples collected from Wammako local government had the highest bacterial counts, followed by samples collected from Dange-shuni local government area while samples collected from Wurno local government area had the least bacterial counts (Table 8).

**Table 1: Frequency of occurrence of the various isolates in the different water sources n(%)**

Isolates	Well (N=20)	Sachet (N=15)	Borehole (N=10)	River (N=5)	Total (N=50)
<i>E.coli</i>	65(11.24)	35(6.05)	68(11.76)	65(11.24)	233(40.31)
<i>Klebsiella</i>	15 (2.59)	26 (4.49)	25 (4.32)	33 (5.70)	99 (17.13)
<i>Salmonella</i>	26 (4.49)	6(1.03)	0 (0)	11(1.90)	43 (7.44)
<i>Pseudomonas</i>	19 (3.28)	37(6.40)	12 (2.06)	20 (3.46)	88(15.22)
<i>Staph.aure</i>	49(8.47)	44(7.61)	0 (0)	22(3.80)	115(19.90)

**Table 2: Mean values of some parameters for well water sources**

Parameters (N=20)	Minimum	Maximum	Mean	Std.deviation
pH	6.56	7.02	6.79	0.32
Temp.	23.50	24.00	28.75	7.42
TCC	7	$3.24 \times 10^2$	$1.46 \times 10^2$	92.92
FCC	1	34	9.15	11.39a

Key: N=number of sample, TCC = Total coliform count, FCC = Faecal coliform count

**Table 3: Mean values of some parameters for sachet water sources**

Parameters (N=15)	Min.	Max	Mean	SD	AL (NSDWQ)
pH	6.50	7.85	7.17	0.95	6.5-8.5
Temp.	23.00	27.00	25.00	2.82	Ambient
TCC	0	$2.0 \times 10^2$	55.40	72.75	10 cfu/ml
FCC	0	48	7.07	11.93	0 cfu/ml

Key: N=number of sample, TCC = Total coliform count, FCC = Faecal coliform count, Min.=minimum, Max=maximum, SD=Standard Deviation, AL=Accepted level

**Table 4: Mean values of some parameters for borehole water sources**

Parameters (N=10)	Minimum	Maximum	Mean	Std.deviation
pH	6.52	6.86	6.69	0.24
Temp.	29.00	35.00	32.00	4.24
TCC	41	$1.68 \times 10^2$	86.40	40.77
FCC	3	56	17	15.59

Key: N=number of sample, TCC = Total coliform count, FCC = Faecal coliform count

**Table 5: Mean values of some parameters for river water sources**

Parameters (N=5)	Minimum	Maximum	Mean	Std.deviation
pH	6.92	7.02	6.97	0.07
Temp.	20.00	22.50	21.25	1.76
TCC	64	$3.28 \times 10^2$	$1.89 \times 10^2$	$1.01 \times 10^2$
FCC	9	92	34	34.63

Key: N=number of sample, TCC = Total coliform count, FCC = Faecal coliform count

**Table 6: Comparison of mean of TCC from different water sources**

Sources	N	Range	Mean	Std.deviation
Well	20	$7-3.24 \times 10^2$	$1.46 \times 10^2$	92.92
Sachet	15	$0-2 \times 10^2$	55.40	72.75
Borehole	10	$41-1.68 \times 10^2$	86.40	40.77
River	5	$62-3.28 \times 10^2$	$1.89 \times 10^2$	$1.01 \times 10^2$

N=number of sample

**Table 7: Comparison of mean of FCC from different water sources**

Sources	N	Range	Mean	Std.deviation
Wells	20	1-34	9.15	11.39
Sachet	15	0-48	7.07	11.93
Borehole	10	3-56	17	15.59
River	5	9-92	34	34.63

N=number of sample

**Table 8: Comparison of mean of counts from the different sampling locations**

Bacteria	N	Location	Min.	Max.	Mean
Faecal coliform count	19	Dange-shuni	2	48	12.53
	16	Wurno	1	40	12.13
	15	Wamako	0	92	13.13
Total	50		0	92	12.58
Total coliform count	19	Dange-shuni	4	$2.28 \times 10^2$	$1.31 \times 10^2$
	16	Wurno	6	$3.28 \times 10^2$	94.69
	15	Wamako	0	$3.24 \times 10^2$	$1.03 \times 10^2$
Total	50		0	$3.28 \times 10^2$	$1.11 \times 10^2$

N=number of sample

## Discussion

This research work which aimed at assessing the bacteriological quality of drinking water sources (well, borehole, sachet water and pond/river/stream) within zones of Sokoto state showed that almost all the water samples were contaminated having total coliform count above that of Nigeria Standard For Drinking Water Quality.<sup>13</sup> This contamination of the water sources may be brought about by various activities including surface run off, animal and human faeces and urine.<sup>14,15,16,17</sup> This finding is in agreement with a study conducted by Raji *et al.*,<sup>18</sup> in Sokoto State where the analyzed water sources were also found to be contaminated by bacteria of human and animal faeces was attributable to the fact that most wells are left open and not well sited as they are prone to contamination from various sources such as dumps and animal pen and with surface water which are prone to contamination mostly from human activities such as bathing and washing. All the water samples analyzed failed to meet WHO standards for drinking water because many potentially pathogenic bacteria were isolated in them, this corroborate the findings by Raji *et al.*,<sup>18</sup> in Sokoto State, who reported that all the different water sources sampled had both total coliform count and fecal coliform count far above the WHO standards and as such the water was unfit for drinking purposes. The isolation of *E. coli* and *Klebsiella* spp in our study strongly suggests that the presence of other members of the family such as *serratia* spp and *citrobacter* spp cannot be completely ruled out as there were a number of unidentified isolates. These bacteria are used as marker of water contamination. *Escherichia coli* a prominent member of this family which had about 233 (40.31%) prevalence in the 50 water samples analyzed, occurring in all the different water sources, while *Klebsiella* spp had a prevalence of 99 (17.13%). This point to faecal contamination of various water analyzed. The presence of *E.coli* also indicates presence of other enteric pathogens such as *Salmonella* spp and *Shigella* spp. In this study, *Salmonella* spp was particularly found to have a total prevalence of 43 (7.44%) of the 50 water samples analyzed, this finding also agree with that reported by Oluma *et al.*,<sup>19</sup> Johnson *et al.*<sup>20</sup> and Isaac *et al.*<sup>21</sup> The well water source was found to be the most contaminated of all sample sources having highest mean value of both total coliform and fecal coliform counts. This may be unconnected with the temperature

and pH as the mean values seemed to be on the favouring side. This is because the survival in soil of bacteria is generally enhanced by low temperatures and neutral or alkaline pH.<sup>22,23,24</sup> It was also observed that *E.coli* had the highest prevalence 233 (40.31%) in all the water samples making them unsuitable for consumption. Sachet water was found to be very much contaminated; there was about 35 (6.05%) prevalence of *E.coli* in the sampled sachet water. This indicates that the so-called 'pure water' which has found a wide acceptance for use as drinking water source is not safe for human consumption, which agrees with findings of Edema *et al.*,<sup>2</sup> in South western Nigeria, which reported that the mean values of total coliform count and faecal coliform count were greater than the international guidelines for drinking water quality.

## Conclusion

From this study, it is obvious that the water sources from the areas under study are bacteriologically unfit for drinking purposes. From the quality and sanitary risk evaluation points of view, the studied water sources could be classified as grossly polluted. The present study has shown that some of the bacteriological data of the different water sources had values beyond the maximum tolerable limits recommended by WHO. Therefore, this calls for an urgent and stringent measures aimed at ensuring a safer drinking water for the populace.

## Recommendations

Effective and affordable water treatment options should be provided and the populace enlightened about the importance of and need for small scale water disinfection and treatment techniques.

The siting of wells and boreholes close to refuse dumps and other waste disposal systems should be discouraged, wells should be properly covered and activities of human and animal should be discouraged near surfaces water sources used for drinking. The government should be more involved in shouldering the responsibility of providing potable water for her citizenry.

Package water companies should particularly improve the packaging process devoid of room for contamination during filtering and packaging processes. Authorities should set up monitoring and surveillance to ensure maintenance of the standard of drinking water in the zones understudied.

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