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Comparing bacteriological parameters of sachet water samples collected in Nassarawa and Tarauni local governments, Kano metropolis, Kano State, Nigeria

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ABSTRACT

Background: Water is a resource that is both invaluable and vital to the existence of all living organisms, but this valued resource is increasingly being threatened as human populations grow and demand more water of high quality for domestic purposes and economic activities. The conducted research was aimed to assessing bacteriological quality of sachet water samples sealed and sold and consumed in some Local Governments. **Methods:** The bacteriological quality assessment of sachet water samples were determined by Aerobic Plate Count (APC), test for Coliforms and Biochemical Test (IMViC). The tests were preceded by staining technique. Moreover, the Aerobic Plate Count results revealed various values (CFU)/100ml of water samples collected and examined. **Results:** Going by zero tolerance levels stipulated by regulatory agencies for coliforms in drinking water, a cumulative figure of twenty five percent (25%) meets the standards of drinking water quality and subsequent percentages were satisfactory, suspicious as well as unsatisfactory which were in conformity with that of world health organization, 2010. The total percentage of good sachet water samples based on this research was 90% and that of bad samples was 10% as indicated. It revealed the presence of *Pseudomonas aeruginosa* in the sample waters. **Conclusion:** It has been concluded that most sachets water samples sold and consumed in Kano metropolis conformed with world health organiztion (WHO) recommended standards for potable water and the consumption of some sachet waters whose values are either above or below WHO and SON permissible limit may pose health hazards to the consumers.

Introduction

Adequate water quality needs seem to have improved greatly in some regions and countries especially in the developed world but for poor nations this is still a major issue [1]. According to [2], the main source of water in these regions includes untreated rain water from roofs, polluted

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rivers and streams, unprotected wells and bore holes. The quality of any surface or groundwater is a function of either or both natural influences and human activities. Without human influences, water quality would be determined by the weathering of bedrock minerals, atmospheric processes of evapotranspiration, and the deposition of dust and salt by wind. Others include the natural leaching of organic matter and nutrients from soil, hydrological factors that lead to runoff, and by biological processes within the aquatic environment that can alter the physical and chemical composition of water [3]. Water quality assessment is a very complex subject, in part because water is a complex medium intrinsically tied to the ecology of the planet [4]. To determine water quality therefore, several parameters must be examined. The complexity of water quality assessment as a subject is reflected in the many types of measurements of water quality. Water related health problems are a growing human tragedy, and according to [2], it kills more than 5 million people a year with infants being the most affected. This figure seems to be the highest as compared to wars and disasters [5]. The problems also prevent millions of people from leading healthy lives, and undermine developmental efforts by burdening the society with substantial socio-economic costs for treatment of water-borne diseases. This problem is of great significance in cities in developing countries, where polluted water, water shortages, and unsanitary living conditions prevail. Information from [6],[7] say although access to water has improved, access to safe water is still a major issue. The sources quoted that about some 1.1 billion people rely on unsafe drinking water sources in developing countries and the lowest drinking water coverage rates are in Sub Saharan Africa (58%) with a corresponding low sanitation coverage rate (36%) which leads to many deaths especially among children through diarrhea among other water-related diseases.

Materials and Methods

Study area

This Meteorologically, Kano metropolis is hot in most time of the year which makes sachet water business very lucrative. Kano is a region located between Latitude 10’03 north and between Longitude 7’10 East and 10’28 East in Northern Nigeria. Kano metropolis with about 9.4 million inhabitants [8] comprises of six local government areas namely: Dala, Fagge, Gwale, Municipal, Nasarawa and Tarauni.

Sample collection

The samples were collected in Nasarawa local government and Tarauni local government. Sixty different sachet water brands were collected from each of the local government areas. Three of every 20 of sachet water of a particular brand and bags were sampled randomly, for bacteriological analysis as described by [9]. This collection was repeated thrice. A total of one hundred and twenty (120) various samples of different brands were purchased at different locations i.e. motor parks, toll gates, schools, hospital and markets with codes allocated for each brand and location of purchase for this study. The sachet water samples used in this study were purchased and collected in labeled clean plastic containers/polythene bags and carried to the laboratory for analysis as described by [9].

Bacteriological analysis

Aerobic Plate Count (APC)

For the enumeration of mesophillic bacteria in treated water samples, the serial dilution method as described by the American Public Health Association [7] was employed. 11ml of water sample was mixed with 99 ml of 1% peptone water. The sample was shaken thoroughly to make a homogenate solution, this give the dilution of 10^{-1}. 1ml of this prepared solution was transferred in to 9ml of the diluents (0.1% peptone water), this give the dilution of 10^{-2}. This procedure was repeated up to the fifth dilution which gave the dilution of 10^{-5}. [10], the dilution bottles were agitated, 1ml of each dilution was then pipette into separate corresponding Petri dish in duplicates. About 15ml of nutrient agar (NA) cooled to 45^0C was poured in to each plate. The sample and the agar medium were mixed by rotating the plate on a flat surface and allowed to solidify. The Petri-dishes were then inverted and incubated at 35^0C for 48 hours. Plates containing between 30-300 colonies were selected and counted. The number obtained was multiplied by the dilution factor. This gave the number of bacterial colony forming unit per ml of the treated water sample, (CFU/ml). This above procedure was repeated for other treated (package) water samples [10].

The following formula used to calculate the number of bacteria colony forming units per mill of the treated (package) water samples [11]

$N(ml) = n/vd$

Where; N= the number of bacterial colony per ml of treated water sample.

n= Number of colonies counted. v= volume of sample (inoculums) used.

d= dilution factor.

N(ml) = $30/1*10^{-1}$ = 3.00cfu/ml Lower limit

N(ml) = $300/1*10^{-1}$ = 30.00cfu/ml Upper limit

Enumeration of Staphylococcus aureus

For the enumeration of *Staphylococcus aureus* in the well water samples, serial dilution methods as described by the American Public Health Association [7,12], was employed. 111ml of treated water sample was aseptically measured and transferred in a clean conical flask containing 99ml of 1% peptone water and stirred to make a homogenate mixture. Decimal dilution of the sachet water ($1\text{-}10^{-1}$, to $1\text{-}10^{-5}$, was prepared by successive transfer of 1ml of the treated water homogenate to 9ml sterile 0.1% peptone water in dilution bottles. 0.2ml of a dilution of the homogenate was pipette onto the surface of previously dried duplicate plate of Baird parker medium and a sterile bent glass rod was used to spread the inoculums. The plates were incubated at 37^0c for 24 hrs. Black and shining colonies were selected and counted. The result was reported as number of Staphylococcal colony forming unit per ml of the water sample (CFU/ml) [10].

Gram staining

A colony from the purified subculture was isolated and emulsified in sterile distilled water and a thin preparation was made on the slide. This was spread on the slide. After the smear, the slide was left on a rack to dry, the smear was then fixed using gentle heat to make sure too much heat is not applied which can affect or even kill the microorganism, [13]. The smear was then allowed to cool before staining. The glass slide containing the smear was placed on the staining rack and covered with crystal violet stain; the stain was then washed off with distilled water. The water was then completely tip off and the smear was covered with Lugol's iodine for 60sec. The iodine was also washed off using distilled water. The smear was then decolorized rapidly with acetone and washed immediately with clean water. The smear was covered with neutral red stain for 2min, and again was washed off using clean water. The back of the slide was wiped clean, and placed on a draining rack for the smear to air-dry, [10].

Biochemical tests/IMViC reactions

IMViC reactions are a set of four useful reactions that are commonly employed in the identification of members of family Enterobacteriacea, [10]. The four reactions are: Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test. The letter "i" is only for rhyming purpose.

i- **Indole test:** Some bacteria produce indole from amino acid tryptophan using the enzyme typtophanase. Production of indole was detected using Kovac's reagent. Indole reacts with the aldehyde in the reagent to give a red color. Example, bacteria: *Escherichia coli*: Positive; *Klebsiella pneumoniae*: Negative

ii- **Methyl Red (MR) test:** This is to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria produced large amounts of acids from glucose fermentation that they overcome the buffering action of the system. Example: *Eschericihia coli*: Positive; *Klebsiella pneumoniae*: Negative

iii- **Voges Proskauer (VP) Test:** While MR test is useful in detecting mixed acid producers. VP test detects butylene glycol producers. Examples: *Escherichia coli*: Negative; *Klebsiella pneumoniae*: Positive

iv- **Citrate utilization test:** This test detected the ability of an organism to utilize citrate as the sole source of carbon and energy. Examples: *Escherichia coli*: Negative; *Klebsiella pneumoniae*: Positive.

Sample Size

[14], provides a simplified formula to calculate sample sizes. This formula used to calculate the sample sizes in scientific researches. A 95% confidence level and p = 0.5 are assumed for the formula.

$$n = \frac{N}{1 + N * e^2}$$

n = $400/1+400*0.05^2$

n = 400/1+9

n = 400/10 = 40

Where n is the sample size, N is the population size, and e is the level of precision.

Results

Presumptive test triple values obtained from various packaged water samples collected from Nasarawa using MPN techniques.

No. of bottles giving positive reaction									
Samples	**1(50/50ml)**	**5(10/10ml)**	**MPN/10ml**	**1(50/50ml)**	**5(10/10ml)**	**MPN/10ml**	**1(50/50ml)**	**5(10/10ml)**	**MPN/10ml**
1	-	-	-	1	2	6	1	2	6
2	-	-	-	-	-	-	-	-	-
3	-	-	-	1	2	6	-	-	-
4	1	2	6	1	1	3	1	2	6
5	1	-	6	1	1	3	1	2	6
6	-	-	2	-	-	-	-	-	-
7	1	2	6	1	2	6	-	-	-
8	1	1	3	1	1	3	1	1	3
9	-	-	-	-	-	1	1	3	9
10	1	2	6	1	-	1	1	1	3
WHO	0	0	0	0	0	0	0	0	0

Triple presumptive test values obtained from various packaged water samples collected Tarauni using MPN techniques.

No. of bottles giving positive reaction									
Samples	**50/50ml)**	**5(10/10ml)**	**MPN/10ml**	**1(50/50ml)**	**5(10/10ml)**	**MPN/10ml**	**1(50/50ml)**	**5(10/10ml)**	**MPN/10ml**
1	1	1	3	-	1	1	-	-	-
2	-	-	-	-	1	1	1	2	6
3	-	-	-	1	1	3	-	-	-
4	1	2	6	-	1	1	1	-	2
5	1	-	2	1	2	6	1	-	2
6	-	-	-	-	-	-	-	1	1
7	1	2	6	-	1	1	-	-	-
8	1	1	3	1	4	16	1	1	3
9	-	2	2	1	1	2	-	-	-
10	1	-	2	-	1	1	1	-	2
WHO	0	0	0	0	0	0	0	0	0

Aerobic Plate Count values obtained from various sachet water samples collected from Nassarawa and Tarauni.

Colony Forming Unit (CFU)/100ml of sachet water samples								
Nassarawa					**Tarauni**			
S/No	A	B	N	10^{-1}	A	B	N	10^{-1}
1	76	84	80	8	67	92	79.5	7.95
2	81	93	87	8.7	92	100	96	9.6
3	69	80	74.5	7.45	100	118	109	10.9
4	53	73	63	6.3	83	99	91	9.1
5	66	69	67.5	6.75	79	85	82	8.2
6	TNC	TNC	---	---	52	67	59.5	5.95
7	73	80	76.5	7.65	93	100	96.5	9.65
8	69	76	72.5	7.25	TNC	TNC	---	---
9	104	109	106.5	10.65	66	73	69.5	6.95
10	TNC	TNC	---	---	69	75	72	7.2

TNC: Too Numerous To Count , APC: Aerobic Plate Count , CFU: Colony Forming Unit

Classification of sachet water samples collected and assessed according to WHO (2010) criteria for drinking water.

Class	**Grade**	**Presumption Count (per100mL)**	**Number of samples (n=100)**	**Percentage (100%)**
First	Excellent	0	45	25.00
Second	Satisfactory	1-3	72	40.00
Third	Suspicious	4-9	48	25.33
Last	Unsatisfactory	10 and above	15	09.66

Types of bacteria isolated from various sachet waters collected from different location in Nasarawa and Tarauni, Kano metropolis

Samples No.	**Location**	**Organisms isolated**
121 – 150	Nassarawa	*Staphylococcus aureus, Klebsiella spp*
151 – 180	Tarauni	*Salmonella typhi, Klebsiella spp, Bacillus subtilis and Streptococcus feacalis,*

The results of various biochemical tests carried out to determine the type of bacteria present in the sachet sample water collected within Kano metropolis.

Location	**Fermentation on MacConkey agar**	**Growth on EMB agar**	**Growth on Nutrient agar**	**Growth on DCA agar**	**Indole test**	**Citrate test**	**Urea test**
Nassarawa	+ve	+ve	+ve	-ve	-ve	-ve	+ve
Tarauni	+ve	+ve	+ve	+ve	-ve	+ve	-ve

Positive (+ve) growth, Negative (-ve) growth

Morphology, Gram staining and biochemical properties of bacterial isolates in sachet water samples sold in Kano metropolis.

Colonial Morphology	Microscopic Examination	Indole test	Methyl red	Voges Proskauer	Citrate test	Gram Staining	Suspected Organisms
Small circular colonies	**Short rod in singles**	-	-	+	+	-	**Salmonella spp**
Opaque cream yellow growth	**Gram positive cocci in clusters**	-	-	+	+	+	***Staphylococcus aureus***
Shiny viscous Colonies	**Gram negative short rod**	-	-	+	+	-	**Klebsiella spp**
Green metallic sheen colonies	**Gram negative rods**	-	-	+	+	-	**Pseudomonas spp**
Green metallic sheen colonies		+	+	-	-	-	***E. coli***

Discussion

This study has presented the bacteriological analysis of some sachet water samples taken from different residential areas and locations in Kano metropolis at random from August, 2015 to March, 2016. Almost all the sachet water were registered with appropriate regulatory agency (NAFDAC) and very few of the sachet water producers indicated manufacturing date, expiring date and batch number on the sachet, therefore not complying with the labeling compliance as stipulated by the [15]. Bacteriological analyses were based on Coliform count using the Most Probable Number (MPN) technique. The results of coliform count using the MPN which defined the degree of contamination and the bacteriological quality of the collected sachet drinking water sample brands in the study area. Previous studies in other parts of the country and Kano itself reported similar bacterial load indicative of poor water quality [16]. Relatively high aerobic colony counts are indicative of poor, unhygienic handling and processing [17].

Bacterial growth in water may be unnoticed even in transparent packaged water and the presence of some of these microorganisms may pose a potential risk to consumer [15]. This indicates that the consumer would not have any clear information of knowing if the water is within the standard limit for drinking water. It can be seen that most samples of the water brands were found to contain coliform species, which is about 64% of the total number of the sachet water brands examined. Therefore, the presence of species as well as *E. coli* which is also a member of the *coliform* group found in the water sample brands, suggests that these sample of water brands have been contaminated with feces either of human or animal origin [16]

Going by the zero tolerance levels stipulated by regulatory agencies for coliforms in drinking water, a cumulative figure of twenty five percent (25%) meets the standards of drinking water quality and is subsequent percentages were satisfactory, suspicious as well as unsatisfactory as shown in **table (3)**, which were in conformity with that of [18]. Moreover, the presence of *Pseudomonas aeruginosa* in the sachet sample water brands suggested that the contamination of the water was either through decay of wastes, improper sanitization and sterilization of the factory equipment or instrument used in the production processes. It can also result from the use of unsterile polythene which was used for the packaging of the water meant for human consumption. The presence of *Salmonella typhi* in the sachet water samples suggested that there was serious pathogenic water borne threat, capable of causing disease to consumers. The aerobic plate count (APC) result indicated that most of the pure water samples are in conformity with [19] set values for drinking water

quality. Based on APC results, Sample number 8 was the only contaminated sample from Tarauni with too numerous colony to count. All the samples collected from Nassarawa presented various values of minimal bacterial load. The total percentage of safe sachet water samples based on this research was 90% and that of unsafe samples was 10%.

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