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Original Research Article

Bacteriological Assessment of Some Sachet Water Samples Sold In Dala Compared With That of Fagge, Kano Metropolis

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Abstract: Water is one of the most significant or vital resource for human life and welfare, contamination of water sources is one of the areas of major concern in the public health. Bacteriological assessment and physicochemical analysis of sixty (60) brands of sachet water samples sealed and sold in Kano metropolis was conducted. The bacteriological evaluation of the sachet water samples collected were determined by test for Coliforms using Most Probable Number, presumptive test, confirmatory test and completed test for coliforms. This was continued by Biochemical test, staining technique and concluded with microscopy were some members of Coliforms were viewed. The bacteriological quality of the water samples were examined using multiple tube techniques (MPN). Majority of the sixty (60) brands of sealed sachet water samples contained bacteria**.** Going by result obtained and presented in table 4.4.7 only 06.66% samples were unsatisfactory, 28.33% were suspicious samples, 40.00% were satisfactory and 25.00% were excellent samples. This can be presented in ascending order as follows; unsatisfactory (06.66%) < excellent (25.00%) < suspicious (28.33%) < satisfactory (40.00%). Going by the zero tolerance levels stipulated by regulatory agencies for coliforms in drinking water, accumulative figure of 25% meets the standards of quality water and a cumulative figure of 75% (n = 100) of all the identified packaged water did not meet the existing Standard stipulated by regulatory agencies for coliforms in drinking water. The isolates were identified using culture, morphology and biochemical characterization method by using samples collected from different sites of study areas. The identified isolates include: *Escherichia coli*, *Streptococcus feacalis*, *Bacillus subtilis*, Staphylococcus spp, *Pseudomonas aeruginosa*, Klebsiella spp and *Salmonella typhi*. \Subsequently, the proper settings of water sources for public consumption, treatment and maintaining the existing water supply facilities is of great importance toward reducing and eliminating health threats so as to improve public health.

Keywords: Bacteriological, physicochemical, isolates and public health.

INTRODUCTION

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 population grow and demand more water of high quality for domestic purposes and economic activities. There is a growing concern everywhere that in the coming century, cities will suffer imbalances in quality water supply, consumption, and population. Many regions of the world are already limited by the amount and quality of available water According to WHO (2004).

In the next thirty years alone, accessible water is unlikely to increase more than ten percent (10%), but the earth's population is projected to rise by approximately one-third. Unless the efficiency of water use rises, this imbalance will reduce the quality of water, reduce the conditions of health of people and deteriorate the environment and the world. Micro-organisms such as faecal coliform bacteria (*Escherichia coli*), cryptosporidium and *Giardia lamblia*; nutrients (fertilizers), dissolved metals and metalloids (lead, mercury, arsenic, etc.) and dissolved organics. Water related health problems are a growing human tragedy, and according to WHO (2004), Pollutant represents any biological, chemical and physical residue present in

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the groundwater in excess of WHO and SON limits that may be harmful when consumed. Groundwater pollution can result from the contamination through physical, chemical or biological processes facilitated by man or nature. Water Quality describes the amount of biological, chemical and other residues in a sample of 75ml, with specific reference to WHO & SON standards. The objectives of this study are: To determine the bacteriological quality of sampled sachet water sold in Dala and Fagge and also to determine some of the physical parameters of sampled sachet water sold in Dala and Fagge, Kano metropolis.

MATERIALS AND METHODS

Site of sample collection

The samples were collected in six areas as: A = Dala local government, and B = Fagge local government,

Bacteriological Examination

Most Probable Number

The Microbiological procedure was used by employing Most Probable Number technique as described (Cowan and Steel, 2003). Primary isolation was done by using MacConkey broth, a sterile 10ml syringe and needle was used in withdrawing from the sample sachet water and also dispensed aseptically into 5 bijou bottles each containing 10ml of the media broth and then 50ml of the samples was dispensed into 1 bijou bottle containing 50ml of the media broth. Durham tube was also inverted in each and every bijou bottle of both 10 by 10 (ml) and that of 50 by 50 (ml) accordingly. Then the bottles were closed tightly, shaken and distributed the samples uniformly throughout the medium and to make sure the inverted Durham tube was full of broth and there's no air bubbles trapped inside it. The bottles will then be incubated at 37ºC for 24hrs. This procedure have been carried out in a clean-lighted flow hood and repeated for the remaining 179 brands of samples. The corresponding Most Probably Number (MPN) index has been determined using the probability/statistical table (Cowan and Steel, 2003).

Determination of Total Coliform

The total coliform in pure water samples was determined, three basic principal tests has been employed, which are: the presumptive, the confirmed and the completed test of coliform.

Presumptive Coliform Test

The test is called presumptive because the reaction observed may occasionally be due to the presence of some other organisms and the presumption that the reaction is due to coliform organisms and this has been confirmed. An estimated number of coliform organisms usually made by adding varying quantities of water (10ml and 50ml) to double strength MacConkey's broth and single strength MacConkey's broth containing bromocersol blue sterilized in bijou bottles containing Durham's tube (for indication of gas production). This has been done with the help of sterile pipettes/syringes all the two sets of bijou bottles have been filled with 50ml and 10ml quantities of water sample. Then, incubation will be made possible at 37^{o}C for 24 hours to 48 hours for estimation of total coliforms and for faecal coliforms at 44^{o}C for 24-48 hours and observation for the production of acid and gas will be done. Broth color will change to yellow from reddish purple which indicates the production of acid, while gas entrapment in the Durham tubes will be an indication of gas production. Then the number of bottles that shows positive result will be recorded and referred to MPN statistical table (Cowan and Steel, 2003).

Confirmed Test

Confirmed test was done by transferring a loopful of culture from a positive tube of the presumptive test into a tube of brilliant green lactose bile broth (oxoid) with Durham tubes. The tubes were incubated at 37°C for 24-48 hours for total coliforms and 44.5°C for 24-48 hours for fecal coliform and gas production were observed.

Completed Test

To complete this test, complete test step was applied as in accordance with World Health Organization, (2012) by streaking a loopful of broth into Eosin Methylene Blue agar plate from a positive tube for pure colonies. Then plates should be incubated at 37^{o}C for 24 to 48 hours. Growth of colonies on MacConkeys agar or EMB agar should be observed. Then, by using culture characteristic, morphology and biochemical test colonies will be categorized as coliforms or faecal coliforms (*E. coli*). For fecal coliforms, colonies with green metallic sheen are said to be Gram stained and the IMVIC test should be carried out to recognize the colony as *E*. coli. Preparation and fixing of smear: A colony from the purified subculture was isolated and emulsified in sterile distilled water and a thin preparation should be made on the slide. This was evenly spreaded on the slides to cover an approximated area of about 15-20mm in diameter. After the smear, the slide left on a rack to dry making sure the slide was protected from dust and sunlight. The smear was then fixed using gentle heat, by rapidly passing the slide with the smear uppermost, three times through the flame of a Bunsen burner. The slide will then be placed on the back of the hand just to make sure too much heat is not applied, which can affect or even kill the microorganism. The smear should then be allowed to cool before staining Cheesebrough (2004).

Staining: The glass slide containing the smear should be placed on the staining rack and covered with crystal violet stain and allowed for 60sec. The stain will then be washed off with distilled water. The water will then completely tip off and the smear should be covered with Lugol's iodine for 60sec. The iodine will also be

washed off using distilled water. The smear will then be decolorized rapidly with acetone and to be washed immediately with clean water. The smear will be covered with neutral red stain for 2min, and again should be washed off using clean water. The back of the slide should be wiped clean, and placed on a draining rack for the smear to air-dry Cheesebrough (2004).

Microscopy: Using a microscope the smear should be examined with 40x objective lens, to check the staining and to see the distribution of the material (Microbes).

Biochemical test: various media, broth, reagents, agar etc. was used in the biochemical test of sachet water, these include; MacConkey Broth, MacConkey agar, Nutrient agar, Deoxycholate agar (DCA), Kligler Iron Agar (KIA), Peptone broth, Eosin Methylene Blue Agar (EMB), Citrate agar, Urea agar and Oxidase reagent, Antisera, Crystal violet stain, Lugol's iodine, Acetone-alcohol decolorizer and Neutral red. The isolated organisms were subjected to biochemical tests as described by Cheesebrough (2004).

Kligler Iron Agar (KIA) test: Using a sterile wire loop, a colony from the purified subcultures was isolated and stabbed straight down in the slanted agar medium. The wire loop was removed, flamed, sterilized and the inoculum was streaked on the surface of the slant. The test tube was covered tightly with a screw cap and labeled accordingly before it was placed into the incubator where it will be left for 24h at 37ºC. It then be removed and observed for fermentation, this could be shown by a change in color from bright red to amber yellow Cheesebrough (2004).

Urease test: Using a sterile wire loop, a colony from the purified subculture will be isolated, stabbed and streaked on the surface of the media. The bijou bottle will be then covered tightly with a screw cap and labeled accordingly before it will be placed into the incubator for 24h at 37ºC. It will then be removed and observed for growth Cheesebrough (2004).

Citrate Test: The Procedure Is The Same As In Urea Agar.

Indole test: Using a sterile wire loop, a colony from the purified subcultures will be isolated and inoculated into the bijou bottles containing 3mL of the sterile peptone water. The mouth of the bijou bottles will then be flamed sterilized and to be covered tightly with a screw cap and labeled accordingly, it will be incubated for 24h at 37ºC. The bijou bottles will be then removed and 0.5mL of Kovac's reagent was added. The bijou bottles will then be shaking gently and left standing for 10min. Examination for positive result is done by the formation of red color in form of a ring on the surface layer of the culture media Cheesebrough (2004).

Oxidase test: The oxidase test is used in the identification of pseudomonas species will produce the enzyme cytochrome oxidase (Chessbrough, 2004). A piece of filter paper will be placed in a clean Petri dish and 2 or 3 drops of the oxidase reagent will be added to it. Using a sterile wire loop, a colony from the purified subculture nutrient agar was removed and a smear will be made on the filter paper. The filter paper will be left for 10sec after which it will be observed for the development of a blue purple color as a positive oxidase test Cheesebrough (2004).

Serotyping for salmonella; Glass slide was cleaned and sterilized using 70% ethanol. Two drops of saline suspension were dropped on the left side of the slide and two drops of salmonella antiserum were also dropped on the right side of the slide. Using a sterile wire loop, a colony from the purified subculture (Kligler Iron Agar) was isolated and a smear was made on both the saline suspension and the salmonella antiserum. The glass was then brought under an electric fluorescent lamp for observation of agglutination. A positive result was observed when agglutination forms only on the salmonella antisera smear Cheesebrough (2004).

RESULTS

Table 1: Presumptive test triple values obtained from various packaged water samples Collected from site A (DALA) using MPN techniques

	No. of bottles giving positive reaction								
Samples	**1(50/50)**	**5(10/10ml)**	**MPN/10ml**	**1(50/50)**	**5(10/10ml)**	**MPN/10ml**	**1(50/50ml)**	**5(10/10ml)**	**MPN/10ml**
1	1	2	6	1	2	6	0	1	1
2	0	0	0	0	0	0	0	0	0
3	1	4	16	1	3	9	0	1	1
4	1	2	6	1	0	2	1	2	6
5	1	2	6	1	0	2	1	3	9
6	0	0	0	1	5	18+	0	1	1
7	1	2	6	1	2	6	0	0	0
8	1	1	3	1	1	3	1	1	2
9	0	0	0	0	0	0	1	3	9
10	1	1	3	1	0	2	1	4	16
WHO	0	0	0	0	0	0	0	0	0

Source: Laboratory Assessment, 2016

Table 2: Presumptive test triple values obtained from various packaged water samples collected from site B (FAGGE) using MPN techniques.

	No. of bottles giving positive reaction								
Samples	**1(50/50ml)**	**5(10/10ml)**	**MPN/10ml**	**1(50/5)**	**5(10/10ml)**	**MPN/10ml**	**1(50/50ml)**	**5(10/10ml)**	**MPN/10ml**
1	- 0	1	1	1	3	9	1	0	2
2	0	0	0	0	1	1	0	0	0
3	0	1	1	1	4	16	1	2	6
4	1	2	6	1	2	6	1	1	3
5	1	3	9	1	2	6	1	2	6
6	0	1	1	0	0	0	1	3	9
7	0	0	0	1	2	6	1	3	9
8	1	1	2	1	5	18+	1	1	3
9	0	1	1	0	0	0	0	0	0
10	1	4	16	1	2	6	1	2	6
WHO	0	0	0	0	0	0	0	0	0

Source: Laboratory Assessment, 2016

Table 3: Class, Grade, Percentage (%) and Classification of Sachet water samples collected and assessed according to WHO (1997) 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	51	28.33
Last	Unsatisfactory	10 and above	12	06.66

Source Lab. 2016

Table 4: Shows water quality in terms of percentage of the bacterial load between the sampling areas from the most excellent to the most unsatisfactory.

	SAMPLE GRADE					
Locations	Excellent	Satisfactory	Suspicious	Unsatisfactory	Good samples in percent (%)	Bad samples in percent (%)
Nassarawa	10	9	11	0	100	0
Tarauni	8	17	4	1	97	3
Municipal	7	13	8	2	93	7
Gwale	7	14	6	3	90	10
Dala	6	10	10	3	90	10
Fagge	6	9	12	3	90	10
Total	30	30	30	30	93	7

Table 5: Morphology, Gram staining and Biochemical properties of bacterial isolates in sachet Water samples sold in Kano metropolis.

Colonial Morphology	Microscopic Examination	Fermentation on MacConkey agar	Growth on EMB agar	Growth on Nutrient agar	Growth on DCA agar	Indole test	Citrate test	Urea test	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*
		+	+	+					*Streptococci faecalis*
		+	+	+					*Bacillus Subtilis*

Source: Laboratory Analysis, 2016

DISCUSSION

Bacteriological analyses in some sachet water sample shows that Bacteria and *E. coli* were detected in some water samples indicating recent human or animal fecal contamination of the sachet water sampled and analyzed. This is attributed to either fecal contamination or poor sanitation during purification, sealing, transportation selling or consumption. All the sachet water were registered with appropriate regulatory agency (NAFDAC) but none of the sachet water producers indicated manufacturing date, expiring date and batch number on the sachet, therefore not complying with the labeling guideline as stipulated by the SON, (2009). The result of coliform count using the Most Probably Number (MPN) is shown and which defined the degree of contamination and the bacteriological quality of the sachet drinking water sample brands. Going by result obtained and presented in table 4.4.7 0nly 06.66% samples were unsatisfactory, 28.33% were suspicious samples, 40.00% were satisfactory and 25.00% were excellent samples. This can be presented in descending order as follows; unsatisfactory (06.66%) < excellent (25.00%) < suspicious (28.33%) < satisfactory (40.00%). Going by the zero tolerance levels stipulated by regulatory agencies for coliforms in drinking water, accumulative figure of 25% meets the standards of quality water and a cumulative figure of 75% (n = 100) of all the identified packaged water did not meet the existing Standard stipulated by regulatory agencies for coliforms in drinking water. The most bacteriologically Unsatisfactory samples are sample 6* > 3 and 10 from Dala, 8* > 3 and 10 from Fagge, 10* > 6 from Municipal and sample 8* from Tarauni. The satisfactory samples are 2 > 6 from Nassarawa, 6 > 3 from Tarauni, 2 > 3 > 4 from Municipal, 2 from Tarauni Dala and Fagge and samples number 9 > 1 > 8. Previous studies in other parts of the country reported similar bacterial load indicative of poor water quality (Olayemi, 1999; Itah and Akpan, 2005).

The result of coliform count using the Most Probably Number (MPN) is shown and which defined the degree of contamination and the bacteriological quality of the sachet drinking water sample brands. From Nassarawa which is the first ranking of good water quality, 10 samples were examined Excellent, 9 samples Satisfactory, 11 samples Suspicious and zero samples Unsatisfactory. The second class is Tarauni, Excellent 8 samples, Satisfactory 17 samples, Suspicious 4 samples, and Unsatisfactory1 sample. Third class is Municipal, Excellent 7 samples, Satisfactory 13 samples, Suspicious 8 samples, and Unsatisfactory 2 samples. Fourth class is Gwale, Excellent 7 samples, Satisfactory 14 samples, Suspicious 6 samples, and Unsatisfactory 3 samples. The Fifth is Dala, Excellent 6 samples, Satisfactory 10 samples, Suspicious 10 samples, and Unsatisfactory 3 samples. The last and sixth is Fagge, Excellent 6 samples, Satisfactory 9 samples, Suspicious 12 samples, and Unsatisfactory 3 samples.

Going by result obtained and presented in table 4.4.7 0nly 45 samples were Excellent, 72 samples were Satisfactory, 51 samples were Suspicious and 12 samples were and Unsatisfactory. The most bacteriologically Unsatisfactory samples are sample 6* > 3 and 10 from Dala, 8* > 3 and 10 from Fagge, 10* > 6 from Municipal and sample 8* from Tarauni. The satisfactory samples are 2 > 6 from Nassarawa, 6 > 3 from Tarauni, 2 > 3 > 4 from Municipal, 2 from Tarauni Dala and Fagge and samples number 9 > 1 > 8. Previous studies in other parts of the country reported similar bacterial load indicative of poor water quality (Olayemi, 1999; Itah and Akpan, 2005). Relatively high aerobic colony counts are indicative of poor, unhygienic handling and processing, bacteria 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 (Geldriech, 1996).

The result obtained and presented in table 4.4.8 the whole total of 06.66% samples were unsatisfactory, 28.33% were suspicious samples, 40.00% were satisfactory and 25.00% were excellent samples. This can be presented in descending order as follows; unsatisfactory (06.66%) < excellent (25.00%) < suspicious (28.33%) < satisfactory (40.00%). Going by the zero tolerance levels stipulated by regulatory agencies for coliforms in drinking water, accumulative figure of 25% meets the standards of quality water and a cumulative figure of 75% (n = 100) of all the identified packaged water did not meet the existing Standard stipulated by regulatory agencies for coliforms in drinking water.

The result of Morphology, Gram staining and Biochemical properties of bacterial isolates in sachet Water samples sold in Kano metropolis presented in table 4.2.9. The isolated bacteriaincludes; *staphylococcus aureus* which was the common, *Pseudomonas aeriginosa, E.coli and Klebsiella spp.* in sachet Water samples sold in Kano metropolis.

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